Another piece of the puzzle: is there a "nodal" monoclonal B-cell lymphocytosis?

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The surface phenotype of chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) cells ($CD19^+$, $CD20^{dim}$, $CD5^+$, $CD23^+$, sIg^{dim}) is so characteristic that for a long time it has been considered due to the process of neoplastic transformation. This notion was somehow corroborated for many years by the lack of any evidence of the existence of a population with a similar phenotype in healthy individuals. The subset of B1 lymphocytes ($CD19^+CD5^+$) present in the cord blood, in the mantle zone of the lymph nodes and in the peripheral blood of adults, where they constitute 5-15% of all B lymphocytes, though considered for some time as a potential counterpart of CLL cells,[1] turned out to be likely unrelated to CLL based on the differences in the actual surface phenotype (e.g. different levels of the CD20 molecules – Figure 1A) and in their gene expression profiles.[2]

It is now a few years that the use of more sensitive flow-cytometric techniques allowed the detection of tiny populations of B lymphocytes, with a phenotype identical to that of CLL, circulating in the blood of otherwise healthy individuals (Figure 1B).[3,4] This entity has been named CLL-like monoclonal B-cell lymphocytosis (MBL) and the formal distinction from CLL/SLL is based essentially on the following two features:[5] (i) a B-cell count less than 5×10^9/L (to distinguish it from Rai stage 0 CLL); and (ii) the absence of palpable lymphoadenopathy and/or organomegaly (to distinguish it from SLL).

What we (do not) know about monoclonal B-cell lymphocytosis

CLL-like MBL is a rather common finding in the peripheral blood of adults, especially above 65 years of age, and the frequency largely depends on the sensitivity of the cytometric procedure utilized, ranging from 3.5% to 6.7% to 12% among people over 45 years of age.[4,6,7] That notwithstanding, it is also now clear that no matter how hard one searches for MBL, it cannot be found in every individual.[8] In contrast, a subject reaching 90 years of age will very likely be carrying a MBL, present in 50-75% of individuals in that age group.[7,8] The concentration of MBL cells in the peripheral blood can be strikingly different ranging from less than 1 to more than 1000 cells/μL. The size of the population has recently been suggested to be a potential discriminator between two forms of MBL.[9,10]

On the one hand, the previously defined "low-count" MBL[6] have an aberrant B-cell count lower than 50 cells/μL, are usually detected in the general population through screening studies utilizing high sensitivity flow cytometric analyses and have distinctive biological and molecular features from CLL (*"general population MBL"*). The risk of general population MBL evolving into CLL has been recently reported to be very low if any (Ghia *et al.*, ASH 2010). General population MBL may represent a form of senescence of the immune system, closely resembling the occurrence of B-cell expansions in aging mice[11] and it may arise from chronic stimulation due to persistent infectious agents[12] or chronic exposure to auto-antigens. It is possible that it is a para-physiological manifestation, as suggested also by the detection of a few cases that are indeed polyclonal/oligoclonal although still carrying an identical surface phenotype.[6,13]

On the other hand, the form that is attracting more interest among clinicians is the so-called *"clinical MBL"*, which have an aberrant B-cell count higher than 1000-1500 cells/μL, are usually detected during clinical work-ups using standard flow cytometric approaches, and have the same biological and molecular features as CLL, at least those with a good prognosis.[14] The risk of clinical MBL progressing into CLL requiring treatment is low, but appreciable, occurring at a rate of 1-2% per year[14,15] as compared to the 5-7% risk in patients with Rai stage 0 CLL. This difference is statistically significant but both entities appear to be a mixture (though in different proportions) of individuals who will never develop clinical complications and real patients whose life expectancy will be affected by the disease. Indeed the B-cell count correlates with the risk of progression as a continuous variable, indicating that the distinction between Rai stage 0 CLL and clinical MBL is somehow artificial and no numerical cut-off value will be able to clearly segregate the individuals who will eventually develop a CLL needing treatment from those who simply need to be reassured on the non-progressive nature of their affection. Ideally, this distinction would avoid the necessity of long-term clinical follow-ups and the related economic and psychological burden in a large number of individuals who will not indeed need them. It is likely that a better understanding of the biology of MBL/CLL will identify crucial molecular features that may predict the fate of CLL-like B cells in each affected individual.

