Review

Staying alive: regulation of plasma cell survival

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On describing the catastrophic effect of the plague during the Peloponnesian War, Greek historian Thucydides (c ~450 BC) made the prescient observation that the ''same man was never attacked twice – never at least fatally''. This is probably the first description of the mammalian immune systems' remarkable ability to elicit a pathogen-specific response that potentially protects the host for its lifetime. This protection is largely mediated by plasma cells (PCs) that produce copious quantities of antibodies for extended periods of time, even after pathogen clearance. Here, I review the requirements for PC longevity in mice and humans, in particular the roles of survival niches in bone marrow and other tissues, and the ''dialogue'' between PCs and other cells that are crucial for long-lived humoral immunity.

B cell differentiation into memory and plasma cells (PCs)

B lymphocytes develop from bone marrow (BM) multipotent stem cells. A series of maturation stages yields naïve B cells expressing a functional B cell receptor [1,2] (Figure 1). When naïve B cells traffic through secondary lymphoid tissues and encounter foreign antigen (Ag), they can differentiate into multiple fates depending on the type, strength and timing of signals received within the lymphoid microenvironment. Thus, both T-independent and T-dependent (TD) Ags induce naïve B cells to become short-lived antibody (Ab)-secreting plasmablasts that localize to extrafollicular regions of lymphoid tissues [3–6]. TD Ags also induce naïve B cells to seed germinal centers (GCs) in lymphoid follicles. Within GCs, B cells undergo somatic hypermutation, isotype switching and affinity-based selection, which collectively result in the generation of long-lived memory and PCs [3–5,7] (Figure 1). Both of these cells then migrate from the GC to distinct sites, such as the splenic red pulp, medullary cords of lymph nodes (LNs) or mucosal-associated lymphoid tissues (MALT) of the gut for PCs, or splenic marginal zone or tonsillar epithelium for memory B cells [3,6–8]. Alternatively, the cells can egress from their tissue of origin, enter the circulation and take up residence in distal sites: for PCs, this is predominantly the BM [8,9] (Figures 1 and 2). During inflammatory or autoimmune responses, PCs can also home to inflamed tissues [10,11]. Migration of PCs from GCs is achieved by alterations in expression of chemokine receptors: they downregulate the B- and T-zone homing receptors chemokine CXC receptor (CXCR)5 and chemokine CC receptor (CCR)7, respectively, and increase CXCR4 [12–16], thereby facilitating recruitment to sites of production of its ligand CXC ligand (CXCL)12. Although PCs express receptors for other chemokines (e.g. CXCR3, CXCR6, CCR25, CCR28, the latter two being important for homing to MALT; [8,13]), whereas the CXCR4–CXCL12 axis is important for their initial migration to the BM [12,17]. However, production of CXCR3 ligands in inflamed tissue probably underlies the ability of PCs to localize to such sites, including tissues under autoimmune attack [11]. PCs can also be generated from memory B cells following re-exposure to the initial Ag [18] (Figures 1 and 2). Thus, PCs can arise from numerous precursors at different stages during an immune response, for example, naïve B cells, which generate short-lived plasmablasts that provide the first line of protection; GC B cells, which yield high-affinity long-lived PCs; and memory B cells, which potentially replenish the pool of long-lived PCs on re-encounter with specific pathogens/Ags.

Although B cells have multiple functions including Ag presentation, $CD4^+$ T cell stimulation and immune regulation via cytokine production [1], their most important function is unquestionably the production of Abs that efficiently neutralize and/or clear invading pathogens from the host. Indeed, the ability of naïve B cells to differentiate into Ab-secreting cells underlies the success of most – if not all – current vaccines [19]. The continual secretion of neutralizing Ab by PCs, and the rapid differentiation of memory cells into PCs following recurrent exposure to the initial Ag/pathogen, provide mechanisms by which these cells maintain long-term humoral immunity and host protection against recurrent infections [18,19]. This fundamental function of B cells is evident from the primary immunodeficiences X-linked and autosomal recessive agammaglobulinemia, in which B cell development is blocked in the BM, resulting in a severe reduction (100–1000-fold fewer than healthy donors) in mature recirculating B cells and a lack of serum Ab of all isotypes [20]. Affected individuals can be successfully treated by Ig replacement therapy, despite the perpetual absence of B cells [20]. Thus, although individuals can survive without B cells, their mortality is greatly compromised by an absence of secreted Ab. Although the importance of Ab to long-lived humoral immunity has been appreciated for several decades, and the existence of immunological memory known for centuries, we are only now starting to

