The Longevity of the Humoral Immune Response: Survival of Long-lived Plasma Cells

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The production of antibodies by terminally differentiated plasma cells is the central humoral response that mediates host immunity to infections. Many plasma cells are short-lived and die by apoptosis after several days. Long-term production of antigen-specific antibodies is usually attributed to memory B cells. However, the central paradigm of plasma cell biology is changing with the discovery of long-lived plasma cells which contribute to life-long humoral immunity. Long-lived plasma cells have only been recognized as a part of immunological memory in the last decade – there is much to learn about the complexities of their development and survival. In this article, I review the extrinsic signals involved in long-lived plasma cell longevity, with an emphasis on how the bone marrow microenvironment contributes to the survival of long-lived plasma cells. Insights into these mechanisms will have a profound impact on plasma cell biology and provide novel therapeutic targets in autoimmune diseases and plasma cell malignancies.

KEYWORDS: Humoral Immunity; B cells; Long-lived Plasma Cells; Antibody

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The main function of the immune response is to generate an appropriate and robust response to defend against foreign stimuli, i.e., infection by viruses or bacterial pathogens. As the main producers and secretors of antibodies, terminally differentiated B-cells, also called plasma cells (PCs), represent the humoral arm of this response [1, 2]. Antibodies can directly neutralize microbes by blocking factors essential for their survival and also mark the pathogen for clearance by other cells of the immune system [3, 4]. A remarkable property of humoral immunity is the long-lasting ‘memory’ of the response, providing decades and sometimes life-long protection against previously encountered pathogens or antigens [5, 6, 7, 8, 9]. The longevity of humoral immunity is provided by two different types of B-cells: memory B cells (Bmem) and long-lived plasma cells (LPCs). The role of Bmem in long-lived humoral immunity has been extensively reviewed elsewhere [10, 11]. Here, I will review recent developments which have led to a renewed understanding of the longevity of PCs and provide a detailed mechanistic overview of factors in the bone marrow microenvironment that sustain their survival. LPCs persist after the resolution of an immune reaction and contribute to an enhanced protective state during re-infection. They are substantially different from Bmem cells as they remain non-dividing in the bone marrow and secrete abundant amounts of antibodies for extended periods of time [8, 9]. LPC persistence do not require presence of the antigen [12].

Before the discovery of LPCs, PCs were considered to be differentiated B-cells which survive for less than a week [1, 10, 13] and antibody levels were presumed to be maintained by continuous generation of PCs from activated B cells or Bmem after antigenic challenge [14, 15]. This principal dogma was derived from several lines of evidence. Very early in vivo studies suggested PCs

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were short-lived [16, 17, 18] and these findings were substantiated by later studies that observed that PCs rapidly die after ex vivo isolation as PCs fail to survive after three days of in vitro cell culture [19, 20]. PC numbers were also observed to decline to very low numbers in secondary lymphoid organs after their initial peak response to an antigen [21, 22].

This textbook paradigm was re-examined when two different research groups, using mouse models, found evidence that PCs can be long-lived. In 1997, Manz et al. [8] used proliferation markers to differentiate between short- and long-lived anti-ovalbumin-secreting PCs in the bone marrow of mice secondarily immunized with ovalbumin. Seventy percent of these anti-ovalbumin-secreting PCs migrated to the bone marrow within the first month after immunization, and persisted for at least 2 months without proliferation (characterized by a lack of cell division and DNA synthesis) while constantly secreting antibodies. The same group then further demonstrated that the persistence of these cells did not require the presence of antigen [12]. The second group, Slifka et al. [9], employed a different approach. Using lymphocytic choriomeningitis (LCMV)-infected mice, they demonstrated that these mice continued to produce specific LCMV-antibodies for 250 days after irradiation. Irradiation depletes memory B cells and prevents generation of new PCs. Adoptive transfer of these plasma cells into naïve non-immunized mice also resulted in consistent anti-LCMV antibody titres.

Direct evidence for LPCs in humans has not been demonstrated thus far, but several studies point to their existence. The first observation was obtained from patients with autoimmune diseases who were treated with the therapeutic antibody rituximab, which depletes all B-lineage cells except those that secrete antibodies [23]. Total serum antibody titres of these patients were found to remain within normal range and stable in the long-term after rituximab treatment even though naïve and memory B cells were confirmed to be absent [24, 25, 26]. In other cases, specific serum antibody titres remain stable for decades in humans even without repeated antigenic challenge for more than 70 years [6]. This phenomenon was also independent of the number of vaccine administrations [6, 27]. In addition, recent findings from Mesin et al. [28] suggests that LPCs are present in the human small intestine. As the evidence for LPCs contributing to long-lived humoral immunity continues to increase, these new cells provide novel targets and new therapeutic opportunities for autoimmunity and PC malignancies such as multiple myeloma.

