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Review

The microenvironment in chronic lymphocytic leukemia (CLL) and other B cell malignancies: Insight into disease biology and new targeted therapies

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ABSTRACT

Over the last decade, the active role of the microenvironment in the pathogenesis of B cell lymphomas has been recognized, delivering signals that favor clonal expansion and drug resistance. We are only beginning to understand the complex cross talk between neoplastic B cells and the tissue microenvironment, for example in secondary lymphoid organs, but some key cellular and molecular players have emerged. Mesenchymal stromal cells, nurselike cells (NLC) and lymphoma-associated macrophages (LAM), in concert with T cells, natural killer cells and extracellular matrix components participate in the dialog with the neoplastic B cells. B cell receptor signaling, activation via TNF family members (i.e. BAFF, APRIL), and tissue homing chemokine receptors and adhesion molecules are important in the interaction between malignant B cells and their microenvironment. Disrupting this cross talk is an attractive novel strategy for treating patients with B cell malignancies. Here, we summarize the cellular and molecular interactions between B cell lymphoma/leukemia cells and their microenvironment, and the therapeutic targets that are emerging, focusing on small molecule inhibitors that are targeting B cell receptor-associated kinases SYK, BTK, and PI3Ks, as well as on immunomodulatory agents and T cell mediated therapies. Clinically relevant aspects of new targeted therapeutics will be discussed, along with an outlook into future therapeutic strategies.

1. Introduction to the microenvironment in CLL and selected other B cell lymphomas

The secondary lymphatic tissues are the principal site for expansion of normal mature B cells, ultimately leading to the generation of antigen-specific B cells and maturation into antibody-producing plasma cells. Normal B cell growth in germinal centers is based on antigen selection and clonal expansion, which is reinforced by antigen specificity, along with co-stimulatory signals from T cells and antigen-presenting cells (APC) [1]. Generally, the pathways responsible for the growth of antigen-specific normal B cells appear to be also functional in their malignant counterparts. CLL cells interact with different types of stromal cells, such as mesenchymal stromal cells [2], monocyte-derived nurselike cells (NLC) [3–5], as well as T cells [6,7], collectively referred to as the "microenvironment", and they proliferate in the context of microanatomical tissue sites called proliferation centers (pseudofollicles), a hallmark histopathology finding in CLL [8]. Early evidence of microenvironment-dependency came from the notion that CLL cells normally undergo spontaneous apoptosis in suspension culture unless they are co-cultured with bone marrow stromal cells (BMSC) [3,9,10] or NLC [3]. Microenvironment-dependence is also implied by the difficulty to establish CLL cell lines in the absence of EBV [11]. In follicular lymphoma (FL), a similar pattern of microenvironment-dependency has unfolded. The neoplastic B cells are also highly difficult to grow ex vivo, and microarray-based gene expression profiling (GEP) [12] and immuno-phenotyping of the accessory cells [13] revealed that the composition of the microenvironment has major impact on disease prognosis. The proposed mechanisms how the microenvironment impacts FL outcome include recruitment of lymphoma growth-promoting monocytes/macrophages and suppression of cytotoxic T cells. In the lymphatic tissues from FL patients, T cells display impaired formation of immunologic synapses, an immune evasion mechanism which can be restored by lenalidomide [14]. In diffuse

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large-cell B cell lymphoma (DLBCL), GEP and immunohistochemistry also revealed that the microenvironment significantly impacts disease prognosis. Based on GEP, DLBCL can be sub-classified into "germinal center B cell-like" (GCB) and "activated B cell-like" (ABC) DLBCL [15]. Further GEP analyses revealed two stromal signatures, the favorable stromal-1 signature reflecting infiltration by monocyte/macrophage lineage cells and extracellular-matrix deposition, while the unfavorable stromal-2 signature reflects high endothelial cell density [16]. Based on these concepts, CLL and other mature B cell malignancies are expected to be responsive to microenvironment-directed treatment approaches, provided that critical molecular pathways can be identified and targeted.

2. Tissue microenvironments in the bone marrow and secondary lymphoid organs

The BM and secondary lymphoid organs have entirely different, distinct microenvironments, supporting lymphocyte maturation and differentiation. B cell lymphopoiesis in the marrow results in the generation of B cells with functional antigen (Ag) receptors (BCRs). Mature B cells then migrate to secondary lymphoid organs where they are exposed to Ag within germinal centers (GC) of secondary lymphoid follicles [1]. The microenvironment of GC allows maturing B cells to interact with $CD4^+$ T-cells for the necessary help upon Ag recognition and with specialized stromal cells (follicular dendritic cells/FDC) for the required quality control following affinity maturation [17,18]. Neighbor cells in the microenvironment are critical at all stages of B cell maturation, as they provide growth support signals and assistance in selection of Ag-specific B cells. Mesenchymal stromal cells are important during B lymphopoiesis in the marrow, as well as for B cell positioning and territoriality in GC. Monocyte-lineage cells, such as NLC, provide various growth-promoting signals and appear to be involved in BCR activation in the lymphatic tissues. T cells can either suppress or promote B cell expansion, and their function depends on activation status, T cell subset, and micro-anatomical location.