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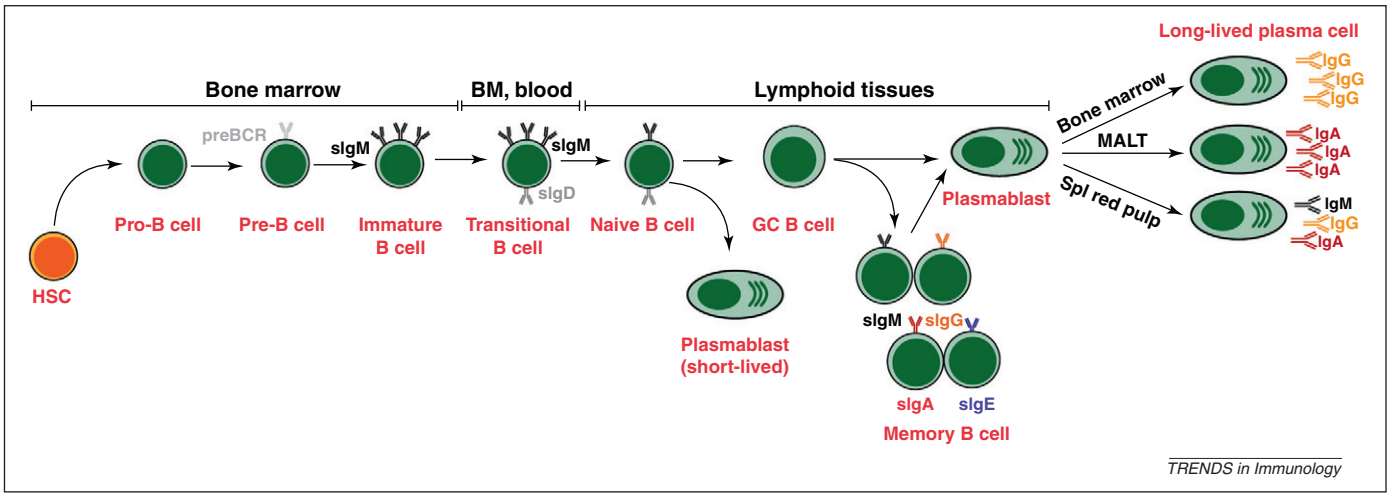

Figure 1. B cell development and differentiation: from hematopoietic stem cells to PCs. B cells develop from multipotent stem cells present in BM. Following encounter with Ag, naïve B cells can differentiate into short-lived plasmablasts, secreting predominantly IgM. These cells provide a first line of defense against infection. Alternatively, activated naïve B cells can seed a GC, where affinity maturation and differentiation into long-lived memory and plasma cells occur. Following their generation, memory cells and PCs can then migrate into niches in distinct sites (MALT, BM, splenic red pulp) where they receive survival cues from neighboring cells.

understand the requirements and mechanisms responsible for PC longevity. This has largely come about by recognizing the importance of survival niches for PCs not only in BM but also other lymphoid tissues, and the underlying 'dialog' between PCs and other cells that occupy the survival niche.

Longevity of protective serological immunity following infection of vaccination

Memory B cells versus PCs

A fascinating feature of the mammalian immune response to TD Ags is the ability to detect protective Abs for an entire lifetime following initial immunization or infectious exposure [21–23]. The half-life of serum Ab against non-replicating protein Ag (e.g. tetanus or diphtheria) is 10–20 years, whereas the half-life of Ab specific for live viruses [e.g. measles, mumps, rubella, vaccinia (smallpox), varicella, Epstein–Barr virus) is 50–200 years [21]. Although it has been hypothesized that memory B cells maintain humoral immunity by differentiating into PCs polyclonally following exposure to Toll-like receptor (TLR) ligands present in many pathogens [24], the data supporting this proposal are limited. For instance, the numbers of Ag-specific memory B cells does not always correlate with