The importance of antibodies and long-lived humoral immunity has been acknowledged for decades [29, 30]. Indeed, the ability of naïve B cells to differentiate into antibody-producing cells is the basis of using vaccines [31, 32]. The emergence of a new player (long-lived plasma cells) contributing to this process brings about a need to understand its role and importance in immunological memory. Importantly, we need to appreciate how the regulation and formation of LPCs is controlled, as failure to balance between a continued production of antibodies targeting foreign antigens and choosing when to turn off this response may result in collateral damage to host cells [33]. In severe cases, dysregulation of this response may result in the development of multiple myeloma [34, 35] or autoimmunity diseases [36, 37, 38]. There is increasing evidence that the survival of LPCs is

**Figure 1. Development of B cells and generation of short- and long-lived plasma cells in mice and humans.**

B cells develop from hematopoietic stem cells (HSC) present in bone marrow and migrate to lymphoid tissues upon maturation (naïve/follicular B cell). Upon encountering an antigen, naïve B cells can differentiate into plasmablasts and short-lived PCs which provide a first-line defense against infections. Some of these activated naïve B cells can enter germinal centre (GC) reactions. In GCs, B cells first undergo several rounds of expansion before beginning somatic hypermutation (SHM) and class switch recombination (CSR) (for further details, see [39, 50, 51, 52]). These cells are in a state of activated apoptosis and will die unless they obtain a survival cue from dendritic cells or T cells in the GC environment. In this GC selection process, B cells with BCRs that have a higher affinity for the unique antigenic epitope are selected to survive whereas B cells with low affinity BCRs are out-competed and die. With a survival signal, these B cells then leave the GC where they develop into plasmablasts and PCs and begin to secrete antibodies. PCs become LPCs if they are able to migrate into specific survival niches in the bone marrow and receive survival signals from cells in the microenvironment. CLP: Common lymphoid progenitor.
influenced by extracellular conditions which regulates their lifespan. Below, I will briefly summarise the developmental pathway of LPCs and review the current findings and interpretation of the extrinsic factors which regulate the formation and survival of LPCs. The bone marrow microenvironment as a survival niche for LPCs will also be discussed.

Development of long-lived plasma cells

Plasma cells are the differentiated result of a series of cellular maturation steps beginning from a bone marrow hematopoietic multipotent stem cell (fig. 1). This multistep developmental pathway yields a naïve B cell expressing a functional B cell receptor (BCR) and is antigen-independent [1, 59]. Activation of B cells via the BCR induces naïve B cells to differentiate into short-lived antibody-secreting plasmablasts (fig. 2) [1, 14]. Plasmablasts are larger cellular precursors to mature plasma cells which retain the ability to proliferate and migrate into the bone marrow or during autoimmune and inflammatory conditions, immigate to inflamed tissues [10, 14, 40]. The migratory ability of plasmablasts is achieved by alterations in the expression of chemokine receptors. Chemokines are specific chemical signals that are upregulated or secreted in tissues to attract and recruit specific immune cells which express the matching chemokine receptor [43]. Upon arrival at tissue niches, the plasmablasts terminally differentiate into non-migratory PCs which either reside there for several days before dying by apoptosis or persist as LPCs over years [14, 40, 44, 45]. Few intrinsic differences have been found between PCs and LPCs, as genes involved in B cell to PC differentiation, adhesion and survival have been found to be essential for all PCs [40]. (cf. a recent manuscript demonstrates that LPCs in the bone marrow were able to respond to signalling via the receptor molecule CD28 which was not observed in short-lived PCs from the spleen [46]). Instead, current data suggests they are regulated by extrinsic factors. The extrinsic determinants which govern the survival of PCs are discussed in further detail in the next few sections.