3. Mesenchymal stromal cells (MSC)

In the BM, stromal "feeder" cells maintain hematopoietic stem cells (HSC) in specialized "niches" which are close to the marrow vasculature (vascular niche) or to the endosteum (osteoblast niche) [19]. The importance of stromal cells for hematopoiesis was initially demonstrated in long-term BM cultures [20,21]. In vitro, CLL cells are attracted to BMSC, and the protective effects of BMSC require the close proximity between CLL and the stromal counterparts [3,10,22,23]. The high affinity of CLL cells for stromal cells is exemplified by a striking in vitro phenomenon termed pseudoemperipolesis [22]. Pseudoemperipolesis describes the spontaneous migration of a fraction of CLL cells beneath BMSC, which occurs within a few hours of co-culture. Pseudoemperipolesis describes symbiotic complexes of leukemia cells with their stromal cell component [24,25]. Co-culture systems of CLL cells with BMSC, typically BMSC cell lines, have been standardized [23] and represent a useful tool for studying CLL cell activation by BMSC, as well as stroma-mediated drug resistance. Intrinsic qualitative and quantitative abnormalities of CLL patient-derived primary BMSC have recently been characterized [26], as well as the effects of more physiologic hypoxia present in the marrow microenvironment on BMSC function [27]. Interestingly, CLL cell activation by BMSC is bi-directional, and BMSC in turn also become activated by the CLL cells [28]. CLL cell supernatants activate platelet-derived growth factor receptors (PDGFRs) in BMSC [29], and contact with CLL cells causes expression of protein kinase C (PKC)-βII and subsequent activation of NF-κB in BMSC [30]. Pro-survival effects of MSC also have been described in FL [31], and this cell type appears to be generally present, to variable degrees in all types of B cell lymphomas [2].

4. Nurselike cells (NLC) and lymphoma-associated macrophages (LAM)

NLC were named after thymic nurse cells that nurture developing thymocytes [3]. In vitro, NLC differentiate from blood monocytes co-cultured with CLL cells in high-density culture conditions after 7–14 days [3]. In vivo, NLC can be found in the spleen and lymphoid tissues of CLL patients [4,32], and the importance of NLC for CLL disease progression was highlighted in recent CLL animal models [33,34]. NLC attract CLL cells by secreting CXCL12 [3] and CXCL13 [4] and protect CLL cells from spontaneous or drug-induced apoptosis via CXCL12 [3,35], BAFF, APRIL [35], CD31, plexin-B1 [36], and activation of the BCR signaling cascade [37]. In other B cell malignancies, NLC are termed tumor-associated macrophages or lymphoma-associated macrophages (LAM). In classic Hodgkin's lymphoma, presence of a LAM GEP signature was associated with primary treatment failure and shorter survival [38]. Similarly, in DLBCL [16] and FL [12], monocyte/macrophage GEP signatures impact the disease prognosis, indicating the LAM are a critical component in the microenvironment in these diseases. The molecular cross talk between monocyte-macrophages and malignant B cells in the tissue microenvironment is a new area of study and likely will guide us toward new targets. GEP has again provided us with insight into these complex interactions. In vitro GEP analyses revealed that NLC activate CLL cells in a different fashion than BMSC [37,39]. Specifically, BMSC induced a GEP pattern with prominent upregulation of the lymphoid proto-oncogene TCL1, paralleled by decreases of TCL1-interacting FOS/JUN [39]. In contrast, NLC induced a GEP response in CLL cells with characteristic induction of genes in the BCR- and NFκB pathways [37] that is strikingly similar to the GEP of CLL cells isolated from lymph nodes of CLL patients [40]. Several other genes of potential importance were also differentially up-regulated by BMSC (for example TNFRSF17, VPREB3, TNFSF10) and NLC (i.e. TNFRSF17, EGR2 and 3, MYCN), but their precise functions in the CLL microenvironment remain to be explored (Fig. 1).

5. T cells

The T-cell compartment is abnormal in CLL, with an increase in absolute numbers of peripheral blood T cells, particularly $CD8^+$ T cells, with a fall in the CD4:CD8 ratio. Despite their increased numbers, these T cells show profound functional defects. CLL T cells show evidence of chronic activation, with upregulation of CD69, HLA-DR and CD57, downregulation of CD28 and CD62L, and expansions of oligoclonal T cells. These oligoclonal expansions are primarily restricted to populations with an activated $CD57^+$ phenotype, suggesting a role for chronic antigen stimulation in their development. T cells exhibit features of "exhaustion", with increased expression of the exhaustion markers CD244, CD160, and PD1, with expansion of a $PD1^+BLIMP1^{HI}$ subset [41]. These T cells have functional defects in cytotoxicity, with impaired packaging of granzyme into vesicles and non-polarized degranulation. In contrast to virally induced exhaustion, CLL T cells showed increased production of interferon-γ and TNFα and increased expression of TBET, and normal IL2 production potentially protecting CLL cells from apoptosis.

T cells in CLL exhibit profound changes in their global gene expression profiles with alterations in expression of genes involved in cytoskeletal formation [42]. Similar defects in gene expression are induced in healthy T cells after co-culture with CLL cells, demonstrating that it is the leukemia cells that are inducing these changes.