Structure and function of the hematopoietic cancer niche: focus on chronic lymphocytic leukemia

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TABLE OF CONTENTS

1. ABSTRACT

Chronic Lymphocytic Leukemia (CLL) is a B cell malignancy characterized by the accumulation of mature monoclonal CD5-positive B cells in the blood, secondary lymphoid tissues, and marrow. The infiltration of CLL cells in lymphoid tissues is a key element of disease pathogenesis. It is in such tissues that are found the microenvironments that provide CLL cells protection from spontaneous and/or drug-induced apoptosis. CLL cells actively shape their microenvironment by producing cytokines and chemokines, and by subverting normal accessory cells to promote leukemia-cell survival, proliferation, and escape from immune detection. In this review, we discuss how CLL cells disrupt the niches required for normal hematopoiesis or immune function and subvert normal cells in the microenvironment to support neoplastic cell growth and survival.

2. INTRODUCTION

CLL is characterized by the accumulation of small, mature-appearing CD5-positive B lymphocytes in the blood, marrow, and secondary lymphoid tissues. Even though the initiating event(s) leading to the onset of CLL is still unclear, the resulting CLL B cells appear resistant to programmed cell death, in part due to intrinsic factors, such as high-level expression of anti-apoptotic proteins (1), and in part due to extrinsic factors derived from the leukemia-cell microenvironment. The inability of CLL cells to survive *in vitro* under conditions that can support the growth of human B cell lines (2) implies that extrinsic signals from the microenvironment play an important role in CLL pathogenesis (3).

CLL cells infiltrate primary and secondary lymphoid organs, causing lymphadenopathy,

splenomegaly, and hypercellularity in the marrow. Such infiltration is thought to be an active process that contributes to disease maintenance and progression, creating niches where CLL cells can survive and proliferate. The homing and invasion of CLL cells into lymphoid structures alter the normal physiology of the marrow and lymphoid tissues.

3. DISRUPTION OF HEALTHY NICHES BY CLL CELLS

3.1. Disruption of lymphoid architecture in CLL

The architecture of primary and secondary lymphoid organs in CLL patients is altered by the presence of leukemic cells. The marrow of CLL patients is invariably infiltrated with CLL cells, following a pattern that can be diffuse and extensive, interstitial, nodular, or a mixture of interstitial and nodular (4-6). A diffuse infiltration refers to a uniform replacement of normal hematopoietic tissues by CLL cells, while in nodular and interstitial infiltrations, there is gross preservation of areas of normal marrow architecture (5). The extent of marrow infiltration by CLL cells correlates with the severity of the prognosis, where extensive marrow replacement is generally associated with advanced clinical stage and/or aggressive disease (5, 7). The lymph nodes and spleen of CLL patients typically are diffusely infiltrated with monomorphic, small, round lymphocytes that efface the normal lymphoid-tissue architecture (8, 9). These invasive patterns result in the displacement of the major resident populations and allow CLL cells to generate microenvironments that apparently support leukemia-cell proliferation.

In contrast to other B cell malignancies, the lymphoid tissues of patients with CLL develop pseudofollicles, which are oftentimes called "proliferation centers". Such pseudofollicles are scattered throughout primary and secondary lymphoid tissues of CLL patients (10). In these pseudofollicles, the B lymphocytes exhibit a paraimmunoblast and prolymphocyte morphology, express high-levels of CD23, and are enriched for cells that express Ki-67, a nuclear antigen present during replicative phases of the cell cycle that can serve as a marker for proliferating cells (8). The number and size of these pseudofollicles are variable amongst patients (11). Although the prominence of such pseudofollicles has not been associated with overall prognosis, it has been associated with lymphocyte doubling time (12), suggesting that such pseudofollicles might represent the compartment in which CLL cells proliferate.

