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Pathogenic Long-Lived Plasma Cells and Their Survival Niches in Autoimmunity, Malignancy, and Allergy

Oliver Winter,*† Christof Dame, ‡ Franziska Jundt, ‡ and Falk Hiepe*

Long-lived plasma cells (LLPCs), first identified in the 1990s (1, 2), provide a persisting Ab titer in the blood and form the humoral memory of the immune system, which is independent of permanent Ag presence (3). Therefore, LLPCs are defined as memory PCs (MPCs) (Table I). PCs differ from their direct precursors, the plasma blasts, by proliferation halt, loss of migratory capabilities, downregulation of characteristic B cell markers such as CD19, CD20, CD22, and HLA-DR/MHC class II, and upregulation of CD138 and BLIMP1. Depending on the primary Ag stimulation, the estimated half-life of the humoral memory varies from 11 y (tetanus) to 200 y (measles, mumps) (4). MPCs, however, are not long-lived per se but need anti-apoptotic stimuli from their microenvironment, the so-called PC niche (5). If a newly generated PC does not successfully enter a survival niche in a competitive process or, vice versa, if an LLPC is displaced from its niche by an immigrating young PC, it undergoes apoptosis (6) (Fig. 1A). In the past 15 y, several soluble survival factors, for example, the chemokine CXCL12, the BCMA ligands APRIL and BAFF (also known as BlyS), IL-6, TNF-α, and membrane-bound PC survival factors, for example, VCAM-1 and the CD44 ligands hyaluronic acid, fibronectin, and collagen, were discovered both in vitro and in vivo (Table II). These factors can synergistically contribute to PC survival (7), but only BCMA stimulation (8), expression of CD93 (9), and for bone marrow PCs expression of CD28 (10) were demonstrated to be essential for PC longevity. However, the role of CD28 might be controversial, as Njau et al. (11) reported increased Ig titters and PC numbers in CD28−/− chimeras. Whereas the large majority of MPCs are localized in the bone marrow (5, 12), LLPCs are also found in lymphatic organs such as spleen (13), lymph nodes (14), mucosa-associated lymphatic tissues (15), as well as in chronically inflamed tissues such as the synovium in rheumatoid arthritis (16), the CNS in induced multiples sclerosis (17), the kidneys in systemic lupus erythematosus (SLE) (18), the salivary glands in Sjögren’s syndrome (19), or the lung during chronic airway inflammation in allergy/asthma (20, 21). In secondary lymphatic tissues, PCs reside in extra-follicular areas such as the lamina propria of mucosa and the red pulp of spleen, and LLPCs are associated with APRIL, BAFF, and IL-6 sources in the vicinity (14, 15, 22, 23). Whereas APRIL is produced by several cell types in the bone marrow (24), only few cells in specific areas (e.g., the subepithelium zone in tonsillar crypts or intestinal villi in the mucosa) secrete this survival factor in other organs (15). Moreover, certain organs have different potential to support PC survival, and induction of angiogenesis seems crucial to maintain PCs in inflamed tissues (25).

In studying the cellular composition of the PC niche, Tokoyoda et al. (26) found that in the bone marrow >95% of all IgG PC are colocalized with CXCL12-abundant reticular (CAR) cells, a subpopulation of mesenchymal stromal cells. According to cell division studies, only 50–70% of bone

Abbreviations used in this article: BCMA, B cell maturation Ag; BlyS, B lymphocyte stimulator; CAR, CXCL12-abundant reticular; HNC, hematopoietic niche component; LLPC, long-lived plasma cell; MCPN, multicomponent plasma cell niche; MM, multiple myeloma; MCPC, multiple myeloma cell; MPC, memory plasma cell; PC, plasma cell; SLE, systemic lupus erythematosus; SLPC, short-lived plasma cell.