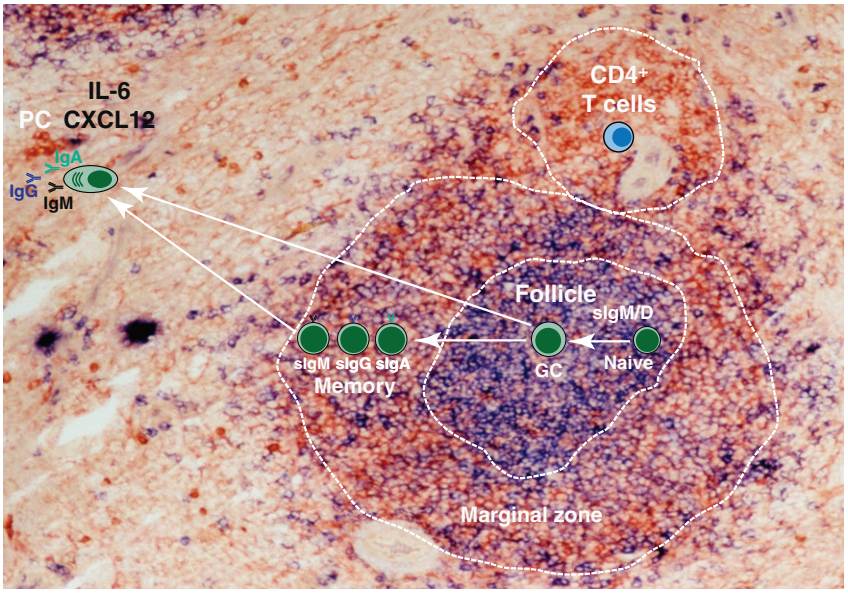

Figure 2. Migration and positioning of B cells at distinct stages of activation and differentiation. Human spleen section was stained for CD27 (orange) and IgD (blue) to delineate naïve B cells in the follicle ($IgD^{hi}CD27^{-}$), memory B cells in the marginal zone ($IgD^{lo/-}CD27^{+}$), PCs in the red pulp ($CD27^{hi}$) and T cells in the T-zone ($CD27^{+}$). The follicle, marginal zone and T cell zone are demarcated by dotted white lines. GC B cells transiently appear within the B-cell follicle (not abundantly present in this tissue section example). PCs migrate away from the GC under the influence of chemokines (i.e. CXCL12; shown in black print) produced by stromal cells in the red pulp. PCs can remain in the red pulp, where their survival is maintained by stromal-cell-derived IL-6 (shown in black print), as well as cell–cell (e.g. adhesion) interactions between the stromal cells and PCs. Alternatively, PCs can exit the tissue of origin and migrate to distant sites, predominantly the BM. This migration is also mediated by the CXCR4–CXCL12 axis. Memory B cells can also remain in the spleen, where they relocate to the marginal zone and are tethered there by interactions with adhesion molecules.

concentrations of Ag-specific Ab in serum [21,25]. Similarly, although anti-CD20 mAb therapies deplete most peripheral B cells, serum titers of Ag-specific Abs are largely unaffected [26]. Lastly, individual with mutations in *MYD88* or *IRAK4*, which are required for signaling through most TLRs, have intact humoral immune response to TD Ag [27]. These findings suggest that memory cells and PCs are subject to distinct regulatory mechanisms and contribute to the longevity of humoral immunity by independent processes. Based on these observations, it is clear that PCs are the primary cell type responsible for sustained production of protective Abs in the absence of repeated Ag exposure.

Plasma cells require specific niches for their long-term survival

Although Abs produced by PCs can be detected for decades, PCs themselves are not intrinsically long-lived. Following isolation from human or mouse spleen, tonsils or BM, PCs undergo rapid apoptosis unless they are co-cultured with stromal-type feeder cells or exogenous cytokines [2,15,28]. These *in vitro* observations are consistent with *in vivo* studies that have discovered that, irrespective of the absolute number of PCs generated during an immune response, the spleen can only support survival of a finite number following resolution of infection and/or clearance of the pathogen [29]. Together, these findings have led to the concept that the long-term persistence and function of PCs depends upon specialized niches within lymphoid tissues that facilitate their survival [30]. In the presence of an excessive number of PCs, only those that successfully compete for these niches will survive. Similarly, newly generated plasmablasts are proposed to displace resident, non-migratory PCs generated during previous immune responses from their survival niches [30]. This hypothesis provides an explanation for the simultaneous detection of both Ag-specific and non-specific PCs in the circulation following immunization – with one population representing recently generated niche-seeking PCs, and the other being 'older' displaced PCs [24,31,32] – as well as the progressive decline in the levels of Ag-specific Ab in serum [21].

The survival niche – what, who and how?