3.2. Impact of CLL cells on the hematopoietic niche

The infiltration of CLL cells into the marrow results in over-crowding and possible production of factors that distort or impair the normal hematopoietic microenvironment. CXCL12 is the main chemokine responsible for the recruitment, maintenance, and survival of hematopoietic cells in the marrow (13-15). CLL cells express high levels of the receptor for CXCL12, namely CXCR4 (3), allowing leukemia cells to migrate into and survive within the marrow. Normal CD34$^+$ hematopoietic stem cells have to compete with CLL cells for CXCL12

elaborated by marrow stroma. As a likely consequence, patients with CLL may come to have a reduced number of CD34$^+$ stem cells that can give rise to granulocytes/macrophages, megakaryocytes, and erythrocytes in the marrow, compared to healthy individuals (16).

The capacity for hematopoietic stem-cell differentiation also appears affected by CLL cells. Particularly, CLL cells may produce Tumor Necrosis Factor (TNF)-alpha, which can inhibit growth of hematopoietic cells *in vitro* (17, 18). Also, CLL patients with disease-associated anemia have been noted to have higher serum levels of TNF-alpha than CLL patients without anemia, suggesting that TNF-alpha may be at least in part responsible for the cytopenias observed in some patients with CLL (18).

In addition, CLL cells may produce or alter the elaboration of factors that affect the marrow stroma, which ordinarily supports hematopoiesis. This might account for the observation that the marrow stroma of CLL patients appears less supportive of normal hematopoiesis *in vitro* than the marrow stroma of healthy individuals (19). This defect is associated with reduced production of interleukin (IL)-6 and increased production of Transforming Growth Factor (TGF)-beta by the marrow stroma of CLL patients compared to that of healthy individuals (19).

3.3. Impact of CLL cells on the immunologic niche

CLL typically is associated with progressive immune deficiency, resulting in hypogammaglobulinemia and impaired cellular immune responses (20, 21). The infiltration of secondary lymphoid tissues by CLL cells depletes resources required for normal immune cells and may be responsible in part for the acquired immune deficiency of patients with CLL.

Like normal lymphocytes, CLL cells are attracted to the lymph nodes by various chemokines. CCL19 and CCL21 are found on high endothelial venules (HEV) (22), which serve as the major site for lymphocyte trafficking into the lymph nodes. CLL cells express CCR7, which serves as a receptor for these chemokines. Stimulation of CLL cells by CCL19 via CCR7 enhances leukemia-cell survival (23, 24), and promotes CLL-cell production of active matrix metalloproteinase-9 (MMP-9) (25). Interactions of hyaluronan (HA) found on the basement membrane of HEV with CD44 expressed on CLL cells also may enhance leukemia-cell production of MMP-9 (26). This production of active MMP-9 potentially contributes to degradation of the matrix and/or basement membranes and facilitates the migration/invasion of CLL cells into the lymph nodes. Similarly, stimulation of CLL cells by CXCL12 via interaction with CXCR4 also can induce a comparable production of active MMP-9 (27), which might facilitate entry of CLL cells into the marrow.

Once in tissues, CLL cells can sequester a fraction of the available T cells. CLL cells can secrete T-cell attracting chemokines (28), which might contribute to formation of pseudofollicles within lymphoid tissues (29,

30). As such, CLL cells can compete with normal B cells for cognate interactions with T cells, thereby reducing the probability for chance encounter of T cells with antigen-reactive normal B cells. CLL cells also can inhibit the generation of effective cognate-interactions between activated T cells and normal B cells *in vitro* (31). Moreover, CLL cells can down-modulate the expression of CD154 on activated T cells (32), and elaborate factors, such as TGF-beta (33) or receptors for IL-2 that can mitigate the activity of helper T cells to stimulate antigen-reactive cells (34).

3.4. Subversion of non-neoplastic cells within the niche

CLL cells not only infiltrate the lymphoid structures; they also subvert surrounding cells to become more proficient in promoting leukemia-cell survival. CLL cells co-localize with numerous CD3$^+$ T cells (29), which may express CD154, the ligand for CD40 (30). Since CLL cells express CD40 and can temporarily be rescued from apoptosis (35) and induce to enter cell cycle (29, 36, 37) by contact with CD154-bearing cells, it is speculated that CLL-T cell interactions in these microenvironments may contribute to pathogenesis.