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marrow PCs appear to be long-lived (27), indicating the existence of another determining factor. Indeed, within the last 3 y, several in vivo studies demonstrated the contribution of hematopoietic cells such as megakaryocytes (27), eosinophils (28), basophils (29, 30), dendritic cells and monocytes/macrophages (14), myeloid progenitors (31), neutrophils (15), and monocytes (22) to the PC niche. In vitro, osteoclasts also stimulate PC survival (32). Of note, in the intestinal mucosa, which contains large numbers of mainly IgA-secreting PCs, induced titers and LLPC numbers were stable for at least 100 d under germ-free conditions but declined under nonsterile conditions, indicating rapid adaptation of the humoral memory to the intestinal microbiota and intense competitive pressure in the mucosal PC niche (33). As such, some cell types may specifically support LLPCs in the bone marrow sustaining stable/long-term humoral memory, whereas others provide PC survival in secondary lymphoid organs or inflamed tissues during chronic inflammation or infection, maintaining temporary/short-term humoral memory. Considering recent findings, we extended the classical stroma/plasma cell model of the survival niche with a hematopoietic niche component (HNC) to the model of a multicomponent PC niche (MCPN) (24) (Fig. 1A). The HNC enriches the concentration of PC survival factors in the niche but may also add specific factors. Thus, different hematopoietic cells form alternative niches that compensate the loss of one particular cell type but may also harbor distinct PC subpopulations, for example, TNF-α and inducible NO synthase producing IgA PCs (34) or nerve growth factor and neurotrophin-3–dependent PCs (20).

Interestingly, all hitherto reported HNCs (Table II) are of myeloid origin and most express CD80/86. Thus, PCs can stimulate IL-6 production in these cells via CD28 (10). The finding that myeloid cells not only cooperate with lymphocytes during early phases of the immune response but sustain humoral memory defines a new hallmark of interaction between the innate and adaptive immune systems.

The understanding of the MCPN should be considered in processes involving long-lived autoreactive, malignant, and allergen-specific PCs. Such an approach may open new avenues to overcome therapy resistance and may be helpful to improve future therapeutic concepts.

**Autoreactive PCs**

Genetic, hormonal, and environmental factors contribute to the pathogenesis of autoimmune diseases such as chronic immune thrombocytopenia, SLE, rheumatoid arthritis, Sjögren’s syndrome, or multiple sclerosis (12). In these disorders, autoreactive MPCs continuously secrete Abs, resulting in chronicity or relapse of autoimmunity (12) (Fig. 1B).

Current therapeutic strategies primarily target the inflammatory processes and activated immune cells (12, 35). Nonsteroidal anti-inflammatory drugs, antimalarials, glucocorticoids, conventional immunosuppressive or cytotoxic drugs, IVIg, as well as cell depletion Abs such as anti-CD20 (rituximab) targeting B lymphocytes can efficiently reduce the acute or chronic inflammation. They can also abrogate temporary PC survival niches in inflamed tissues and thus eliminate LLPCs in these organs, whereas LLPCs in the bone marrow are not affected by them. Patients with severe refractory autoimmune disorders may benefit from splenectomy or immunodepletion followed by autologous stem cell transplantation (36). The complete elimination of the autoreactive memory PCs by immune ablation with polyclonal antithymocyte globulin is crucial for the success of the autologous stem cell transplantation (37). Bortezomib, a proteasome inhibitor directly targeting PCs, eliminates both short- and long-lived autoreactive PCs (38, 39). Targeting the survival niche (see Table III) for autoreactive LLPCs may synergize with the primary therapy and thus improve outcome.

**Targeting migration (chemokines and their receptors).** The CXCR4 antagonist AMD3100 dislocates malignant PCs from their niche and enhances the susceptibility to bortezomib (40). Additionally, CXCR4 inhibitors disturb the migration of newly formed PCs and may prevent CXCR4+ hematopoietic cells from entering the PC survival niche (41). As recently demonstrated, natural (CXCL11) and synthetic (CCX771) ligands for CXCR7 block the CXCR4-mediated transendothelial migration 100-fold more efficiently than does AMD3100 (42) and they interfere with the CXCL12-directed migration (43). Suppressing the migration of CXCR3+ leukocytes toward CXCR3 ligands (CXCL9/10/11), expressed in inflamed tissues, could also serve as a therapeutic target in various autoimmune diseases. Consistent with this hypothesis, CXCR3−/− MRL/lpr mice displayed nephritis with reduced albuminuria and less glomerular tissue damage, which was associated with diminished infiltration of Th1 and Th17 cells into the kidneys (44). In contrast, CXCR3-deficient NZB/W mice had reduced IgG1 titers but did not show clinical improvement (45). This might be explained by the finding that infiltration of CD4, CD8 T cells and IgG, IgM PCs in the kidneys was not disturbed and suggest the presence of CXCR3-dependent and -independent pathways, pointing out the diversity in the pathogenesis of lupus nephritis. Accordingly, some, but not all, SLE patients might benefit from anti-CXCR3 treatment.

