Inflammation, the microenvironment and chronic lymphocytic leukemia

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Normal cells develop, differentiate, proliferate, exert their specific function or simply survive in specific microenvironments, where a team of bystander elements ensures a functional three-dimensional scaffold that allows cell-cell contacts and active molecular cross-talk. In cancer a unique microenvironmental organization is instrumental in the development and thriving of malignant cells: chronic inflammation exposes cells to growth factors, newly formed vessels provide nutrients and immune tolerance avoids immune-mediated elimination. The dissection of the molecular basis of the interactions between cancer cells and their microenvironment is leading to the development of new treatment modalities which are aimed at manipulating the communication of tumor cells with their milieu. Chronic lymphocytic leukemia (CLL) is an instructive example of how these relationships influence the natural history of a disease. Like other mature B-cell malignancies, CLL is characterized by a redirection and reinforcement of malignant cell/microenvironment interactions and new findings concerning these interactions are being translated into clinical practice.[1]

Antigen stimulation/inflammation and the natural history of chronic lymphocytic leukemia

CLL is a low-grade, B-cell tumor with monoclonal $CD5^+$ B cells that relentlessly accumulate in peripheral lymphoid organs and bone marrow and flow into the peripheral blood.[2] As most circulating CLL cells are in the G_0/early G_1 phase of the cell cycle, it was long thought that CLL clones hardly proliferate and die infrequently. CLL was, therefore, considered primarily a disease of accumulation. On the contrary, several data show that the proliferative rates of CLL cells can be higher than expected and indicate that CLL cells have a dynamic kinetic behavior.[3,4] At least two aspects account for this behavior. First, CLL cells retain the capacity to respond to stimuli primarily provided by their interactions with stromal cells and T cells within specific microenvironmental niches.[2] These interactions favor cell proliferation, up-regulate apoptosis-regulatory proteins and modulate the expression of chemokines and surface molecules. Secondly, the concept of the microenvironment being a regulator of CLL cell growth is tightly linked to a promoting role of antigen stimulation through the B-cell antigen receptor (BCR) on the surface of leukemic cells. Numerous observations indicate a prominent role of antigenic pressure: (i) at least half of patients have somatically mutated immunoglobulin heavy chain variable genes (*IGHV*) that track the clonal history to *in vivo* BCR activation;[5,6] (ii) more than 20% of cases express closely homologous, if not identical, stereotyped BCR which may recognize auto-antigens or bacterial components;[7] (iii) in the *TCL1* transgenic murine model of CLL[8] leukemic immunoglobulins are autoreactive and bind polysaccharides found in bacterial cell membranes. Autoantigens and molecular structures normally involved in scavenging debris, apoptotic cells and pathogenic bacteria appear relevant in triggering and/or facilitating the evolution of at least some CLL clones.[9,10] It is also appropriate to consider that inflammatory receptors such as Toll-like receptors (TLR) can be engaged concomitantly with the BCR: hence it becomes reasonable to presume that TLR may also play a role in BCR co-stimulation of CLL cells. Indeed, it was recently shown that bacterial lipopeptides protect CLL cells from spontaneous apoptosis mediated by TLR signaling.[11] The relationship between antigen stimulation/inflammation and the natural history of CLL is not surprising considering that inflammation is involved in the initiation and progression of several chronic lymphoid malignancies of B-cell type.

Tissue events

CLL cells circulating in the peripheral blood are the tip of the iceberg. The most significant pathophysiological events occur in tissues[2] (Figure 1A,B) where leukemic cells: (i) are activated by exposure to antigens, although it is still unclear where and how this exposure takes place. It is also unclear how BCR stimulation may translate into either cell proliferation or cell anergy and how these processes are mediated by various signal transduction pathways; (ii) receive the proper T-cell help, if and when needed, to be selected for clonal expansion; (iii) proliferate in specific niches, the pseudofollicular proliferation centers, which are not detected in any other B-cell malignancy, but are observed in inflamed tissues of patients with systemic autoimmune/inflammatory disorders; and (iv) interact with stromal cells that favor cell accumulation. CLL cells not only interact with but also shape their conducive microenvironments by recruiting activated T cells and stromal cells by means of chemokines and chemokine receptors.[1,2]