Infiltrating CLL cells also interact with other accessory cells in lymphoid tissues. CLL cells can come also in contact with follicular dendritic cells (FDCs), which are found in the marrow (38, 39) and lymph nodes (9), where they may enhance CLL-cell survival (40). In addition, myelomonocytic cells can differentiate into "Nurselike cells" (NLCs) when cultured with CLL cells (41). Such NLCs can promote leukemia-cell survival through the production of CXCL12 (42), B cell-activating factor of the TNF family (BAFF; CD257), and a proliferation-inducing ligand (APRIL; CD256) (43, 44). Mesenchymal stromal cells (MSCs; also known as reticular stromal cells) have been found in the marrow and around the white pulp of the spleen (8). The contribution of MSCs to CLL-cell survival has been shown *in vitro* using stromal cell lines (3, 42) or primary stromal cells derived from the marrow (45-47). CLL cells have been shown to affect these stromal cells through the release of factors, resulting in MSC activation and proliferation (48, 49).

3.4.1. NLCs in CLL and other lymphomas

An important modification induced in the leukemia-cell niche is the generation of a distinct type of supportive cells called "Nurselike cells" (NLCs). First identified as large, round cells that developed from the blood mononuclear cells of CLL patients after several days in culture (42), these cells were found to be derived from CD14-positive hematopoietic cells, indicating that they originated from the myelomonocytic lineage (41). Distinct from normal macrophages and hematopoietic-cell-derived dendritic cells, these cells can be found in the lymphoid tissues of patients with CLL (41), where they apparently promote CLL-cell survival (42, 44). In follicular lymphoma, immunohistochemistry studies found that patients who have lymphoid tissues with relatively high numbers of such cells (referred to as "lymphoma-associated macrophages") have greater resistance to therapy and shorter survival (50). In addition, gene expression profiling of lymphoid tissues found that the presence of the gene-signature derived from such non-malignant myelomonocytic cells appears to be more strongly associated with poor outcome than the gene-signature of the lymphoma cells themselves (51, 52), providing support for the importance of such accessory cells in the clinical behavior of patients with indolent B-cell lymphomas and leukemias.

3.4.2. T cells in CLL and other lymphomas

CLL cells can impair the capacity of T cells to recognize putative leukemia-cell-associated antigens. Following direct contact with CLL cells *in vitro*, T cells from healthy donors lose their ability to form an immunological synapse, which is the cell-cell interaction that T cells form with antigen-presenting cells. In particular, they lose their capacity to polymerize F-actin or recruit accessory molecules to the sites at which they contact antigen-presenting cells (53). T cells isolated from patients with CLL already manifest these defects (53), suggesting that CLL cells similarly impair the capacity of T cells to form such immunological synapses *in vivo*. The interaction between CD200 and Programmed Death 1 ligand (PD-L1; CD274) expressed by CLL cells with their respective receptors, CD200R and PD1, expressed on T cells, could be involved in this T cell defect, as preventing their interactions *in vitro* was recently reported to restore the ability of T cells to form immunological synapse with antigen-presenting cells (54).

In addition, a relatively high number of regulatory T cells can be found in the lymphoid tissues of patients with CLL (55). Comparably, an increased number of regulatory T cells have been found in lymphoid tissues of patients with follicular lymphoma compared to that of healthy individuals (56). Such regulatory T cells are either attracted to lymphoid tissue by lymphoma-cell production of CCL22, or generated *in situ* by contact with lymphoma cells that express high levels of CD70 (56, 57). These cells may suppress other T cell functions within the tumor (56, 58, 59), and, when present in a follicular pattern in lymph nodes, are associated with poor survival (60). Similarly, there often are relatively high numbers of such regulatory T cells in the lymphoid tissues of patients with CLL, where they might impair normal immune function.

