The Bone Marrow Microenvironment in Waldenström Macroglobulinemia

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INTRODUCTION

Waldenström macroglobulinemia (WM) is a rare low-grade B-cell lymphoproliferative disorder defined by infiltration of lymphoplasmacytic lymphoma in the bone marrow (BM) and an increase synthesis by malignant cells with subsequent accumulation in the serum of monoclonal immunoglobulin M (IgM) that can cause hyperviscosity and other symptoms in the affected patients.1 Recently, substantial research has been focused on understanding the pathogenesis of WM and to define the underlying molecular mechanisms involved in the disease. This work has confirmed not only the importance of mutations within the malignant cells but also highlighted the role for the BM microenvironment in promoting malignant B-cell growth, tumor cell survival, and IgM production. Using whole-genome sequencing analysis, initial work identified a somatic variant in the Myeloid Differentiation factor 88 (MYD88) gene that results in an amino acid leucine substitution by proline (L256P), and this mutation is seen in the vast majority of patients with WM.2

MYD88 is integral in processing signals external to cell and acts as an adaptor protein for interleukin-1R (IL-1R) and Toll-Like receptor (TLR) signaling pathways. Recent

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data have also shown a role for MYD88 in B-cell receptor signaling. Overall, its role is to recruit signaling molecules and kinases that ultimately converge in nuclear factor (NF)κB activation. TLRS are involved in regulating the innate immune response, and stimulation of TLR, induced by environmental signals, recruits MYD88 to the cytoplasmic domain of TLR. It then forms a complex with IL-1R associated kinase 4 (IRAK4) and IRAK1 and initiates a signaling cascade that subsequently activates NFκB. Bruton tyrosine kinase (BTK) is also a component of TLR signaling that specifically binds to MYD88 and activates signaling cascades in response to environmental signals. In WM, the mutant form of MYD88 is shown to form a complex with BTK and induces constitutive activation of TLR, which amplifies the signaling process, and results in increased survival and proliferation of the tumor cells. In addition to promoting NFκB signaling, the MYD88 mutation has been shown to trigger Janus kinase/Signal transducer and activator of transcription 3 (JAK/STAT3) signaling and promote the secretion of cytokines, including IL-6, IL-10, and interferon-β (IFN-β) in the BM microenvironment. The accumulation of these cytokines in the tumor microenvironment enhances the survival of malignant cells via an autocrine mechanism, implying that the MYD88 mutation not only dysregulates malignant cell growth, but also amplifies signals from the BM environment, thereby further promoting uncontrolled lymphoma cell growth and increased IgM production.

A number of recent studies have focused on understanding the role of the BM microenvironment in WM, and these studies are exploring the role of other nonmalignant cells, cytokines, and other growth factors in WM pathology. Studies have suggested a reciprocal interaction between WM cells and the elements of the BM and shown that this interaction provides a growth support for WM cells. In this review, the authors summarize data regarding the key elements in the BM microenvironment that support the growth of lymphoplasmacytic lymphoma cells and stimulate monoclonal IgM production.

COMPONENTS OF THE BONE MARROW NICHE

The physical environment surrounding the malignant cell is important, and the architecture of the BM milieu incorporates both cellular and noncellular components. A wide variety of cell types, including blood cells and their lineages, including fibroblasts, mesenchymal cells, osteoblasts, osteoclasts, adipocytes, and endothelial cells are present, as well as noncellular components, such as extracellular matrix, cytokines, chemokines, growth factors, and metabolites. Various studies have shown that a complex, yet coordinated array of the interactions exists between the cellular and acellular entities of the BM microenvironment. These interactions are a key determinant of survival and self-renewal of the hematopoietic stem cells (HSCs) in the BM and also in the differentiation of various cell types under physiologic conditions.