These facts indicate that CLL cells are antigen-experienced B cells that traffic and home to and from specific microenvironmental niches, such as the still mysterious pseudofollicular proliferation centers. Lymphocyte localization appears to depend on the sequential engagement of adhesion molecules and activation through chemokine receptors. CLL cells express functional CXCR3, CXCR4, and CXCR5 chemokine receptors that direct leukemic cell chemotaxis *in vitro*.[2] Within pseudofollicular proliferation centers, proliferating leukemic lymphocytes are in contact with numerous $CD3^+$ T cells, most of which are $CD4^+$, with many expressing CD40L that can support the growth of CLL cells through CD40 ligation.[12,13] *In vitro* (and likely *in vivo*) CLL cell apoptosis can be prevented by an interaction with stromal and nurse-like cells.[14] The interaction between CD38 on the surface of CLL cells and its natural

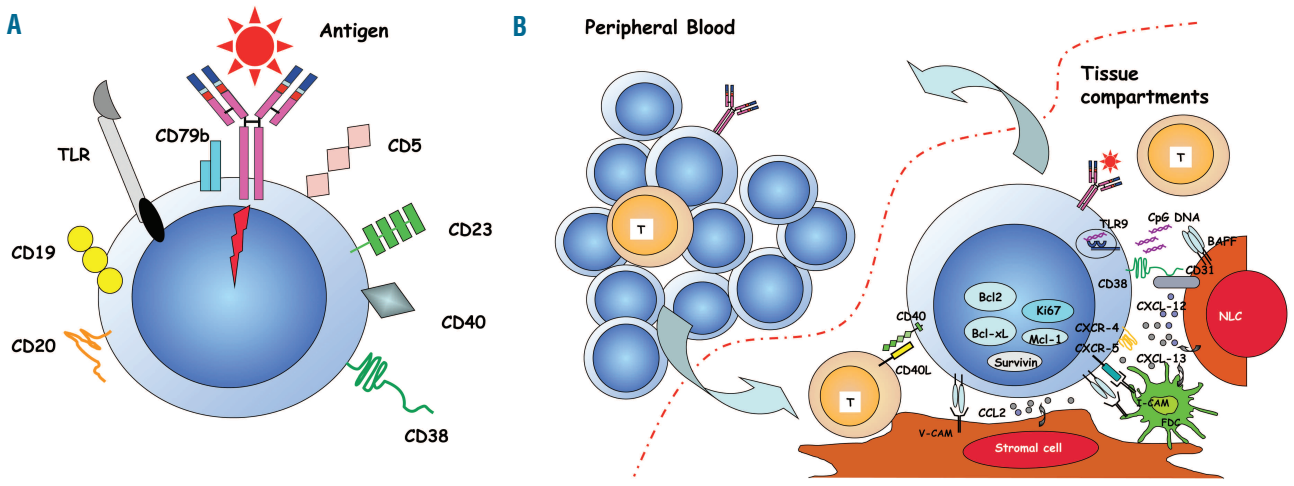

Figure 1. (A) A CLL cell is an activated antigen-experienced B cell. (B) A proposed model of CLL clonal expansion from a CLL cell/microenvironmental perspective that includes the main actors. NLC: nurse-like cells. FDC: follicular dendritic cells.

ligand CD31[15] also favors the survival of leukemic cells. Cytokines such as interleukin-4 and chemokines such as CXCL13/SDF-1 might support the expansion of CLL clones by promoting the up-regulation of anti-apoptotic genes including *BCL2*, *SURVIVIN*, and *MCL1*. Taken together several findings suggest that T-cell subsets provide short-term support that influences malignant B-cell proliferation and that different subpopulations of stromal and accessory cells provide long-term support that favors prolonged survival and accumulation.[2] The exposure of malignant cell subclones to microenvironmental stimuli results in increased proliferation, a prerequisite for the occurrence of new genetic abnormalities that lead to the development of a more aggressive disease.