3.5. The cytokine/chemokine prolife in CLL niches

CLL cells can engage in cross-talk with accessory cells within the microenvironment. Such cross-talk is mediated by the elaboration of chemokines, cytokines, and other factors that may distort the normal function of the lymphoid tissue. The established and proposed interactions that occur between CLL cells and cells in the microenvironment are depicted in

3.5.1. CLL–cell factors shaping the niche

CLL cells express multiple factors and receptors that function in the cross-talk between leukemia cells and their microenvironment (Figure 1). Both resting CLL cells in blood and cells homing to tissues can produce such factors. However, CLL cells exposed to antigens that may be sequestered in lymphoid tissues may be more active in
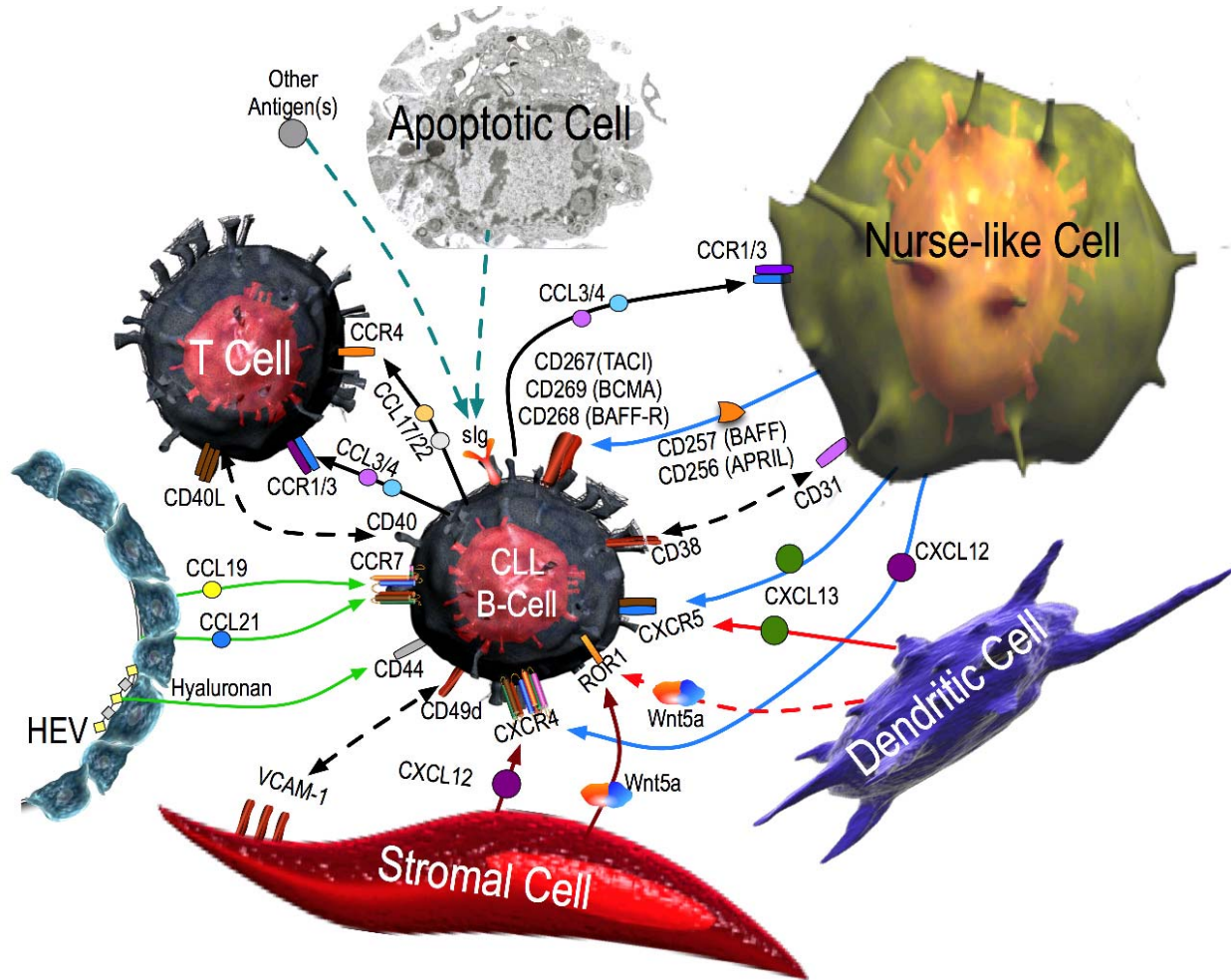
Figure 1. CLL microenvironment: the supportive cells and factors. CLL cells are recruited into the marrow and secondary lymphoid tissues following chemokine gradients. The migration of CLL cells into the marrow is primarily mediated through CXCR4 in response to CXCL12 (3), which is secreted mainly by NLCs (42) and MSCs (86, 87). CLL cells also are attracted to lymph nodes via CCR7 in response to the chemokines CCL19 and CCL21, which are produced by High Endothelial Venules (HEV) (22). The basement membranes of HEV also express hyaluronan (HA), which can interact with CD44, a signaling glycosoaminoglycan expressed by CLL cells that can recruit membrane-associated receptor-tyrosine kinases or their substrates and thereby facilitate cell signaling (118-120). It also might enhance the production of active MMP-9 (25), which could facilitate entry through the marrow. Follicular dendritic cells (FDCs), on the other hand, can secrete CXCL13 (94), which can attract CLL cells via CXCR5. Once in tissues, CLL cells can derive survival support from the same chemokines, as well as from additional factors elaborated by accessory cells in the CLL microenvironment. For instance, CLL cells come in contact with NLCs that can promote CLL cell survival through the production of CXCL12 (42). NLCs also express BAFF (B cell activating factor of the TNF family) and APRIL (a proliferation-inducing ligand), which can complement the survival stimulus afforded by CXCL12 (44). NLC-CLL interactions also can promote CLL-cell survival through cognate interactions between CD31 and CD38, which are expressed on NLC and CLL cells, respectively (80). In turn, CLL cells may secrete chemokines, such as CCL3 and CCL4 (81), which can recruit T cells and NLC-precursor cells to the CLL niche. T cells in that microenvironment might become activated and provide CLL cells with proliferative signals through CD154-CD40 interactions (30), as well as through the secretion of multiple cytokines, such as IL-2, IL-4, and IL-10 (92). In return, activated CLL cells secrete CCL12 and CCL22 (30, 93), chemokines that can attract more CCR4^{+} T cells into the CLL niche. MSCs also can contribute to CLL cell survival via the secretion of CXCL12, and in contrast to NLCs, also via the production of Wnt5a (Wingless-type MMTV integration site family, member 5a) (121), which can interact with ROR1 (Receptor tyrosine kinase-like Orphan Receptor 1) expressed by CLL cells (90). CLL-MSC contact also can be established through VCAM-1–CD49d interactions that contribute to CLL-cell survival (122). In tissues, CLL cells can be exposed to environmental and/or self antigens that might trigger cell activation through interactions with the surface immunoglobulin (sIg) expressed by CLL cells. Stimulation from ligation of sIg with antigen could amplify the responsiveness of CLL cells to the signals and factors provided by the CLL microenvironment.