Previous research has shown that 2 distinct specialized microenvironments, described as an endosteal niche and a vascular niche, are present in the BM, and these niches are shown to provide support for HSCs. The endosteal niche is localized at the interface of the trabecular bone with the BM elements, where the HSCs are in close proximity to cells such as osteoblasts. These cells maintain the self-renewal capacity of the HSCs and regulate their function. Interaction of HSCs with osteoblasts is mediated either directly through cell-cell contact or indirectly via secretory factors that are produced by osteoblasts and bind to their cognate receptor on HSCs. The endosteal niche therefore plays an essential role in sustaining HSCs, thereby allowing them to maintain the self-renewal capacity of the BM. In contrast, the vascular niche supports HSCs that are located near sinusoidal blood vessel structures, and the vascular
niche facilitates dissemination of cells into the vascular system and also promotes “homing” of circulating cells from blood back to the BM.\textsuperscript{11,12} Although the BM is the primary site for normal hematopoiesis, it may become a permissive environment for the location and growth of malignant cells due to these favorable BM niches. BM infiltration is a commonly seen in WM, and BM involvement in WM is commonly in a paratrabecular pattern,\textsuperscript{5,11,13} suggesting that the BM niche supports and even promotes malignant cell growth.

**MOLECULES THAT REGULATE HOMING OF MALIGNANT CELLS TO THE BONE MARROW**

Previous studies have investigated the mechanisms by which WM cells home to the BM. This work has shown that stromal derived factor-1 (SDF-1; also known as CXCL12) is highly expressed in the BM of patients with WM, and the presence of SDF-1 results in dose-dependent migration of WM cells in vitro. The SDF-1–induced migration by WM cells is mediated by C-X-C chemokine receptor type 4 (CXCR4) on the cell surface, and inhibition of CXCR4 by the small molecule inhibitor plerixafor or by knockdown techniques substantially reduces WM cell migration.\textsuperscript{14} These findings are highly relevant, as approximately 30\% of the patients with WM have somatic mutations in the \textit{CXCR4} gene, and this mutation is the second most prevalent somatic mutation in WM after mutations in \textit{MYD88}.\textsuperscript{7,8} Initial research studied the cell surface expression of mutant CXCR4 and found that mutant CXCR4 is highly expressed on WM cells. This study found that increased CXCR4 expression, together with increased SDF-1 levels in patients with WM, could augment the CXCR4/SDF-1 interaction and significantly promote homing of WM cells to the BM.\textsuperscript{15} SDF-1 has also been shown to be involved in the adhesion of WM cells to the fibronectin. WM cells have increased expression of Very Late Antigen-4 (VLA-4), an integrin dimer that co-interacts with CXCR4 and mediates adhesion of WM cells to the BM stromal cells (BMSCs) and endothelial cells in response to SDF-1. Furthermore, adhesion of WM cells to stromal cells from the BM has been found to make WM cells resistant to chemotherapy, suggesting that stromal cells play an important role in drug resistance providing a potential opportunity to exploit this therapeutically.\textsuperscript{14}

Furthermore, activation of survival signaling pathways impacts WM cell homing to the BM. Akt activity has been found to be constitutively upregulated in malignant WM cells. In vivo and in vitro studies have demonstrated that activation of Akt not only induces proliferation but also influences migration and homing of the WM cells to the BM.\textsuperscript{16} MicroRNA (miRNA), including MiR-155, regulates Akt, and WM cells have altered miRNA expression profiles compared with their normal cellular counterparts. Using miRNA expression profiling, it has been found that several miRNAs including MiR-155 are upregulated in malignant WM cells. MiR-155 plays an important role in B-cell malignancies and regulates the BMSC-induced proliferation of WM cells, modulates the adhesion of WM to the fibronectin, and induces the migration of the cells in response to SDF-1, thereby resulting in homing of the WM cells in the BM.\textsuperscript{17}

**CELLS IN THE BONE MARROW THAT FACILITATE TUMOR CELL GROWTH**

WM cells do not grow in isolation in the BM; instead, normal cells that typically facilitate hematopoiesis, immunity, and bone formation surround them. The BM typically consists of T cells (CD4\(^+\), CD8\(^+\), and T\(_{\text{reg}}\) cells), B cells, dendritic cells, natural killer cells, myeloid-derived suppressor cells, mesenchymal stem cells, osteoclasts, osteoblasts, and endothelial cells. Although the number and/or function of these cells can vary under pathologic circumstances, the contribution of these cells to the
pathogenesis of WM has not been fully evaluated. Recently, several studies have reported that mast cells, T cells, monocytes, and endothelial cells may contribute to the pathogenesis of WM (Fig. 1).