Non-hematopoietic stromal cells

Survival niches for PCs have been best characterized in BM. For PCs to access survival niches, they need to exit lymphoid follicles and traffic to distal sites. CXCR4 is important for PC recruitment to the BM. Indeed, mice

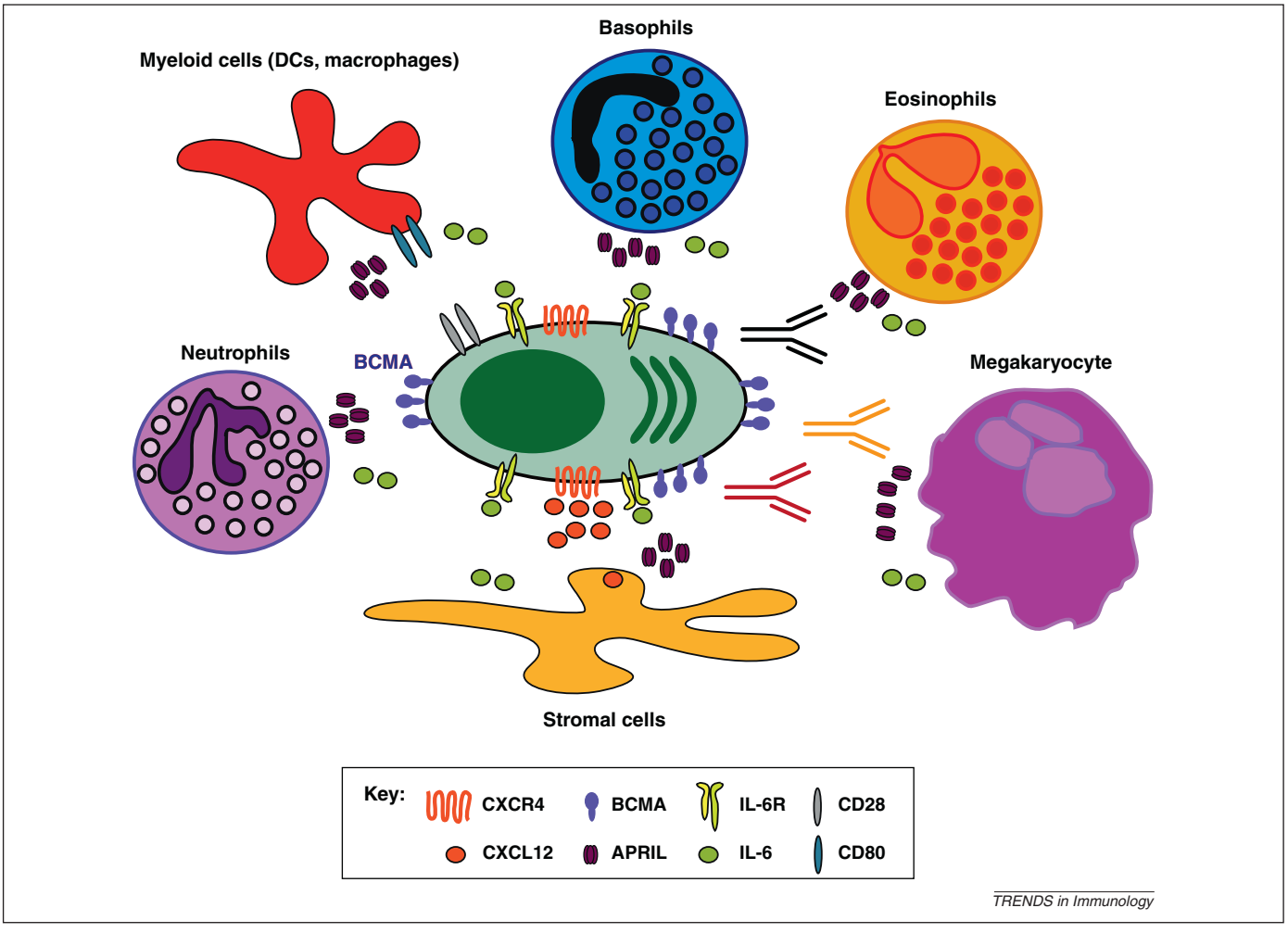

Figure 3. PC survival niche. PCs are recruited to survival niches under the chemotactic influence of CXCL12 produced by stromal cells. The survival niches can potentially comprise many cell types including not only stromal cells, but also myeloid cells (DCs and macrophages), granulocytes (basophils, neutrophils and eosinophils) and megakaryocytes. Each of these cell types promotes PC survival by producing APRIL (and probably also BAFF) and IL-6, which act through BCMA (and possibly also TACI) and the IL-6 receptor, respectively. Note: basophils only appear to exert a survival effect in the spleen, but not BM, whereas all the other types of cells predominantly promote survival of BM PCs.

whose B cells lack CXCR4 have reduced numbers of BM PCs [12,17]. However, this is not absolute because some CXCR4$^-$ PCs do reach the BM, especially at later times [17,33]. Despite this, a role for CXCR4 in attracting PCs to the BM is supported by the positioning of PCs in close proximity to BM stromal cells expressing CXCL12 [34].