In this issue of the journal, Schulz *et al.*[16] touch upon the issue of inflammatory cytokines and signaling pathways associated with CLL cell survival. Their investigation started from the well known notion that while CLL cells have prolonged survival *in vivo*, they tend to die rapidly *in vitro* unless they are co-cultured in the presence of stromal accessory cells. The authors tried to characterize the molecular basis of the survival-inducing cross-talk provided by these interactions with the long-term goal of identifying potential novel therapeutic targets. To this end they established different survival-supportive culture conditions which were essentially based on the use of stromal cells or of stromal cell conditioned medium and investigated the gene expression changes of leukemic cells by means of microarray-based profiles and the composition of soluble factors by means of cytokine antibody arrays. Their findings show that an inflammatory microenvironment, including TLR, is at the basis of the survival support provided by the culture system. Consistent with this possibility they found that inflammatory cytokine genes are up-regulated. Among these genes chemokine (C-C motif) ligand 2 (*CCL2*) was shown to be induced in monocytes by the presence of CLL cells *in vitro*. Increased serum levels of this chemokine were detected in patients. CCL2, also known as monocyte chemotactic protein-1 (MCP-1) and small inducible cytokine A2, is a chemokine structurally related to the CXC family of cytokines which binds to the chemokine receptors CCR2 and CCR4.[17,18] CCL2 is a very attractive potential microenvironmental actor within the cancer microenvironment as it is primarily secreted by monocytes, macrophages and dendritic cells, has chemotactic activity for monocytes and basophils, appears to recruit monocytes, memory T cells and dendritic cells to the sites of inflammation, is tethered on endothelial cells by glycosaminoglycan side chains of proteoglycans and is cleaved by metalloproteinases. Within lymphoid malignancies CCL2 has been implicated in the migration and localization of follicular lymphoma cells.[19]

Novel perspectives

We are starting to understand which genes, molecules and accessory cell subsets are involved in CLL cell/microenvironment interactions and what roles they play. Despite the significant progress made to date, we still have to elucidate the molecular mechanisms through which these cells promote the accumulation of leukemic cells. Further work needs to be done to define the role of cytokines, chemokines and chemokine receptors in shaping a supportive microenvironment, to characterize the still incompletely defined stromal cells and to elucidate the proper place of functionally different T-cell subsets. Three important avenues of investigation are now being used to tackle these issues. The first is the progressive development of experimental approaches, such as those delineated by Schulz *et al.*,[16] which mimic *in vitro* the interacting events that occur *in vivo*. The second is represented by animal models which have been invaluable tools to understand leukemogenesis. Numerous animal models of CLL have become available recently and many more are under development. The third major area is based on the observation that monoclonal B-cell lymphocytosis, a condition that occurs frequently in the elderly, may be a

preleukemic situation as virtually all CLL patients appear to have a preleukemic phase of monoclonal B-cell lymphocytosis.[20] Understanding which genes are involved in the transition of monoclonal B-cell lymphocytosis into overt CLL and investigating to what extent antigen stimulation and an inflammatory proactive microenvironment favor this transition may provide a clue to many unanswered questions.

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Bernard-Soulier syndrome

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Bernard-Soulier syndrome (BSS) is an inherited, usually autosomal recessive, platelet bleeding abnormality, characterized by a prolonged bleeding time, large platelets and thrombocytopenia.[1] In 1975, Nurden and Caen reported that platelets from BSS patients lacked a major surface membrane glycoprotein complex,[2] subsequently demonstrated to be the component subunits of the glycoprotein (GP)Ib-IX-V complex.[3,4] In this issue of the journal, Savoia and colleagues describe 13 patients with BSS from ten unrelated families with causative mutations in GPIbα, GPIbβ and GPIX, and attempt to relate the severity of the bleeding phenotype with genotype.[5]

Structure and function of the GP Ib-IX-V complex

The GPIb-IX-V complex is a pivotal receptor complex in hemostasis and thrombosis. In binding von Willebrand Factor (VWF), it mediates the initial contact adhesion of platelets to exposed vascular subendothelium or ruptured plaque in damaged vessels at high shear flow rates