producing such factors. Such antigens may activate CLL cells by cross-linking the surface immunoglobulin (sIg) present on the leukemia cell.

Indeed, the sIg plays a critical role in determining B cell fate, dictating cell death or survival, depending on quality and magnitude of sIg crosslinking by antigen. Similarly in CLL, the immunoglobulin (Ig) expressed by leukemic cells may dictate the fate of the cell and participate in the primary events involved in leukemogenesis (61-63). The frequent non-stochastic use of particular Ig heavy chain variable region genes (IGHV) and IGHV alleles (64) and the stereotypic Ig receptors of CLL cells (65) strongly argue that the Ig expressed in CLL are selected by some common antigen(s). Indeed, the CLL cells of many patients express Ig that can bind self antigens, such as human IgG (rheumatoid factor), myoglobin, single-stranded DNA, thyroglobin (66, 67), or non-muscle myosin heavy chain IIA, which are exposed on the surface of apoptotic cells (68). The Ig expressed by CLL cells also can bind cytoskeletal proteins (vimentin, cofilin-1, filamin B), phosphorylcholine-containing antigens found in *Streptococcus pneumonia* polysaccharides, or oxidized low-density lipoproteins (LDL) (69). Conceivably, such antigens not only may play a role in leukemogenesis, but also may stimulate CLL cells constitutively in the leukemia microenvironment.