Although it is clear that PCs are differentiated end-products of activated B cells, the origin from which PCs arise can differ. The route from which PCs arise depends on the type, dose and form of antigen as well as the current location of the B cell [39, 47]. This, in turn, affects the strength and timing of signals propagated by the BCR. Further, PCs can emerge from naïve or memory B cells upon encountering antigen and be dependent or independent of T cell help [1, 39]. Although both dependent and independent T cell help can induce naïve B cells to become plasmablasts, T cell-dependent help induces B cells to seed germinal centres in lymphoid follicles. Germinal centres are specific, defined structures in secondary lymphoid tissues where mature B cells proliferate [33, 48, 49]. In addition, antibodies at germinal centres undergo affinity maturation (a situation whereby antibodies that are produced against a specific antigen display increasingly greater affinity for the antigen over the time of the immune response) by somatic
hypermutation (SHM) [50, 51] as well as antibody class switching [52]. The development of the germinal centre has now been well-defined and is outside the scope of this review. For further reading, I direct readers to an excellent review by Klein and Dalla-Favera [48].

Why is the route of PC differentiation important? The current literature suggests that PCs generated outside of germinal centres (i.e., extrafollicularly) are thought to be mostly short-lived [1, 10]. They provide a rapid initial response to pathogens but undergo apoptosis in situ within a few days [13, 53]. In contrast, PCs generated from germinal centres with T cell-dependent help mostly migrate to the bone marrow [10, 54] with a small proportion remaining in the spleen [53]. Additional studies have also demonstrated that PCs can migrate and persist in inflamed tissues under chronic inflammatory conditions [58, 55]. This migration to bone marrow (and/or other sites) plays an important role in determining whether a PC becomes long-lived or not, as little evidence exists to demonstrate that LPC generation is dependent on intrinsic or internal cell signals. Consistent with this idea, LPCs harvested from bone marrow exhibit affinity-matured antibodies [8, 9, 40] suggesting that they emerged from germinal centre reactions. At the moment, current evidence suggests that the lifespan of a PC is dependent on multiple extrinsic survival factors and cell-cell interactions. Therefore, being a LPC is conditional upon locating a dedicated plasma cell survival niche such as the bone marrow - if a plasma cell arrives at the right place, it can become long-lived.

**Extrinsic factors for survival secreted by the bone marrow microenvironment**

Bone marrow hosts the majority of LPCs and plays an important role in their survival. Increasing experimental data points towards the idea that the bone marrow functions as an ecological ‘niche’ where the microenvironment provides survival signals to LPCs through ‘cellular survival factors’. Here, I will first review the extrinsic factors involved in the regulation of LPC survival, before discussing how the bone marrow microenvironment provides a survival niche to maintain a long-lived plasma cell.

The removal of PCs from bone marrow for in vitro cell culture led investigators to observe that PCs had a poor survival rate [19]. This brought about studies which attempted to isolate soluble factors that could sustain PCs and contribute to the longevity of LPCs. The cytokines IL-5, IL-6, TNFα and CXC-chemokine ligand 12 (CXCL12, also known as stromal cell-derived factor 1) were identified as individual factors which improve LPC survival in vitro, promoting the survival of seeded LPCs for at least 3 days [19]. Intriguingly, the role of IL-6 as an essential survival signal for LPCs was initially contradictory. In vitro studies indicated that it is a crucial factor for the survival of bone marrow derived plasma cells [19, 56, 57]. In addition, IL-6 had the most pronounced effect on PC survival, as addition of this cytokine rescued 70% of cultured PCs [19]. However, LPC numbers and persistence were similar in IL-6-deficient mice as compared to wild-type mice [19] which indicates that IL-6 was not essential for LPC survival in vivo. Interestingly, the presence of PCs alone could induce the production of IL-6 from bone marrow stromal cells (BMSCs), which suggests that PCs themselves could contribute to the formation of a survival niche [56]. BMSCs are a collection of adherent cells (endothelial cells, reticular cells, adipocytes, osteoblasts, stromal fibroblasts and smooth muscle cells) in bone marrow with a variety of immunological roles including the maintenance of immunological memory and LPC survival [56, 58, 59]. The observation that PCs themselves contribute to a survival niche is not unlike the tumorigenesis of multiple myeloma (MM), whereby BMSCs stimulated by MM cells secrete abundant amounts of IL-6 which then promotes the growth of malignant MM cells [55, 60]. Thus, the production and effector function of IL-6 appears to result in a positive feedback loop for PC survival.