**Mast Cells**

Increased numbers of mast cells in the BM is a characteristic of WM. It has been demonstrated that the mast cells in the BM of patients with WM promote the proliferation of malignant cells, and this support of malignant growth is mediated by the interaction of CD154 (CD40 L) and CD40, which are expressed on mast cells and malignant cells, respectively.\(^{18}\) Although the signaling mechanisms regulating cell surface expression of CD40 and CD40 L are not well understood, previous work has described a role for soluble CD27 (sCD27), a member of the tumor necrosis factor (TNF) family. These studies have found that sCD27 is increased in the serum of the patients with active WM and induces the expression of CD40 L on mast cells, thereby promoting the CD40-CD40 L interaction. Of note, increased sCD27 in WM correlates with the serum IgM level, implying that it could serve as a marker for disease progression.\(^{19}\)

**T Cells**

T cells present in the BM of patients with WM are thought to possibly represent part of an antitumor immune response; however, expression of immune checkpoint molecules on the surface of T cells is associated with an exhausted T-cell phenotype and recent work has shown that immune checkpoint molecules are expressed in both normal and WM marrows. The expression of programmed death-1 (PD-1) and its ligands, PD-L1 and PD-L2, has been found to be increased in WM, and the increased expression of PD-L1 and PD-L2 in the BM of the patients with WM modulates the proliferation of both the malignant B cells and normal T cells.\(^{20}\) These findings suggest that T cells present in the BM are inhibited by the expression of immune

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**Fig. 1.** Schematic representation of a malignant cell interacting with neighboring BM cells in WM.
checkpoint molecules on malignant cells and the PD-1/PD-1 ligand interactions may favor the growth and survival of the malignant clone.

**Endothelial Cells**

WM cells have also been shown to interact with endothelial cells in the BM microenvironment. Ephrin receptor-B2 (Eph-B2), a transmembrane receptor tyrosine kinase, is upregulated on WM cells, and its interaction with the ligand Ephrin-B2 on the endothelial cell surface induces WM cell adhesion and proliferation. Co-culture of WM cells with endothelial cells promotes Eph-B2/EphrinB2 interaction and induces phosphorylation of Eph-B2, resulting in downstream signaling including activation of Akt, cyclin D3, cyclin E, focal adhesion kinase, Src, P130, paxillin, and coflin. Activation of these molecules has been found to induce WM cell proliferation and adhesion. In contrast, downregulation of Eph-B2 on WM cells reduces the BM infiltration by malignant cells and inhibits tumor progression.

**CYTOKINES IN THE BONE MARROW MICROENVIRONMENT**

Soluble factors, particularly cytokines, are critical regulators of B-cell differentiation to plasma cells and also facilitate plasma cell homeostasis and stimulate immunoglobulin secretion in the BM. For example, IL-21 is integral to the regulation of normal B-cell function and facilitates B-cell differentiation to immunoglobulin-producing plasma cells. B-cell activating factor (BAFF), also known as B-lymphocyte stimulator (BLyS), is a member of the TNF superfamily that binds to its receptor on B cells (BAFF-R), promotes B-cell survival, and induces B-cell proliferation. IL-6 also induces B-cell differentiation, stimulates immunoglobulin production, and promotes plasma cell survival. Although cytokines are required to maintain normal B-cell function, dysregulation of their production or function will result in increased malignant cell growth.