CLL cells also secrete different soluble factors *in vitro* that potentially alter the normal hematopoietic cell niche. For example, compared to normal CD5$^+$ B cells, CLL cells constitutively express high levels of the chemokine CXCL8, as well as the receptor for CXCL8 (70), suggesting a potential autocrine loop (71). CLL cells also can express CXCL9 (72), as well as BAFF (73, 74), factors that also may enhance CLL-cell survival. In addition, CLL cells express high levels of immune-suppressive factors including TGF-beta and IL-10 (33, 75), and growth-promoting factors including TNF-alpha (76, 77) and IFN-gamma (78). CLL-cell secretion of these molecules might shape the microenvironment and influence the CLL cell cross-talk with other cells in this leukemia cell niche.

3.5.2. The cross-talk between CLL cells and NLCs

NLCs secrete CXCL12 (42) and CXCL13 (79), which can attract CXCR4$^+$ CLL cells to lymphoid tissues. NLCs also promote CLL-cell survival through the production of CXCL12 (42), BAFF and APRIL (44). CXCL12 mediates signaling via CXCR4. On the other hand, BAFF interacts with transmembrane activator and calcium-modulator and cytophilin ligand interaction (TACI; CD267), BAFF receptor (BAFF-R; CD268), or the B cell maturation antigen (BCMA; CD269), whereas APRIL interacts TACI or BCMA (43, 73, 74). Upon contact with NLCs, CLL cells secrete CCL3 and CCL4 (28), chemokines that attract other accessory cells, such as monocytes/macrophages and T cells, which in turn may be recruited to the CLL niche.

The expression of CD31 on NLCs also may interact with CD38 on CLL cells (80). Such engagement of CD38 increases CLL-cell production of CCL3 and CCL4, which in turn can recruit CCR1/5$^+$ monocytes and macrophages (81). This might account for the large numbers of infiltrating CD68$^+$ macrophages (NLCs) in marrow biopsies of patients with CLL cells that express high levels of CD38 and CCL3 (81). The stimulation of these macrophages with CCL3 also can upregulate endothelial-cell expression of vascular cell adhesion molecule 1 (VCAM1, CD106), which can provide another survival stimulus to CLL cells via CD49d (81). The co-localization of endothelial and stromal cells that express high levels of VCAM1 in the marrow of patients with CLL cells that express high levels of CD38 supports the notion of such a dynamic *in vivo* (81). The engagement of CD38 on CLL cells may increase their capacity to migrate towards CXCL12 by enhancing signaling via CXCR4 (82), revealing how these intricate signals can interact with one another to enhance the support that CLL cells derive from their microenvironment.

3.5.3. The cross-talk between CLL cells and MSCs

CLL cells derive survival support from MSCs. MSCs can produce many cytokines that can affect hematopoietic cells, such as IL-6, IL-7, IL-8 IL-11, IL-2, IL-14, IL-15, leukemia-inhibitory factor (LIF), macrophage colony-stimulating factor (M-CSF), FMS-like tyrosine kinase 3 (Flt-3) ligand, and stem-cell factor (83-85). MSCs also produce chemokines such as CXCL12, which can support leukemic cell survival *in vitro* (86, 87). In addition to soluble factors, the survival support provided by MSCs can be mediated in part by cell-cell contact. Like endothelial cells, MSCs express high levels of VCAM1 (88), which can interact with CD49d expressed by CLL cells (89). These interactions, still poorly described, can alter the production of factors by MSCs, thereby shaping the cytokine/chemokine profile within the niche. Finally, MSC express high level of Wnt5a, which might promote CLL-cell survival by stimulating signaling through Receptor tyrosine kinase-like Orphan Receptor 1 (ROR1) expressed on CLL cells (90).

