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Are we getting closer to understanding the pathogenesis of chronic lymphocytic leukemia?

Mature B-cell tumors, such as chronic lymphocytic leukemia (CLL), are characterized by a redirection and reinforcement of microenvironment interactions that allow mature malignant B cells to avoid apoptosis and acquire better growing conditions. These interactions may be taken to explain why despite major therapeutic advances chronic B-cell malignancies are still incurable.

CLL B cells are endowed with a functional B-cell receptor (BCR) that allows Ag interaction. Hence the concept of microenvironment as a regulator of malignant B-cell growth is tightly linked to the possible role of antigen (Ag) stimulation. The hypothesis that a BCR-mediated stimulation plays a relevant role in the natural history of CLL is strengthened by the occurrence of somatic mutations of immunoglobulin heavy chain variable (IGHV) genes as well as by phenotypic and expression profiling signatures. CLL patients have a biased use of IGHV genes and subsets can be identified that carry stereotyped complementarity-determining region 3 (CDR3) sequences on heavy and light chains. The probability that two individual B-cells may express identical BCR is extremely low (10^{-9}-10^{-12}), indicating that the remarkable BCR similarity detected in more than 25% of unrelated and geographically distant CLL cases cannot be accounted for by pure chance. Further, as stereotyped CDR3 are more frequent in unmutated than in mutated CLL (about 40% *vs.* around 10% of the cases) it follows that antigenic exposure may be relevant in the pathogenesis of CLL, irrespective of IGVH mutational status. A whole range of investigation lead to believe that CLL B cells are Ag-experienced and suggest a central role for the recognition of a limited set of structurally similar epitopes in the selection and growth of leukemic clones.

It still has to be properly defined which are the Ag involved, where and how. Most if not all CLL produce polyreactive monoclonal antibodies (Abs) that react with a number of novel (auto)Ag targets including cytoskeletal proteins, phosphorylcholine-containing structures and oxidized low-density lipoproteins. Also we have to clarify whether target cells have experienced Ag stimulation before the occurrence of the transforming events that lead to malignancy or whether they are continuously exposed to Ag stimulation. Were this the case not only the onset but also the progression of CLL would be influenced by Ag intervention.

All relevant events of CLL occur in tissues where small lymphocytes, pro-lymphocytes and paraimmunoblatsts give rise to

two major compartments. Small lymphocytes form the "accumulation" compartment that flows in the peripheral blood (PB). Presumably accumulating small lymphocytes are the offspring of an upstream "proliferation" compartment essentially represented by pro-lymphocytes and paraimmunoblatsts that cluster to form the pseudofollicular proliferation centers (PC) scattered in lymph nodes and to a lesser extent in the bone marrow (BM). The prevailing opinion is that PC are the source of most cellular generation in CLL. Several findings suggest the possibility that T cells provide a short-term support which influences malignant B cell proliferation, while stromal cells and accessory cells including the nurse-like cells provide a long-term support that favours the extended survival and accumulation of leukemic cells. Even if all these data indicate the importance of tissue microenvironments for malignant B cells to avoid apoptosis and acquire better growing conditions our understanding of the role of T-cells and especially of stromal cells is just in its infancy. We also have to understand which mechanisms cause the patchy and scattered development of PC in lymph nodes and BM and which is the fate of proliferating cells in the PC, how many of them die *in situ* and how many transit into the accumulation compartment. Data are gathering to indicate that in terms of proliferation and apoptosis the clone is more dynamic than anticipated. Finally it is important to establish which mechanisms promote the flow of resting cells from the tissue accumulation compartment into the PB and which the tissue compartmentalization in small lymphocytic lymphoma. As the microenvironment is an attractive target for innovative treatment strategies it has to be considered that BM and secondary lymphoid organs have entirely different microenvironments, each finely tuned to serve the specific organ function through the activity of different cell types and the expression of different genes. This heterogeneity dictates the necessity to establish proper culture systems to reproduce *in vitro* the *in vivo* situation of different microenvironments. The microenvironment-specific culture systems may become useful tools for testing new drugs.

Phenotypically hyperactivated CLL cells are in a paradoxical situation as in kinetic terms they are mostly in the G0/early G1 phase. Ag stimulation might be able to continuously tickle individual cells without promoting their further entrance into the cell cycle thereby leading cells at the decisional crossroad between apoptosis and proliferation. Accordingly Ag stimulation would be a "preparing", not the ultimate triggering event to enter the cell cyle and differentiation. A potential abnormality may involve the signal transduction system and especially the connections that link BCR stimulation, cell activation and the cytoskeleton modification that the cell has to acquire in order to proliferate and move. New developing animal models may allow to shed light onto this aspect.

Refined cytogenetic studies are documenting the clinical importance of genetic subtyping CLL. Still it is not yet clear which chromosomal abnormalities are primary and which secondary events. The long sought CLL-specific gene alterations are gradually coming to light thanks to mouse models and to the discovery that the main genetic alterations of CLL entail the deregulation of specific microRNAs (miRNAs) that lead to transcriptional/post-transcriptional abnormalities. Distinct microRNA signatures may map different subsets of patients classified according to disease progression implying that specific miRNA (and the genes they control) may be at the basis of the different variants of CLL.

One possibility to approach the central problem of the relationship between (auto)Ag-triggered events of cell activation and malignant

transformation is offered by the investigation of the tiny monoclonal B-cell populations phenotypically very similar to CLL that have been demonstrated in the PB of about 3.5% of healthy individuals and are named monoclonal B lymphocytosis (MBL). It appears logical to hypothesize that MBL might be a precursor state for CLL, somehow reminiscent of the relationship between monoclonal gammapathy of undetermined significance and multiple myeloma. It is not unreasonable to suggest 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.

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