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Cytokines in the Microenvironment of Waldenström's Macroglobulinemia

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Abstract

Waldenström's macroglobulinemia (WM) is a lymphoplasmacytic lymphoma characterized by the overproduction of a monoclonal IgM protein that accumulates in the serum. Although the pathologic findings in this disease entity are reasonably well defined, the mechanisms that regulate malignant B-cell growth and monoclonal protein synthesis are less well understood. Cytokines are known to regulate many biologic processes in normal lymphocyte development including immunoglobulin production, and the presence of cytokines within the tumor microenvironment of WM is likely to contribute to malignant cell growth and survival as well as immunoglobulin production. Several studies have suggested that cytokines are potentially dysregulated in WM, however the precise role played by cytokines in WM biology is not clearly defined. This report therefore highlights our current understanding of the role of cytokines in the microenvironment of WM and how they affect malignant cell persistence and function in the bone marrow of patients.

Keywords

Angiogenin; Angiopoietin; B-lymphocyte stimulator; Vascular endothelial growth factor; Waldenström

Introduction

Waldenström's macroglobulinemia (WM) is an indolent B-cell disorder characterized by infiltration of the bone marrow by lymphoplasmacytic cells and associated symptoms such as anemia, lymphadenopathy, and serum hyperviscosity¹ related to the overproduction of a monoclonal immunoglobulin (Ig) M (IgM) protein by malignant B cells. In recent years, there have been some advances in our understanding of WM biology and significant clinical advances in the treatment of WM. However, WM remains an incurable disease and most patients progress and develop complications of the disease. Clearly there is an increasing need for the development of new targeted therapies and one important element in developing targeted therapies for patients with this disease is a better understanding of the biologic signals that regulate malignant cell growth, survival, and Ig production.

The Tumor Microenvironment as a Source of Dysregulated Cytokine Production

The tumor microenvironment is composed of many elements that contribute to homeostasis. These include the cellular components such as stromal/endothelial cells and cells of the immune system and the acellular components such as the cytokine milieu containing

cytokines and soluble factors produced by those cells. With regard to the cellular component of the tumor microenvironment, mast cells have been shown to be present in the bone marrow of patients with WM in higher numbers compared with healthy controls.² These cells play important roles in the innate and adaptive immune responses and their activation leads to proinflammatory cytokine secretion. Although the precise role of mast cells in cytokine production in WM has not been studied, they have been shown to promote malignant cell growth in WM.² One possibility for the mast cell mediated support of malignant cell growth in WM is via their cytokine production. However, no studies to date have addressed the contribution of cytokine secretion by mast cells to WM biology. Another important cellular component of the tumor microenvironment, particularly in the bone marrow, is the stromal cell. Stromal cells represent a cytokine factory that constantly supplies malignant cells with the necessary signals required to grow. Their role in supporting malignant cells has been studied in many cancers.^{3,4} In fact, the efficacy of many new potential therapies are tested in the presence of stroma, further supporting the notion that stroma is an important component of the tumor microenvironment.

Cytokines with a Role in Normal B-Cell Biology

Several cytokines are known to be key regulators of B-cell homeostasis and play an important role in B-cell development. IL-7 is a well-described cytokine that plays an important role in B-cell development⁵ and B-cell specification and commitment in the bone marrow is driven by IL-7 signaling. The chemokine CXCL12 (also known as SDF1) also plays a role in B-cell development and homing to the bone marrow, and mice deficient in CXCL12 have severely reduced numbers of B-cell precursors.⁵ B-lymphocyte stimulator (BLyS) is a tumor necrosis factor (TNF) family member expressed by several cell types including monocytes, macrophages, dendritic cells, and neutrophils. B-lymphocyte stimulator has been shown to be critical for the maintenance of normal B-cell development and homeostasis⁶ and has been suggested to co-stimulate B-cell proliferation and Ig secretion.⁷ Another cytokine IL-6, is known to play an important role in B-cell proliferation and maturation⁸ and has also been shown to induce Ig secretion by normal B cells.⁹ Overexpression of any of these cytokines, however, may stimulate dysregulated B-cell proliferation or immunoglobulin production.

Dysregulated Cytokine Production in Waldenström's Macroglobulinemia

Recent studies have provided some insight into the role of cytokines in WM and have found that cytokine levels in the sera of patients with WM were dysregulated compared with nonmalignant controls. Serum levels of macrophage inflammatory protein-1 alpha (MIP-1α) were elevated in patients with WM, with higher levels in untreated patients.¹⁰ MIP-1α levels correlated with increased bone resorption, beta2-microglobulin and splenomegaly¹⁰ suggesting a role of MIP-1α in WM. Angiogenic cytokine levels in WM patient sera have also been studied. Serum levels of vascular endothelial growth factor (VEGF), VEGF-A, angiogenin, angiopoietin-1 and -2 and basic fibroblast growth factor (bFGF) were found to be increased compared with healthy controls.¹¹ Angiogenic cytokine levels correlated with various clinical disease characteristics and represent possible targets for the development of new therapies for patients with WM. However, a better understanding of the role those cytokines play is fundamental to the development of targeted therapies.