### Table I. Plasma cell stages

<table>
<thead>
<tr>
<th>Plasma Cell Stage</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma blasts/newly</td>
<td>Capable of dividing and migrating, still expressing declining levels of typical B cell</td>
</tr>
<tr>
<td>developed PCs</td>
<td>markers (e.g., CD19, CD20, CD22, HLA-DR/MHC class II).</td>
</tr>
<tr>
<td>SLPCs</td>
<td>Mature PCs that might lack the capability to become long-lived or were not able to</td>
</tr>
<tr>
<td></td>
<td>enter the survival niche.</td>
</tr>
<tr>
<td>LLPCs/MPCs</td>
<td>Survive for extended periods of time and persistently secrete Abs without need of Ag</td>
</tr>
<tr>
<td></td>
<td>presence, thus providing humoral memory, LLPCs usually arise from T cell–</td>
</tr>
<tr>
<td></td>
<td>dependent germinal center reactions and secrete affinity-maturated and Ig class–</td>
</tr>
<tr>
<td></td>
<td>switched Abs. A large majority of LLPCs are generated in secondary immune</td>
</tr>
<tr>
<td></td>
<td>responses.</td>
</tr>
</tbody>
</table>
Targeting soluble survival factors (cytokines and their receptors). Because several inflammatory cytokines also act as PC survival factors, therapeutic Abs directed against such cytokines modulate the milieu within the PC niche (Table III). The anti-BAFF Ab belimumab reduces anti-dsDNA, anti-Smith, anticardiolipin, and anti-ribosomal P autoantibodies, normalizes C3/C4 complement levels, and decreases the amount of naive and activated B cells and PCs, but it does not deplete memory B cells (57). As such, the fraction of anti-dsDNA Abs secreted by short-lived PCs (SLPCs) (58), whose numbers correlate with flares (12), is targeted by belimumab whereas the Ab titers against pneumococci, tetanus, and influenza representing LLPCs are not reduced (59). This underscores the necessity to block both compensating BCMA ligands, that is, APRIL and BAFF, to deplete LLPCs (8, 60). Thus, the novel TACI-Ig fusion protein atacicept, which binds APRIL and BAFF, might be a promising therapeutic option (Table III).

Targeting attachment (adhesion molecules). The anti–VLA-4 Ab natalizumab, originally generated to suppress the transmigration of activated leukocytes into inflamed tissues, is approved for the treatment of multiple sclerosis and dislocates PCs from their survival niche, leading to the depletion of autoreactive MPCs. Targeting both adhesion molecules VLA-4 and LFA-1 facilitates the reduction of LLPCs in the bone marrow (48).

Targeting the HNCs. The replacement of pathogenic LLPCs by protective PCs could further improve the therapy. Our data show that in mice the approach of combined vaccination and short-term manipulation of the HNC (stimulation of megakaryopoiesis by recombinant thrombopoietin) decreases the amount of pre-existing LLPCs and enlarges the pool of novel emerged protective MPCs (27). Nevertheless, in individuals with autoimmune disorders a booster immunization with live attenuated vaccines or strong adjuvants may lead to a bystander activation of autoreactive lymphocytes and to an increase of autoreactive cells and an enhancement of the autoimmune memory.

Malignant PCs

Multiple myeloma (MM) is characterized by osteolytic lesions and severe renal failure owing to elevated monoclonal Ig production through malignant PCs, so-called MM cells (MMCs). Furthermore, expansion of MMCs leads to progressive competition and replacement of the hematopoietic compartment in bone marrow (61). Survival of MMCs depends, similar to that of protective PCs, on antiapoptotic stimuli, such as IL-6, IGF-1, APRIL, and BAFF, with osteoclasts being a major source for APRIL and BAFF in MM (62). The receptors for APRIL and/or...
BAFF (BCMA, TACI, and BAFF-R) are heterogeneously expressed on MMCs and could not be correlated with disease prognosis (63). Whereas TACI+ MMCs respond to APRIL and BAFF stimulation, BCMA+/TACI− MMCs only proliferate upon APRIL stimulation (63), reflecting the fact that BAFF has a 1000-fold weaker affinity for BCMA than APRIL. Furthermore, CD138 on PCs is an important costimulatory molecule for APRIL–TACI but not for BAFF–TACI or APRIL–BCMA signaling, as it harvests APRIL from the environment and presents it to TACI (63).