A comprehensive analysis of the cytokine composition of the BM microenvironment in patients with WM found that it is very different from that of normal BM. Using a multiplex bead-based array analysis to screen the cytokine expression in healthy controls and patients with WM, cytokines including chemokine (C-C Motif) ligand 5 (CCL5), granulocyte colony-stimulating factor (G-CSF), soluble IL-2 receptor (sIL-2R), epidermal growth factor, and IL-8 were found to be most different in their expression, with CCL5 showing the greatest increase in the patients with WM as compared with healthy subjects. In this study, increased expression of CCL5 in the BM of patients with WM correlated with high IgM secretion and increased infiltration of the BM by malignant cells. To understand the function of CCL5 and define its potential role in modulating the BM microenvironment in WM, further research found that CCL5 does not directly impact the survival, growth rate, or IgM secretion in malignant WM cells. Rather, CCL5 indirectly modulates WM cell function by stimulating BMSCs to secrete IL-6 and the increased secretion of IL-6 stimulates WM cells to secrete IgM by a mechanism that involves JAK/STAT signaling. The role of IL-6 in promoting IgM secretion also involves GLI2, an oncogenic zinc finger transcription factor, which is expressed in the BM of patients with WM. GLI2 induces transcription and cell surface expression of IL-6 receptor-α (IL-6Rα) on WM cells and promotes IgM secretion. All told, increased expression of both IL-6 and IL-6Rα result in increased IgM secretion.

Similarly, IL-21 plays an important role in promoting WM cell growth and survival. Increased IL-21 has been found in the BM of patients with WM compared with healthy controls and IL-21 appears to be predominantly produced by T cells in the BM microenvironment. The receptor for IL-21 (IL-21R) is highly expressed on the surface of WM
cells, suggesting that IL-21 may regulate the function of malignant WM cells. Further investigation revealed that IL-21 in the WM BM microenvironment stimulates cell proliferation and IgM secretion by malignant WM cells via an STAT-3–dependent pathway. STAT5 was also found to be constitutively activated in WM and this activation is likely due to persistent cytokine signaling. Inhibition of STAT5 signaling was found to significantly decrease IgM production.

BAFF is a TNF family member that is critical for maintenance of normal B-cell development and homeostasis, and BAFF has been found to be upregulated in the serum and BM of patients with WM. BAFF is expressed by monocytes, macrophages, and dendritic cells, and potently induces B-cell proliferation and immunoglobulin secretion. Malignant B cells in WM were found to bind soluble BAFF and variably express the receptors for B-cell activating factor (BAF), namely BAFF-R, TACI, and BCMA. BAFF, alone or in combination with cytokines, including IL-6, that induce immunoglobulin production, was found to increase IgM secretion by malignant B cells. Furthermore, BAFF was found to increase the viability and proliferation of malignant B cells from patients with WM.

A further cytokine that has relevance in WM is macrophage inflammatory protein-1 alpha (MIP-1α). MIP-1α is increased in the serum of patients with WM and serum levels differ for different stages of the disease. MIP-1α is a member of CC chemokine family and is secreted by various cell types, including macrophages, lymphocytes, and dendritic cells. MIP-1α also activates osteoclast activity and is associated with bone disease in other plasma cell disorders. Although the presence of abnormal bone remodeling and increased bone resorption has been reported in WM, patients with WM do not typically have lytic bone disease, similar to that seen in multiple myeloma. In WM, the MIP-1α–induced osteoclast activity may be countered by an increase in osteoprotegerin, which has a role in bone formation. However, increases in MIP-1α in WM were found to correlate with disease activity, including increased serum β2-microglobulin and splenomegaly, implying that MIP-1α is relevant in the biology of WM.

Finally, CXCL13 is a chemokine that is expressed in lymphoid organs by follicular dendritic cells and macrophages and is also produced by lymphoplasmacytic cells. Notably, CXCL13 attracts mast cells to the BM microenvironment. Recent data have suggested that CXCL13 levels vary in WM and appear lower among CXCR4 frameshift but not CXCR4 nonsense mutated patients versus those who were CXCR4WT. An increased CXCL13 level was a strong predictor of achieving a response to BTK inhibition, and major responses to ibrutinib therapy were associated with deep suppression of CXCL13 levels.

ANGIOGENESIS AND STROMAL ELEMENTS

Angiogenesis has been associated with disease progression and a poor prognosis in several hematological cancers. Initial studies in symptomatic patients with WM reported that 30% of patients had increased microvascular density (MVD), whereas patients with IgM-monoclonal gammapathy of undetermined significance (IgM-MGUS) and patients with asymptomatic WM had reduced MVD in their BM. This suggested an angiogenic switch between asymptomatic and active phases of the disease. Unfortunately, an increase in the MVD in the BM in WM correlated poorly with the extent of malignant cell infiltration and lacked a prognostic association with survival of patients with WM. Although angiogenesis appeared important once WM was present, it was unclear whether angiogenesis played a significant role in the progression of the disease.