However, even when these factors were collectively provided in vitro, their synergistic operation did not result in a complete recovery of plasma cells [19]. Even the co-incubation of these cells with bone marrow supernatant did not result in a prolonged PC survival rate [19]. These observations suggest that non-soluble factors (i.e., cell-cell interactions) most likely provided additional signals for LPCs survival. To determine which bone marrow cell types could provide those interactions, Roldan et al. [61] showed that adherent cells (but not non-adherent cells) fractionated from bone marrow cell suspensions were able to support PC survival in vitro. To investigate if cellular interactions between LPCs and BMSCs play a role in LPC survival, BMSCs were used as feeder cells for LPCs in vitro, and significantly increased the survival of LPCs for up to 4 weeks [56]. Further investigations revealed that VLA-4 and VCAM-1 interactions between LPCs and BMSCs provided the supportive effect [56, 62, 63]. Signalling via CD44 was also shown to promote a modest survival response [19]. CD44 is a cell surface glycoprotein involved in cell-cell interactions, cell adhesion and migration [64]. Its principal ligand is hyaluronic acid
(HA) which is a common component of the extracellular matrix [64, 65].

Another group of important survival factors is the tumour necrosis factor subfamily of ligands and receptors. The receptors in this subfamily include the transmembrane activator and calcium modulator ligand interactor (TACI), B-cell maturation antigen (BCMA) and B-cell activating factor receptor (BAFFR). The ligands are B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL). The precise interactions between each ligand and receptor have been difficult to identify [66, 67, 68, 69] because the ligands are able to adopt monomeric or oligomeric forms. These forms or complexes can also be soluble or insoluble. As a result, the different ligand forms greatly affect their in vitro interactions with the receptors (reviewed in [70]). Nevertheless, it is clear that BAFF-BAFFR interactions do not play a role in LPC survival [70, 71]. In contrast, interactions between the ligands (BAFF, APRIL) with the receptors (BCMA, TACI) have been shown to be important for the survival of LPCs. In vitro studies demonstrated impaired LPC survival in BCMA−/− mice [72], and that blockage of TACI with TACI-IgG caused a decay in the quiescent LPC population [71, 72]. TACI was later shown to be necessary for the sustained expression of a B-cell gene that regulates the differentiation of long-lived antibody secreting cells [73]. Noelle’s group [71] also demonstrated that eradication of the LPC population from bone marrow required the neutralization of both BAFF and APRIL ligands. The presence of either BAFF or APRIL is sufficient for long-term PC survival which indicates that these two ligands have redundant supportive roles.

The bone marrow microenvironment as a survival niche for LPCs

How do these soluble and insoluble factors contribute to a specific survival niche in bone marrow? With knowledge of the factors involved and their role in LPC survival, a representation of the interactions occurring within the bone marrow microenvironment can be envisioned (Fig. 3). Following activation in the GC and differentiation, plasmablasts migrate to the bone marrow [49]. As with most immune effector cells, the homing of the PC to bone marrow is controlled by a potpourri of adhesion molecules, chemokines and receptors [74]. Plasma cell homing to the bone marrow is dependent on three chemokine receptors: CXCR3 [21, 75], CXCR4 [41, 76] and CXCR6 [77] which bind to CXCL9, CXCL12 and CXCL16 respectively. Following the migration of plasmablasts to bone marrow, these cells bind to BMSCs which express vascular cell-adhesion molecule (VCAM-1) and VLA-4 [62, 65]. Endothelial-cell selectin (E-selectin) on the surface of BMSCs will also retain these PCs in bone marrow by binding to polysaccharides on the PC’s surface [78]. The interaction of PCs with BMSCs will signal these cells to produce and release IL-6 as a survival signal [56]. BMSCs will also secrete other soluble factors such as IL-5 and TNFα, which also act as survival signals [19]. In addition, interaction of the PC with HA in the extracellular matrix activates CD44 signalling which provides another survival stimulus for PCs [19]. Further interactions between PCs and BMSCs via BAFF/APRIL with BMCA/TACI would also provide crucial survival signals to LPCs [71, 72]. Therefore, current data suggests that LPCs are regulated by a
survival mechanism, i.e., anti-apoptotic signals. Accordingly, they show a pro-survival phenotype and gene expression profile (expression of anti-apoptotic Bel-2, no CD95 FasR) [10, 11, 47].

A large variety of cells comprise the bone marrow environment and it is likely that other cell types besides BMSCs play a role in the persistence of LPCs. Indeed, a recent series of studies has shown that a variety of non-stromal cells in the bone marrow - megakaryocytes (platelet precursors) [79], basophils [80] and eosinophils [81] all contribute to LPC survival. The contribution of these cells to LPC survival was demonstrated by several in vivo and in vitro approaches. In these studies, the authors first showed that these cells could interact with LPCs. Megakaryocytes were shown to co-localize and interact with LPCs and that megakaryocytes are an important source of two LPC survival factors - IL-6 and APRIL [79]. Co-culture of mouse PCs with either basophils or eosinophils also increases PC survival and antibody secretion which was dependent on IL-4 and IL-6 (from basophils) [80] or IL-4, IL-6, IL-10, TNFα and APRIL (from eosinophils) [81, 82]. Next, depletion of any of these three cell types in mice in vivo reduced the number of LPCs and significantly impaired PC responses [79, 80, 81, 82]. Finally, increasing the number of megakaryocytes by stimulating megakaryopoiesis in mice (a process tightly regulated by thrombopoietin and its receptor c-mpl) resulted in an increased number of PCs and increased PC persistence.