Moreover, gene expression profiles of MMCs (64, 65) and their niche (66) differ from those of protective PCs (e.g., in TRAIL and FAS expression, with both being important mediators of apoptosis). Transendothelial migration and homing of MMCs is regulated by the CXCL12–CXCR4 axis (43), and VEGFR1+ hematopoietic bone marrow cells initiate a premetastatic niche (67). Experimental removal of these VEGFR1+ cells prevents tumor metastasis. MMCs aggressively modulate their microenvironment by inducing angiogenesis via VEGF-1 and attracting mesenchymal stem cells via CCL25 (Fig. 1C). Latter ones synthesize PC survival factors (IL-6, IGF-1) and counterbalance bortezomib-induced apoptosis (68). Additionally, MMCs attract HNCs such as osteoclast progenitors or immature dendritic cells (69) through secretion of chemokines such as CCL3 (MIP-1α) (70). Moreover, macrophages form a multicomponent niche with stromal cells and extend MMC survival compared with stromal cells alone (71). Via abundant expression of CD28, MMCs promote survival of CD80/86+ myeloid cells in their microenvironment and stimulate IL-6 production in these cells (72).

### Table II. Plasma cell survival factors

<table>
<thead>
<tr>
<th>Soluble factors</th>
<th>Membrane-bound factors</th>
<th>Cellular Niche Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemokines and receptors</td>
<td>VCAM-1–VLA-4</td>
<td>CAR cells</td>
</tr>
<tr>
<td>CXCL12 (SDF-1)–CXCR4</td>
<td>ICAM-1–LFA-1</td>
<td>Hematopoietic origin</td>
</tr>
<tr>
<td>CXCL9/10/11–CXCR3</td>
<td>Hyaluronan and fibronectin–CD44</td>
<td>Mesenchymal origin</td>
</tr>
</tbody>
</table>

### Table III. Current therapeutic treatments targeting the survival niche for PCs

<table>
<thead>
<tr>
<th>Target in the Niche</th>
<th>Drug</th>
<th>Disease/Model</th>
<th>Therapeutic/Mechanistic Effect</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migration</td>
<td>CXCL12–CXCR4</td>
<td>AMD3100/NOX-A12 or CCX2066</td>
<td>Increases mobilization into periphery and sensitivity to several antitumor drugs</td>
<td>AMD3100 plus G-CSF is FDA approved for MM (40)</td>
</tr>
<tr>
<td>Adhesion</td>
<td>VLA-4</td>
<td>Natalizumab</td>
<td>Delays MS progression; inhibits and resolves attachment of MMCs, sensitizes MMCs to bortezomib; causes rare but fatal viral infections (PML)</td>
<td>FDA approved with restrictions for MS; preclinical study for MM (47)</td>
</tr>
<tr>
<td>Angiogenesis</td>
<td>VEGF</td>
<td>Bevacizumab</td>
<td>Improves outcome when combined with chemotherapy; rising numbers of bevacizumab-resistant tumors</td>
<td>Phase II trial for MM (49)</td>
</tr>
<tr>
<td>Cytokines</td>
<td>BAFF (BLyS)</td>
<td>Belimumab</td>
<td>Reduces autoantibodies, naïve and activated B cells, and PCs but not memory B cells and LLPCs</td>
<td>FDA approved for SLE</td>
</tr>
<tr>
<td>APRIL plus BAFF</td>
<td>Atacicept</td>
<td>RA, MM, SLE</td>
<td>Slows progression of MM; reduces levels of Ig and rheumatoid factor</td>
<td>Phase II trials for RA (50, 51); phase I trial for MM (52); phase II/III trial for SLE (NCT00624338)</td>
</tr>
<tr>
<td>IL-6</td>
<td>Siltuximab (NTNO 328); sintukumab (NTNO 136)</td>
<td>MM</td>
<td>Enhances cytotoxic effect of dexamethasone and bortezomib</td>
<td>Preclinical study (53)</td>
</tr>
<tr>
<td>IL-6R</td>
<td>Tocilizumab</td>
<td>RA, SLE</td>
<td>Clinical and serological improvement of the disease</td>
<td>FDA approved for RA; phase I trial for SLE (55)</td>
</tr>
<tr>
<td>HNC Osteoclasts</td>
<td>KRNS000</td>
<td>MM</td>
<td>Induces cell death in MMCs and osteoclasts, reduces bone destruction</td>
<td>Preclinical study (56)</td>
</tr>
</tbody>
</table>

FDA, U.S. Food and Drug Administration; MS, multiple sclerosis; PML, progressive multifocal leukoencephalopathy; RA, rheumatoid arthritis; SS, Sjogrens syndrome.
endothelial cells, and the surrounding extracellular matrix leads to the so-called cell adhesion–mediated drug resistance. Current treatment strategies for MM, detailed elsewhere (70, 73), depend on age, secondary diseases, tumor stage, and progression. High-dose chemotherapy and subsequent autologous or allogenic stem cell transplantation is often advised. Additional treatment strategies addressing the interaction between MMCs and their survival niches may improve outcome by enhancing the primary therapy and reducing severe adverse effects (70).