However, increased angiogenesis in a group of patients with WM could be related to hypoxic conditions in the BM. Although hypoxia appears to prevent proliferation of
WM cells, it induces their dissemination and promotes homing in new BM niches. Hypoxic WM cells lose their adhesion to the BMSCs and this effect is mediated by reduced E-cadherin expression on the surface of WM cells. In comparison with normoxic cells, hypoxic WM cells exhibit increased homing to the BM. This effect is mediated by increased expression of CXCL4 on WM cells, which promotes chemotaxis toward areas of increased SDF-1 expression, such as the BM. This suggests that increased angiogenesis may overcome hypoxia in the BM and may thereby promote WM cell proliferation.

Changes in the expression level of angiogenic factors, including vascular endothelial growth factor (VEGF), VEGF-A, angiogenin, angiopoietin-1 (Ang-1), Ang-2, and basic fibroblast growth factor (bFGF), in patients with WM at different stages of their disease have been evaluated. Patients have been assessed before treatment onset, and then at the time of relapse or remission. The data showed that serum levels of VEGF, VEGF-A, angiogenin, Ang-2, and bFGF are elevated, and Ang-1/Ang-2 ratio is reduced in all patients with WM irrespective of their phase of disease. Serum angiogenin levels correlated with the active disease, and patients in remission had lower angiogenin levels when compared with untreated patients. Furthermore, Ang-1/Ang-2 ratios were inversely correlated with β2M and positively correlated with serum albumin levels. These data indicate that angiogenic factors may be used as biomarkers to monitor disease progression. However, the mechanism by which each of these individual angiogenic factors contributes to disease progression in WM needs further investigation.

Furthermore, Eph-B2 may have a role in angiogenesis, as interactions between Eph-B2 on WM cells and EphrinB2 on the endothelial cells in the BM induces Ephrin-B2 phosphorylation. This subsequent phosphorylation of downstream signaling molecules in endothelial cells results in formation of new blood vessels.

**SUMMARY**

Although our understanding of the role of BM microenvironment in WM has significantly increased in recent years, there are still many unknown factors that promote WM cell growth that need to be studied. Recent publications have provided insightful data on the BM constituents that support WM cells and explained in part which ligands or cell subpopulations promote malignant cell growth and proliferation, or stimulate immunoglobulin production (Box 1). WM cells have been shown to be in close contact

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<th>Box 1</th>
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<td>Factors promoting the bone marrow niche in Waldenström macroglobulinemia</td>
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<tr>
<td>• Stromal derived factor-1 (SDF-1) induces increased migration of C-X-C chemokine receptor type 4 (CXCR4) expressing cells to the bone marrow</td>
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<td>• Increased miR-155 modulates adhesion to fibronectin and promotes migration in response to SDF-1</td>
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<td>• Increased hypoxia promotes colonization of the bone marrow niche</td>
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<td>• Eph-B2/ephrin-B2 interaction enhances adhesion to endothelial and stromal cells</td>
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<td>• Increased mast cells promote malignant cell growth through BAFF/APRIL/CD70/CD40 L signaling</td>
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<td>• Cytokines, including CCL5, interleukin (IL)-6 and IL-21, promote malignant cell survival by means of a JAK/STAT/GLI2 signaling loop</td>
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with BMSCs, mast cells, T cells, monocytes, macrophages, and endothelial cells. These cells directly stimulate malignant cell growth and promote IgM secretion by WM cells. Furthermore, cytokines and chemokines, including IL-6, IL-21, BAFF, and CCL5 also promote cell proliferation and induce IgM secretion. Future research will provide a more comprehensive understanding of the role of the BM microenvironment in WM and will allow for the design of effective therapeutic strategies that exploit the complex network of interactions in the environment on which WM cells rely.

REFERENCES