PC survival within BM niches is influenced by direct cell contact with, or exposure to soluble factors released by, stromal cells (Figure 3). Early studies have dissected the contribution of stromal cells by culturing isolated PCs with cytokines or agonistic mAbs to mimic cell/cell interactions, or with normal or gene-deficient stromal cells in the presence or absence of neutralizing mAbs. One of the key cytokines consistently identified as a stromal-cell-derived PC survival factor is interleukin (IL)-6 [2,15,16,28,35,36] (Figure 3 and Table 1). This is consistent with upregulated expression of IL-6 receptor on PCs [37], and the well-known association between IL-6 and PC malignancies [38]. Tumor necrosis factor (TNF)-α has also been implicated in survival of human PCs *in vitro*, however, it is unclear whether this is produced by B cells or stromal cells [2,39]. Interestingly, CXCL12 also promotes PC survival *in vitro* [2]. Thus, the CXCR4–CXCL12 axis plays several important roles in PC biology: first, it attracts PCs to stromal-cell-containing niches in the BM; and second, it functions to maintain their viability and Ab secretion (Figure 3 and Table 1). This bifurcated role of CXCR4 is reflected in the time-dependent reduction in responses of PCs to the chemotactic, but not pro-survival, effects of CXCL12 [2,40]. IL-6 and CXCL12 probably also contribute to the maintenance of long-lived PCs in non-BM sites because these factors are abundantly produced by stromal cells isolated from spleens and LNs [16,41]. Intriguingly, BM stromal cells are more efficient at maintaining PCs than splenic and LN stromal cells. This does not reflect greater production of IL-6, but rather is associated with increased expression of CXCL12 [41]. This observation goes some way to explaining the substantially larger proportion of PCs in the BM relative to spleen and LNs [40,41], because the chemotactic gradient established by elevated levels of CXCL12 in BM would potentially recruit a greater number of PCs than in the spleen. It is also possible that the increased CXCL12 derived from BM stromal cells contributes to prolonged survival of BM PCs, however, this remains to be formally demonstrated.

Adhesion molecules may also be involved in regulating PC trafficking to, and retention in, niches within the lymphoid microenvironments. Engaging CD44 on human PCs significantly prolongs their survival [2,40]. There is impressive synergy when PCs are stimulated through CD44 and IL-6 receptor [2], highlighting the dynamic interplay between cell–cell interactions and soluble mediators that potentially maximize PC survival. A curious feature of human PCs is the restricted expression of the adhesion molecule CD11a on splenic versus BM PCs [16]. This correlates with localization of splenic PCs with intercellular adhesion molecule (ICAM)-1-expressing stromal cells that produce large quantities of IL-6 and CXCL12 [16] (Figure 2). Thus, interactions between lymphocyte function-associated antigen-1 on PCs and ICAM-1 on stromal cells may tether PCs in survival niches established by stromal cell networks within the splenic red pulp.

Hematopoietic cells

Although much attention has focused on the role of non-hematopoietic stromal cells in regulating PC survival and persistence, there is ample evidence that hematopoietic cells also play a role. One of the first studies to propose this reported colocalization of plasmablasts in spleens and LNs with a subset of CD11c^{hi}CD205$^-$ dendritic cells (DCs), and the presence of these DCs correlated with plasmablast survival and Ig secretion [42] (Table 1). This initial observation has now been confirmed and extended by numerous groups, resulting in the consensus view that the survival niche for PCs relies on inputs not just from resident stromal cells but also diverse populations of migratory cells that can position themselves within these niches. Not surprisingly, in addition to DCs, this includes other myeloid cells such as monocytes and macrophages [43–45] (Table 1). However, what is surprising is that cells as diverse as basophils [46], eosinophils [47], neutrophils [48] and megakaryocytes [49] – not previously recognized as major contributors to humoral immune longevity – also represent key components of PC survival niches (Figure 3 and Table 1).

Table 1. Components of PC niches, and mechanisms contributing to PC longevity.