Comparable to the interactions between CLL cells and NLCs, CLL cells may establish a cross-talk with MSCs. Soluble molecules secreted by CLL cells can induce the activation of the Extracellular signal-Regulated Kinase (ERK) and the AKT pathways in these supportive cells (91), and modify their expression of various molecules that in turn affect leukemia-cell survival. CLL cells may release microvesicles that can activate MSCs through the AKT/mammalian target of rapamycin/p70S6K/hypoxia-inducible factor-1alpha pathway, increase Vascular Endothelial Growth Factor (VEGF) production, and in turn promote CLL cell survival and resistance to chemotherapy (49). It appears that the secretion of platelet-derived growth factor (PDGF) by CLL cells and the concomitant activation of its receptor, PDGF-receptor, on MSCs results in enhanced MSC proliferation and VEGF secretion *in vitro (48)*, providing insight into the dynamic communication established between CLL cells and MSCs.

3.5.4. The cross-talk between CLL cells and T cells

Cross-talk between CLL cells and T cells has been studied *in vitro*, where CLL-T cell contact has been

partly recapitulated by CD40 stimulation. Following CD154 engagement by CD40, activated T cells can produce an array of soluble factors that might affect CLL cells, such as IL-2, IL-4, and IL-10 (92). Interaction with T cells also can trigger CLL-cell expression of CCL17 and CCL22 (42, 93), chemokines that can attract more CCR4$^+$ T cells into the CLL niche. In accordance with these observations, CLL cells found in lymph nodes and the marrow express a higher level of these chemokines than CLL cells in the peripheral blood (42), suggesting that such a recruitment mechanism operates *in vivo*.

3.5.5. The cross-talk between CLL cells and FDCs

In lymphoid tissues, CLL cells are also in the proximity of FDCs, which may provide survival support. *In vitro*, FDCs can promote CLL cell-survival via CD44 expressed on CLL cells (40). Because FDCs can secrete CXCL13 (94), they also might play a role in the recruitment of CLL cells to lymphoid tissues. In addition, FDCs, which are of mesenchymal origin, may express Wnt5a (95) which could promote CLL-cell survival through its effects on ROR1 (90).

4. THERAPEUTIC STRATEGIES TO DISRUPT THE DIALOG BETWEEN CLL CELLS AND THE SUPPORTIVE NICHE

The interactive dynamic established between CLL cells and the microenvironment apparently promotes the growth and survival of CLL cells *in vivo*. Targeting these interactions therefore represents an attractive avenue for therapy, with the aim of inducing CLL-cell apoptosis and/or increasing the sensitivity of CLL cells to current therapies. These new approaches fall into two basic categories: (1) strategies that block the interaction of survival/growth factors with their receptors or (2) strategies that disrupt the signaling pathways in CLL cells that are activated by such survival/growth factors.

CXCR4 antagonists fall into the first category. CXCR4 antagonists block the interaction of CXCL12 with its receptor. MSCs and NLCs constitutively secrete CXCL12, which induces CLL-cell trafficking and homing to the marrow through their expression of CXCR4. CXCR4 antagonists, such as plerixafor (AMD3100) or T140 analogs, can mitigate the survival support provided by CXCR4 in the microenvironment and enhance the sensitivity of CLL cells to cytotoxic effects of anti-leukemia drugs, such as fludarabine monophosphate (96). A phase I clinical currently is evaluating the use plerixafor in combination with rituximab (97) for treatment of patients with relapse and/or refractory CLL. Preliminary data show that treatment with plerixafor induces a dose-dependent mobilization of CLL cells into the blood, suggesting that it represents a promising approach to liberate CLL cells from the supportive microenvironment.

Similarly, a soluble form of TACI, named Atacicept, was developed as a decoy receptor to neutralize APRIL and BAFF. BAFF and APRIL made by NLCs (44) enhance the survival and growth of CLL cells (43, 98). In a phase 1b study involving patients with refractory and/or

relapsed CLL, Atacicept promoted stabilization of the disease (99), suggesting that it might interfere with proliferation and/or survival of CLL cells *in vivo*.

In the second category are compounds that target leukemia-cell growth or survival pathways induced by factors in the microenvironment. These reagents include inhibitors of the delta-isoform of phosphatidylinositol-3-kinase (PI3K) (CAL-101), spleen tyrosine kinase (Syk) (fostamatinib disodium/R406), bruton's tyrosine kinase (Btk) (PCYC-1103-CA), or Bcl-2 (obatoclax/GX15-070 and ABT-263).