**Inducing dislocation and targeting migration.** G-CSF mobilizes hematopoietic stem cells (HSCs) and causes evasion of SLPCs and LLPCs from bone marrow (74). Also, AMD3100 dislocates MMCs from their survival niche and renders them more sensitive to therapy (40). Combinatory therapy with G-CSF and AMD3100 results in augmented HSC mobilization compared with G-CSF treatment alone and has been approved by the U.S. Food and Drug Administration for treating MM (75).

**Targeting attachment.** The adhesion molecule CD44 is synthesized by MMCs in alternative variants (e.g., CD44v3, v6, v9), which differently stimulate IL-6 production in stromal cells. CD44 variants are associated with tumor progression and prognosis (e.g., CD44v6′ frequency: 6% monoclonal gammopathy of unknown significance, 17% MM stadium I, 43% MM stadium II/III) and are promising targets in MM treatment (76). Furthermore, interfering with VLA-4–mediated adhesion inhibits accumulation of MMCs, disrupts binding of MMCs, blocks VEGF-induced angiogenesis, and increases sensitivity of MMCs to bortezomib (47).

**Soluble survival factors (cytokines).** The anti–IL-6 Ab silutuximab (CNO328) synergizes with dexamethasone and bortezomib, and it improves outcome in MM with only few adverse effects (56). APRIL and BAFF, whose serum levels in MM patients are 5-fold elevated compared with healthy donors, partially prevent MMCs from dexamethasone- and anti–IL-6–induced apoptosis. Deprivation of APRIL and BAFF by TACI-Ig consequently increases the efficiency of dexamethasone and anti–IL-6 treatment, and it eliminates nearly all MMCs in culture (62). Moreover, atacicept reduces the amount of MMCs in the bone marrow and slows disease progression in 82% of patients with relapsed/refractory MM (77).

**Hematopoietic niche components.** The spicamycin analog KRN5500 causes stress in the endoplasmatic reticulum and induces apoptosis in MMCs and osteoclasts in vitro and in vivo. Reduction of osteoclasts as important HNCs is a valuable feature of KRN5500 treatment (53).

**Allergen-specific PCs**

Allergen-specific IgE Abs contribute to the pathogenesis of type I hypersensitivity. Independent of allergen presence, IgE Ab titers persist for years, indicating that humoral allergologic memory is formed by LLPCs (78). Additionally, basophils and mast cells, loaded with IgEs on FceRI, can provide allergologic memory for weeks, even in the absence of PCs and soluble IgE, although for stable memory IgE supply is necessary (79). Recently, it was demonstrated that inhalation of the potent Ag OVA leads to the accumulation of IgG, IgA, and IgE LLPCs in the bone marrow and lung (21). However, the number of IgE LLPCs is lower than those of other Ig classes according to worse migratory capabilities of IgG PCs in the competition for the survival niche (80). Nevertheless, life-threatening anaphylactic reactions in patients with severe forms of allergy are still a tremendous risk. For curative treatment, the elimination of both immediate effectors and allergologic memory would be essential (78). Assuming that IgE LLPCs also depend on survival factors and an intact niche (78), they are susceptible to niche-targeted therapy. However, because few LLPCs are sufficient to load basophils and mast cells with pathogenic IgEs, only partial reduction of LLPCs may not gain improvement.

**Conclusions**

A supportive microenvironment that supplies soluble and membrane-bound survival factors is essential for the persistence of protective and pathogenic PCs. In autoimmunity, malignancy, and allergy, long-lived memory PCs are protected by their survival niche against most conventional immunosuppressive treatments and maintain a pathogenic Ab titer or in MM a malignant clone that may enable and accelerate the relapse or progression of the disease. Therefore, the survival niche for pathogenic PCs represents an important therapeutic target. The molecular characterization of the PC survival niche and the discovery of a dynamic HNC unveiled potential therapeutic targets for the treatment of PC-associated diseases. Whether alternative niches with different HNCs provide distinct niches for specific PC subpopulations remains an interesting question for future research, especially when considering novel strategies for selective depletion of pathogenic PCs.

**Disclosures**

The authors have no financial conflicts of interest.

**References**