Cell type	Niche location	Survival factor	Evidence	References
Stromal cells	Spleen	IL-6	• Reduced PC function in co-cultures with anti-IL-6 mAb	[16,41]
Stromal cells	BM	IL-6, CXCL12	• Increased PC survival *in vitro* with IL-6, CXCL12 • reduced survival with IL-6-deficient stromal cells	[2,28,40,41]
DCs	LNs Spleen BM	BAFF, APRIL, IL-6, CD28* (*only in BM)	• Colocalization • reduced plasmablasts following treatment with TACI-Ig • impaired Ab responses/PC survival in *Cd28*$^{-/-}$ mice	[42–44,73]
Eosinophils	BM	APRIL, IL-6	• Colocalization • reduced PCs in eosinophil-depleted mice • Reduced PC function in co-cultures with anti-APRIL/IL-6	[47]
Megakaryocytes	BM	APRIL, IL-6	• Colocalization • reduced PCs in megakaryocyte-deficient mice	[49]
Basophils	Spleen (no effect on BM PCs)	IL-6,	• Colocalization • reduced PCs in basophil-depleted mice	[46]
Neutrophils	MALT	APRIL	• Colocalization • induced production of BAFF/APRIL	[48,63]
Macrophages	BM	APRIL	• Impaired APRIL production by neonatal BM cells • reduced PC survival in co-cultures with APRIL-deficient granulocytes	[45,62]

These cells localize with PCs either in the BM (megakaryocytes, macrophages and eosinophils [47,49]), spleen (DCs/myeloid cells/basophils [43,46]), LNs (monocytes/macrophages and neutrophils [44,50]) and MALT (neutrophils [48]). The potential relevance of PCs colocalizing with other supportive cell types has been demonstrated by several approaches. First, *in vitro* co-culture of primary murine PCs with basophils [39] or eosinophils [47] significantly improves PC survival/Ab secretion. Second, depleting eosinophils, megakaryocytes or basophils from mice *in vivo* significantly reduces PC numbers [39,47,49]. Lastly, artificially increasing the number of megakaryocytes *in vivo* by administering thrombopoietin results in a heightened PC response in BM following immunization with specific Ag [49]. It must be noted that, although depleting basophils reduces splenic PC numbers, there is no effect on BM PCs [46], whereas depleting eosinophils, megakaryocytes or basophils individually reduces PC numbers and Ag-specific Ab responses by only 30–60% [46,47,49]. Thus, although individual cells contribute to the survival niche, one single cell type is not absolutely crucial for long-term PC survival. A clear example of this is that, although megakaryocyte-deficiency diminishes PC responses 5–12 days post-immunization, it normalizes at later times (3–4 weeks) [49]. This redundancy is explained by the finding that, despite the disparate nature of these cell types, they all share a common mechanism by which they support PC survival – the combined production of the cytokines a proliferation inducing ligand (APRIL) (and possibly B cell activating factor belonging to the TNF family (BAFF)) and IL-6 (Figure 3).

APRIL – a key survival factor for human and mouse plasma cells

Ligand and receptors of the BAFF/APRIL family

APRIL and BAFF are related members of the TNF superfamily that share the receptors B cell maturation Ag (BCMA) and transmembrane activator of and CAML interactor (TACI) [51,52]. BAFF also binds BAFF-R, whereas heparan sulfate proteoglycans (HSPGs) act as APRIL-binding receptors [53]. BAFF-R, TACI and BCMA are predominantly expressed by B cells at different stages of differentiation. BAFF-R is expressed on immature B cells and is maintained until they differentiate into PCs. TACI and BCMA are absent from naïve B cells [37,54,55], but can be induced following *in vitro* activation [36,56]. However, only TACI is expressed by GC B cells [54]. Memory B cells express TACI, but not BCMA, whereas plasmablasts and some PCs acquire BCMA but downregulate TACI [36,37,44,48,52,54–56]. APRIL binds lymphocytes, including PCs, via HSPG [53].

How BAFF and APRIL regulate B cell survival

Although the requisite role of BAFF–BAFF-R in regulating survival of mature B cells is well established [51,52], the significance of APRIL to the behavior of B cells, and of BAFF to PCs, has been unclear. This is the result of variable phenotypes of independent strains of APRIL-deficient, BCMA-deficient mice, or APRIL transgenic mice [36,57–59], as well as potential redundancies within the BAFF–APRIL and TACI–BCMA signaling pathways [51,52]. This has been readdressed by detailed examination of Ab responses in gene-targeted or manipulated mice. An early study has reported a paucity of Ag-specific BM PCs in BCMA-deficient, compared to wild-type (WT), mice [36]. These findings mirror results from a previous study that has reported that BAFF and APRIL support survival of human plasmablasts in a BCMA-dependent manner *in vitro* [56]. Similarly, *in vivo* blockade of BAFF and APRIL, but not BAFF alone, depletes BM of Ag-specific PCs [53,54,60]. APRIL-deficient mice exhibit normal PC generation following immunization with TD Ag [54], thus, these findings have revealed that APRIL and BAFF are required for PC longevity, operating through BCMA and/or TACI, and these ligands can compensate for one another. Consequently, effective abolition of PC-mediated humoral immunity requires dual neutralization of both ligands. Although this conclusion is supported by several studies, APRIL probably plays a more prominent role in regulating PC survival than BAFF does, at least in mice. This is based on the findings that (i) APRIL binds murine BCMA with greater affinity than BAFF [61]; (ii) BCMA and HSPGs are preferentially expressed on PCs [53]; (iii) PC persistence is severely compromised following transfer into APRIL-deficient/depleted, but not BAFF-deficient, mice [45,62]; and (iv) APRIL is more efficacious than BAFF at promoting PC survival *in vitro* [45].

