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## Chronic lymphocytic leukemia

Chronic Lymphocytic Leukemia (CLL) is characterized by the expansion of monoclonal CD5<sup>+</sup> B lymphocytes that accumulate in lymphoid organs, bone marrow (BM) and peripheral blood (PB). Refined cytogenetic studies are documenting the clinical importance of genetic subtyping CLL. The CLL-specific gene alterations are gradually coming to light thanks to mouse models and to the discovery that microRNA (miRNA) genes frequently reside in hot spots for chromosomal abnormalities in CLL cells: as an miR-15a and miR-16-1 are located at 13q14.3. The emerging view is that the main genetic alterations of CLL entail the deregulation of specific microRNAs (miRNAs) that lead to transcriptional/post-transcriptional abnormalities.

(CLL) is a paradigmatic example of how chronic malignancies of mature B-cells take advantage of redirection and reinforcement of interactions with the microenvironment to avoid apoptosis and acquire better growing conditions. Several evidences indicate that stimuli from the microenvironment contribute to the selection and expansion of the malignant clone and concur with genetic defects in shaping the natural history of the disease.

Irrespective of its phenotypic homogeneity, CLL is clinically heterogeneous. A number of bio-

logically-defined prognostic factors including the mutational status of immunoglobulin heavy chain (IGHV) genes, the expression of CD38 and of ZAP70, are used as markers to dissect the clinical heterogeneity. Again the biological function of these markers is directing our attention toward the microenvironment and its stimuli.

Numerous experimental data indicate that CLL leukemic B cells have some sort of antigenic exposure and that CLL development and progression are influenced by antigenic pressure. These data include the analysis of the somatic mutations of IGHV genes, the finding that CLL cases have a biased use of certain IGHV genes and that subsets of patients carry closely homologous if not identical (“stereotyped”) Ig complementarity-determining region-3 (CDR3) sequences. These findings indicate that the recognition of an unknown limited set of antigens (Ag) likely plays a central role in selecting the leukemic clones. At least in some instances, stereotyped CLL B-cell receptor (BCR) may recognize antigens found in bacterial cell membranes as well as auto-Ag.

CLL cells differ significantly in their *in vitro* capacity to signal through the B cell receptor (BCR), with some cases (most unmutated) carrying more compe-

tent BCRs and others (usually mutated) appearing to be unresponsive. This may depend on the nature of the Ag as well as on the affinity of the receptor and it is not unreasonable to postulate that in some cases a persistent antigenic stimulation might promote CLL survival and growth via sIg-mediated signals, while in other cases Ag interaction might lead to receptor desensitization and to an anergic state. This possibility is consistent with the modalities of expression of ZAP-70 and of HS1, a molecule pivotal in the signal transduction pathway triggered by BCR stimulation. The observation that in CLL high levels of ZAP70 and the presence of HS1 in a prevalently phosphorylated form are associated with an aggressive behaviour again underlines the possibility that circulating CLL cells may have had a recent/persistent Ag (or Ag-like) exposure. Despite extensive investigations the molecular basis of the striking differences in terms of BCR responsiveness in CLL cells are unclear. We examined the expression and activation of key molecules involved in signaling pathways originating from the BCR, and we found that a proportion of CLL patients express constitutively phosphorylated extracellular signal-regulated kinase (ERK)1/2 in the absence of AKT activation, display constitutive phosphorylation of MEK1/2 and increased nuclear factor of activated T cells (NF-AT) transactivation and are characterized by cellular unresponsiveness to sIg ligation. As this molecular profile recapitulates the signaling pattern of anergic murine B cells, our data may be taken to indicate that constitutive activation of mitogen activated protein (MAP) kinase signaling pathway along with NF-AT transactivation in the absence of AKT activation may represent the molecular signature also of anergic human B lymphocytes and that CLL cases with this signature may be considered a human model of anergic B cells aberrantly expanded.

Mature B-cells can recognize microbial antigens via BCR in a specific way and via Toll-like receptors (TLR) in a costimulatory manner at least in the context of autoimmune reactions where the dual engagement appears to be crucial for B cell activation. Little is known regarding the repertoire and function of TLR in CLL cells. The family of TLR includes 10 different transmembrane proteins devoted to recognize specific pathogen-associated molecular patterns and to alarm immunocompetent cells to trigger an immune response. We found that CLL cells express several TLR pattern-recognition molecules and that the specific TLR expressed by CLL cells are functional (Muzio et al, unpublished). Leukemic cells upon stimulation with distinct TLR ligands, such as bacterial lipopeptides and peptidoglycans, upregulate surface expression of CD86 and CD25 activation molecules and are protected from spontaneous apoptosis. These findings suggest a potential role of costimulatory signals in modulating CLL cell response in the context of specific antigen recognition, and further support the hypothesis that CLL cells resemble Ag-activated B-cells.