Some of the cytokines known to regulate normal B-cell homeostasis appear to play a role in the pathogenesis of WM. The CXCR4/SDF1 axis has been shown to be important for the homing of WM B cells to the bone marrow¹² and represents a potential therapeutic avenue to prevent migration of malignant B cells in WM. BLyS has been shown to enhance survival of B-cell malignancies including CLL¹³ and multiple myeloma.¹⁴ In WM, BLyS levels were

elevated in the sera obtained from patients with WM compared with healthy controls and was found to promote growth and survival of malignant B cells.¹⁵ In the presence of a cytokine cocktail that included IL-2, IL-6, IL-10, and IL-12, BLyS increased Ig secretion by WM B cells.¹⁵ This suggests that IL-2, IL-6, IL-10, and IL-12 alone might play a role in WM biology. IL-6, in particular has gained much interest in WM. IL-6 is a pleiotropic cytokine that has been shown to play a role in B-cell malignancies including diffuse large B-cell lymphoma,¹⁶ Hodgkin lymphoma¹⁷ and multiple myeloma.^{18,19} IL-6 has been suggested to regulate growth of malignant cells in Hodgkin disease¹⁷ and multiple myeloma.²⁰ Previous studies have also shown that serum IL-6 levels are increased in patients with WM.²¹ More recently, gene expression analysis of malignant cells from patients with WM compared with B cells from healthy donors, report that the most significantly increased gene in the WM patient group is the IL-6 gene and the most significantly associated pathway for IL-6 is the mitogen-activated protein kinase (MAPK) pathway, suggesting that IL-6 plays a role in WM by signaling through the MAPK pathway.²² Similar to gene array studies, proteomic analysis of malignant B cells from patients with WM confirm the importance of MAPK signaling pathway in WM,²³ further supporting a role for IL-6/IL-6R signaling in WM. We found IL-6 to be expressed in the tumor microenvironment of patients with WM by immunohistochemistry (IHC; Figure 1), further supporting a role for this cytokine in WM. Therefore, identifying the precise role of IL-6 in WM and the signals that regulate IL-6 production will lead to the use of new therapies for patients with WM. We have also reported that Rantes (CCL5) levels are increased in plasma derived from bone marrow biopsy specimens obtained from WM patients by ELISA. Rantes is a chemokine with a well-described chemotactic role. It has also been shown to promote inflammation^{24,25} and might therefore be important in promoting inflammatory cytokine production in WM.

Conclusion

Cytokines regulate many biologic processes in normal lymphocyte function including Ig production, and the presence of cytokines within the tumor microenvironment of WM contributes to malignant cell viability as well as immunoglobulin production. Anti-cytokine therapies are clinically available for many cancers and targeting cytokines in the microenvironment in patients with WM may provide an opportunity for therapeutic advances. Understanding the precise role of cytokines within the tumor microenvironment of WM is therefore critical and will hopefully lead to better targeted therapies and better outcomes for patients with WM.

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Figure 1. Expression of IL-6 in the Tumor Microenvironment

Immunohistochemical analysis of IL-6 expression in WM bone marrow specimens was performed using anti-IL-6 mouse antibody as per manufacturer recommendations. A similar analysis was performed on control bone marrow obtained from healthy donors.

Table 1

Cytokines Dysregulated in Waldenström's Macroglobulinemia

Cytokine	Potential Role	Studied	Study (Year)
Angiogenin	Angiogenesis	–	Anagnostopoulos et al (2007)
Angiopoietin-1	Angiogenesis	–	Anagnostopoulos et al (2007)
Angiopoietin-2	Angiogenesis	–	Anagnostopoulos et al (2007)
bFGF	Angiogenesis	–	Anagnostopoulos et al (2007)
BLyS	Growth, survival, IgM secretion	Yes	Elsawa et al (2005)
MIP-1 α	Chemotaxis	–	Terpos et al (2006)
SDF-1	Homing to the bone marrow	Yes	Ngo et al (2008)
VEGF-A	Angiogenesis	–	Anagnostopoulos et al (2007)
IL-6	Growth and IgM secretion	–	Hatzimichael et al (2001)

Abbreviations: BLyS = B-lymphocyte stimulator; IL = interleukin; VEGF = vascular endothelial growth factor