APRIL is produced by diverse cell types that can comprise plasma cell survival niches

Although these studies highlight the importance of BAFF and APRIL signaling for PC survival, they do not elucidate the source of these cytokines, nor the mechanisms underlying their actions. However, recent studies have documented APRIL production by multiple hematopoietic cell types including eosinophils, megakaryocytes and myeloid precursors in BM [47,49,62], monocytes/macrophages and neutrophils in LNs and MALT [44,48], and activated human basophils [63]. Furthermore, these cells are found in locations adjacent to PCs [44,46–49], thus providing extrinsic sources for PC survival factors (Figure 3 and Table 1). Additional experiments that have antagonized APRIL *in vitro* have directly linked APRIL production by eosinophils or myeloid precursors with their ability to promote PC survival [47,62] (Table 1). Although this has not been established for basophils, neutrophils and megakaryocytes, such an outcome would be expected based on the reported PC survival-enhancing activity of recombinant APRIL *in vitro* [36,45,56,64]. Interestingly, several of these studies have also identified IL-6 production as a feature of these supportive cell types [44,46,47,49], and have found that – akin to stromal cells in survival niches – neutralizing IL-6 in co-cultures of PCs with basophils [46] or eosinophils [47] partially reduces PC survival. Collectively, these studies have redefined the constituents of PC survival niches from non-hematopoietic stromal cells to include granulocytes (basophils and eosinophils), myeloid cells (DCs and monocytes/macrophages) and megakaryocytes, and also have identified the molecular requirements for PC survival (APRIL, BAFF and IL-6) (Figure 3 and Table 1). APRIL, BAFF and IL-6 probably operate combinatorially to achieve maximum PC longevity, as demonstrated by additive effects

on PC survival *in vitro* [36], and conversely, greater apoptosis of PCs in co-cultures with such supportive cells after blockade of APRIL and BAFF and IL-6 [47].

These data also put into perspective previous studies of the cellular sources of BAFF and APRIL. It was initially unclear how substantial production of BAFF (and probably APRIL) by neutrophils stimulated with granulocyte colony-stimulating factor would impact humoral immunity [65,66]. However, the findings that (i) granulo/myelopoiesis is induced in response to adjuvants/inflammation associated with TD humoral immune responses [67]; (ii) neutrophils are required for optimal Ab production; and (iii) neutrophils and myeloid cells are recruited to niches and support PC survival in an APRIL- and BAFF-dependent manner [48,62], rationalizes these observations. Lastly, the finding of a requisite role for APRIL in sustaining PCs provides an explanation for the poor humoral immune responses typical of neonates [68]. Neonatal BM poorly supported PC survival *in vitro*, compared to BM from adult mice. This correlates with significantly reduced expression of APRIL, but not BAFF or IL-6, by macrophages in neonatal BM [45]. Thus, the inability of BM myeloid cells to produce sufficient levels of APRIL compromises humoral immunity in newborns.

APRIL and BAFF support PC survival by modulating expression of antiapoptotic molecules

The mechanisms underlying the pro-survival effects of BAFF on mature B cells involve induction of anti-apoptotic genes [51,52]. An analogous mechanism appears to underpin the pro-survival effects of APRIL on PCs. *In vitro* exposure of PCs to APRIL upregulates expression of the Bcl-2 family members Bcl-xL, Bcl-2 and Mcl-1 [45,48], whereas BAFF only increases Mcl-1 [36]. A recent study has assessed the relative requirements for different sets of pro-survival proteins in lymphocyte development, differentiation and homeostasis by treating mice with the drug ABT-737. ABT-737 induces lymphocyte apoptosis by selectively targeting Bcl-2, Bcl-xL and Bcl-w, but not Mcl-1 or A1 [69]. *In vivo* administration of ABT-737 suppresses the generation of Ag-specific memory B cells and PCs; however, ABT-737 has no effect on the persistence of PCs once these cells have been generated [69]. Thus, the long-term survival of PCs is independent of Bcl-2, Bcl-xL and Bcl-w, but does require Mcl-1 and/or A1. Collectively, these findings predict that BAFF and APRIL sustain the survival of established PCs via induction of Mcl-1 expression. Mcl-1 has a very rapid turnover, therefore, its expression needs to be continually maintained in PCs. This implies that basal production of BAFF and APRIL by cells in survival niches is required for PC survival in the steady state. Interestingly, type I interferons induce BAFF and APRIL production by plasmacytoid DCs, which facilitate the generation of IgA-secreting PCs in mucosal tissues [70]. Whether such a pathway operates in cells within PC survival niches remains to be elucidated. Lastly, it will be interesting to determine whether polymorphisms in *TNFRSF13B* (encoding TACI) associated with increased susceptibility to developing common variable immunodeficiency [52] compromise the survival function of BAFF and APRIL on PC from these patients.