Finally, two studies have implicated that bone marrow dendritic cells (bmDCs) could play a role in the survival of PCs. The first study by Geffroy-Luseau et al. [85] involved co-culturing plasmablasts with bmDCs or osteoclasts (a stromal cell-type) in vitro. The authors observed that dendritic cells only supported the survival of plasmablasts but were unable to support PC survival. In contrast, osteoclasts (a stromal cell-type) could support the survival of both plasmablasts and plasma cells. By inhibiting BCMA with antibodies, they demonstrated that secreted BAFF and APRIL ligands were not involved. Instead, PC survival was dependent on cell-cell contact. In another study, Sapoznikov et al. [84] demonstrated that bmDCs could contribute to the survival of recirculating mature B cells in bone marrow through the secretion of macrophage migration-inhibitory factor (MIF). The authors used CD11c-diphtheria toxin receptor transgenic mice which when treated with diphtheria toxin results in the conditional ablation of bmDCs [85]. Interestingly, the authors also noted that bmDCs were restricted to specific perivascular clusters and speculated that these clusters localized to the same ecological niche as LPCs. However, neither study characterized the role of bmDCs in LPC survival and further experiments are needed to determine if bmDCs can contribute to the LPC persistence.

The current data suggest that microenvironments within bone marrow function as ecological niches to provide specific survival signals promoting the persistence of LPCs. Referred to as the ‘plasma cell competition model’, this model describes how the survival of LPCs depends on their ability to access a survival niche [47]. This ‘search’ is suggested to occur in a completely stochastic weighted-random manner. The model predicts that only a limited number of these ecological niches exist. The number of BMSCs which express VCAM-1 are estimated to be 17% of all stromal cells [86]. This will limit the total pool of LPCs which can be sustained in bone marrow. Indeed, the number of PCs in human has been estimated to be 0.1%-1.0% of bone marrow cells [87] and mouse bone marrow has been estimated to be able to sustain 10^6 LPCs [88]. This raises the question of how the number of LPCs would be maintained in a stable state over years of infections, considering that in a single year, an individual may generate 10^4-10^5 new PCs [8]. Thus, the competition model indicates that LPCs may persist for long periods of time when in a survival niche, but may be displaced through competition with newly formed migratory plasmablasts [47]. These new cells compete with older resident LPCs for the finite space available and any LPCs which are displaced are believed to be unable to regain localization and subsequently die due to ER stress. Multiple myeloma could represent a dysfunction of this system where available ecological niches to support PC survival are overtaken by MM cells [55]. Indeed, a common symptom in MM patients is the decreased production and increased destruction of normal antibodies resulting in diffuse hypogammaglobulinemia [34].

### Conclusion

The importance of long-lived plasma cells in humoral immunity has only been recognized recently [8]. This has opened a new direction of humoral immunity research. Our knowledge of LPCs and how they play a role in humoral immunity has increased greatly in the past decade. Many extrinsic factors (cytokines, chemokines, surface cell-cell molecules) and neighbouring cells within the survival niche microenvironment have now been identified. As described above, the principal factors which govern the immortality of LPCs are the cytokine IL-6 and the TNF-subfamily ligands, APRIL and BAFF. Targeting these molecules to
impair LPC survival could provide novel opportunities for therapeutic intervention in diseases such as autoimmunity or PC cancers such as multiple myeloma. However, the ideal therapeutic option would be the identification of novel LPC-specific molecules which will allow specific depletion of pathogenic LPCs in autoimmune diseases. This remains an important challenge of the future and a central step towards the development of curative therapy. Although great strides have been made in elucidating the regulation of survival of LPCs in the bone marrow microenvironment, detailed characteristics of these BM niches (e.g., cell types, size, accessibility, ontogeny, dynamics) are still not well defined and require further attention. However, as paradigms become known, new data and conclusions emerge to challenge them. No doubt, these are extremely exciting times for research in B cell humoral memory as we await further groundbreaking publications in this area.

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