All relevant events of CLL occur in tissues where two compartments exist. The “accumulation” compartment, which also flows in the PB and is the most evident and most studied clonal component. This compartment is nourished by an upstream “proliferation” compartment represented by focal cell aggregates that form the proliferation centers (PC) scattered in lymph nodes and BM. PC are not detected in any other B-cell malignancy, while they are observed in inflamed tissues of patients with rheumatoid arthritis and multiple sclerosis reinforcing the possible role of Ag (AutoAg ?) in the genesis of CLL. Within and around PC malignant CLL cells are interspersed with and surrounded by numerous CD3<sup>+</sup> T cells, stromal cells and a number of still incompletely defined accessory cells. Many T cells are CD4<sup>+</sup>

CD40L<sup>+</sup> and are in close contact with the proliferating malignant B cells. The *in vitro* stimulation of CLL cells from PB through CD40 mimics some events that likely occur in the PC, as these cells are rescued from apoptosis, increase their proliferation and express molecules typical of PC cells such as Survivin, CCL17 and CCL22. Several data highlight the role of T cells in supporting the clonal growth and suggest that within PC T cells provide a short-term proliferative support to malignant B. Stromal cells and accessory cells provide a long-term support which favours the extended survival and accumulation of leukemic cells. The nature of the stromal component as well as the definition of the interacting molecules and of the mechanisms through which they operate need to be further elucidated.

Tissues can thus be seen as the actual sites where leukemic cells can be exposed to (auto)Ag stimulation and are selected for clonal expansion. In the tissues they can receive an advantageous T-cell help and interact with stromal cells to progressively accumulate before flowing in the PB. The relationships and the balance between CLL cell proliferation and accumulation in lymphoid organs, as well as the mechanisms that favour the scattered distribution of PC and the rules that control the cell migration and re-circulation from lymphoid organs into PB and BM are still unclear. Our model of CLL natural history entails the existence of abnormal functional features of the signal transduction system and especially of the connections that link BCR stimulation, cell activation and the cytoskeleton modification that the cell has to acquire in order to proliferate, move and circulate. We have recently demonstrated that HS1 interacts with Vimentin and with distinct cytoskeleton adapters, such as unconventional Myosins, HIP-55 and, unexpectedly, Cortactin (Scielzo *et al.* unpublished). These findings suggest a role for HS1 in the regulation of B-cell cytoskeleton organ-

ization. We also hypothesize that the signal transduction mediated by the stimulation of BCR (and possibly also by the costimulation of TLR) leads to a hyperactivated leukemic cell that is unable to complete a cell division unless located in the specific microenvironment of PC. However we ignore the fate of proliferating cells within PC. Data are gathering to indicate that in terms of proliferation and apoptosis the clone is more dynamic than anticipated. As an example investigations on telomere length and telomerase activity in PB cells as well as *in vivo* kinetic studies indicate that all CLL (especially IGVH unmutated cases) have an extensive history of cell division. Still, the precise relationships between number/size of PC and disease aggressivity are unclear as are the mechanisms that govern the time schedule of successful cell divisions.

The use of modern multi-parameter flow-cytometric analyses has demonstrated the presence of tiny monoclonal B-cell populations phenotypically very similar to CLL in the PB of about 3.5% of healthy individuals which are now named Monoclonal B Lymphocytosis (MBL). MBL are more frequent in male, increase in frequency with age and have a significantly higher incidence in the relatives of CLL patients. On these bases, it appears logical to hypothesize that MBL might be a precursor state for CLL, somehow reminiscent of the relationship between monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma. It is still unknown whether MBL, especially those detected in CLL relatives, are Ag-specific or polyreactive as in CLL.

It may be suggested that MBL might be a critical step in the development of CLL, possibly increasing the number of cells where the non-dispensable transforming events occur. Still it has to be determined whether MBL are the expansion of the normal counterpart of leukemic B lymphocytes which represent a

potential target for subsequent transforming hits or are already true (although incomplete) malignant elements, which have to wait for the proper stimuli to expand into an overt leukemia.

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