CD28 expressed by PCs contributes to their maintenance and survival

Analysis of WT and $Pax5^{-/-}$ pro-B cells has identified *Cd28* as a gene whose expression is repressed by PAX5 [71]. Consequently, CD28 is expressed on normal murine PCs following downregulation of PAX5 [71]. This is consistent with an early description of CD28 expression on malignant PCs in some cases of multiple myeloma and on human myeloma cell lines [72]. Delogu *et al*. also have demonstrated impaired Ab responses in mice lacking CD28 in the B-lineage [71]. These observations led to the conclusion that CD28 is required in a B-cell autonomous manner for the successful generation of a TD Ab response.

The role of CD28 in PC homeostasis has recently been reinvestigated. *Cd28*-deficient mice cannot generate normal PC numbers following immunization with TD Ag [73]. This effect is unique for BM PCs, because PCs in spleens of $Cd28^{-/-}$ mice are generated in comparable numbers as in WT mice [73]. These results have been recapitulated in a series of experimental mice that have revealed a B-cell intrinsic role for CD28 in regulating PC maintenance/survival [73]. The mechanisms underlying the requirement of CD28 in PC generation are twofold. First, direct engagement of CD28 on PCs improves survival *in vitro* via an NF-κB-dependent pathway. Second, interactions between CD28 on PCs and CD80 on BM-derived DCs induce IL-6 secretion by DCs, which acts on PCs to promote IgG secretion [73] (Figure 3 and Table 1). This is reminiscent of a previous study which reported that PCs facilitate IL-6 production by BM stromal cells [28]. It has also been found that PCs are in direct contact with $CD80^+$ stromal cells, including DCs, in murine BM [73].

Although the studies in mice present a convincing case for CD28 in supporting PCs [71,73], it is unclear whether these findings translate to humans because CD28 is expressed at very low, if at all detectable, levels on human PCs [15,72,74]. This notwithstanding, these findings may well have relevance to our understanding, as well as treatment, of myeloma. BM of myeloma patients contains an increased number of $CD11b^+$ DCs capable of supporting the survival of myeloma cells/cell lines *in vitro* in a CD28- and IL-6-dependent manner [74]. Interestingly, IL-6 produced by DCs in the presence of myeloma cells prevent the death of myeloma cells induced following exposure to dexamethasone, a chemotherapeutic often used to treat myeloma [74]. Thus, impeding access of malignant cells to their survival niche by targeting CD28 may represent an alternative therapeutic approach to the treatment of this malignancy.

Concluding remarks

Our knowledge of humoral memory has increased enormously in the past decade. Many of the genes, transcription factors, cytokines, chemokines, signaling and surface molecules required for PC generation have been identified. Furthermore, the contributions of these factors, as well as of neighboring cells within the microenvironment and/or survival niches of lymphoid tissues and BM, to the longevity of PCs have been revealed. Remarkably, many cell types provide survival signals important for long-lived humoral immunity. However, the dominant players underlying PC

longevity appear to be the TNF ligands APRIL and BAFF and the cytokine IL-6, which operate by maintaining express of key survival molecules, for example Mcl-1. These advances in our understanding provide a framework for further dissecting humoral immunity, and provide opportunities and targets for therapeutic intervention in diseases such as humoral immunodeficiencies, autoimmunity and PC malignancies, as well as in promoting Ab responses during vaccination in general, and in neonates. For example, altering production and/or expression of BAFF or APRIL by cells within PC niches, or targeting Mcl-1 or CD28 to sustain or deplete their expression could greatly modulate PC survival, thereby either improving the desired, or attenuating the pathogenic, function of PCs and subsequent Ab responses. Future studies will hopefully identify strategies that facilitate such therapeutic manipulation.

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