PI3K plays a role in signaling pathways triggered by several factors produced by cells within the microenvironment. *In vitro*, inhibiting the activity of the delta isoform of PI3K using the compound CAL-101 has been reported to impair CLL-cell survival induced by either CD154, BAFF, TNF-alpha, or stromal cells (100). Treatment of patients with relapsed CLL with this compound reduced the size of the lymph nodes, which was associated with increasing lymphocytosis (101). These results suggest that inhibition of PI3K might interfere with retention of CLL cells in lymphoid tissues, possibly reducing the capacity of CLL cells to remain sequestered within the microenvironment.

Syk plays a critical role in signaling via the B cell antigen receptor (BCR) (102). *In vitro*, the inhibition of Syk using the compound R406 has been shown to induce apoptosis of CLL cells, either by abrogating the survival support induced by BCR ligation, NLCs, or MSCs. Inhibition of Syk also might inhibit the migration and adhesion of CLL cells to the marrow stroma (103-105). In a clinical trial evaluating one Syk inhibitor, fostamatinib disodium, patients with CLL or small lymphocytic lymphoma (SLL) experienced reductions in lymphadenopathy associated with an apparent mobilization of malignant cells into blood (106). The kinase Btk, which is downstream of Syk and activated by BCR signaling has also been identified as a therapeutic target. The Btk inhibitor PCYC-1103-CA is currently under investigation in clinical trials.

CLL cells express higher level of the pro-survival molecule Bcl-2 than normal B cells (107), and the level of expression has been shown to correlate with resistance to apoptosis, and poor response to chemotherapy (1, 108, 109). *In vitro*, the small molecule pan-Bcl-2 inhibitor GX15-070 has been shown to induce apoptosis of CLL cells (110). *In vivo*, a phase I study targeting CLL patients with advanced disease demonstrated that GX15-070 has biologic and modest clinical activity (111), suggesting its usage in a combinatorial therapeutic approach. ABT-263 is another compound targeting not only Bcl-2, but also Bcl-XL and Bcl-w by binding to their common BH3 domains (112). This binding prevents the sequestration of pro-apoptotic proteins, resulting in the induction of cell death. *In vitro*, ABT-263 has been shown to rapidly induce apoptosis in various cell lines (112) as well as primary CLL cells (113). In a phase I clinical

study, this compound was reported to be safe and active in patients with relapsed CLL (114).

Finally, there is lenalidomide, a derivative of thalidomide, which is an immunomodulatory drug that has been approved for the treatment of various hematologic malignancies, such as multiple myeloma or myelodysplastic disease. In clinical studies, lenalidomide had activity in patients with relapsed CLL (115, 116). However, the mechanism of action of this drug remains unknown. Because this drug is not directly cytotoxic for CLL cells (unpublished observations), its activity instead may be directed at cells within the microenvironment. A potential mechanism of action appears to revolve around T cells, since lenalidomide treatment of CLL patients has been shown to reduce the proportion of regulatory T cells (CD4$^+$CD25^{hi}FOXP3$^+$), and to increase the proportion of helper T cells (TH17) (117). In addition, a change in the activation status of CD8$^+$ T cells has also been reported, transitioning from a proliferative to a cytotoxic phenotype (117). Further investigation is required to elucidate the mechanism of action of lenalidomide in CLL.

4. CONCLUDING REMARKS

In CLL, monoclonal B cells accumulate in blood, secondary lymphoid tissues, and marrow. This infiltration disrupts normal hematopoietic and immunologic niches, leading to the establishment of two hallmarks of CLL, namely myelosuppression and immunosuppression. In tissues, CLL cells subvert normal cells to fashion their own microenvironment, which apparently promotes leukemia-cell growth and survival. The support that CLL cells derive from the microenvironment is increasingly being recognized as a major contributor to pathogenesis, as well as a potential target for development of new therapies. Accordingly, new strategies are being evaluated that disrupt the interactions between CLL cells and the cells within the microenvironment that promote CLL-cell survival. Such strategies might prove therapeutic by themselves or be used in combination with standard therapies to improve the outcome of patients with this disease.

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