How does small lymphocytic limphoma fit into the game?

Since the definition of the guidelines, it was easy to predict that having only a mathematical value to distinguish between clinical MBL and Rai stage 0 CLL would have created problems in clinical practice. In contrast, no one could have foreseen that also the distinction from SLL would have become at risk. Theoretically, SLL appears to be easily distinguishable from MBL, as its diagnosis is based on a B-cell count less than 5×10^9/L with the concomitant presence of palpable lymphoadenopathy and/or organomegaly, as assessed by physical examination. This is recommended by the guidelines in order to avoid radiological assessment in patients with potentially no active disease.

In this issue of the journal, Gibson *et al.*[16] show that an

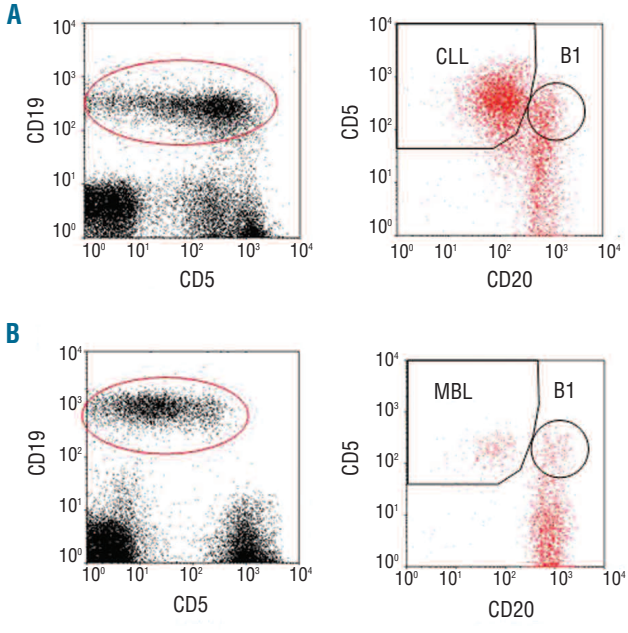

Figure 1. A distinct phenotype for MBL/CLL cells *versus* B1 lymphocytes. (A) All CD19$^+$ B lymphocytes (both CD5$^+$ and CD5$^-$) from the peripheral blood of a CLL patient were gated in a representative dot plot analysis (left panel); CD20 expression is shown in the right panel. CLL cells can be differentiated from normal B1 lymphocytes based on the levels of CD20 expression. (B) A similar strategy was applied to analyze CD19$^+$ B lymphocytes in the peripheral blood of a representative individual carrying a MBL clone (left panel). MBL cells showed distinctly different levels of CD20 expression compared to normal B1 lymphocytes (right panel).

overlap between SLL and MBL may potentially exist. They report on a total of 36 patients who underwent biopsies of extramedullary (mainly lymph node) tissue containing CLL-like B cells and had less than 5×10^9/L peripheral blood monoclonal B cells with identical phenotype (when tested). Interestingly 16 of these cases had a biopsy for reasons other than palpable lymphoadenopathy (mainly staging of solid tumors) and two did not have detectable lymphoadenopathy even after combining a physical examination and radiological studies. Morphological and immunophenotypic examination of the tissues showed a diffuse infiltration of CLL-like B cells involving more than 50% of the lymph node area in all cases but four patients showed a more subtle and/or focal infiltration. From a clinical point of view, it is interesting to note that, after a median follow-up of 23 months, 12 patients progressed (5 needing treatment) but three patients had no detectable lymphoadenopathy (2 cases of post-surgical excision of a single enlarged lymph node) and three other patients experienced regression of lymphoadenopathy without therapy.

Though few, these cases are indeed interesting as they appear to lie in a so far unappreciated diagnostic gray zone between MBL and SLL, characterized by the presence of a subtle and/or focal abnormal lymphoid population, with no detectable lymphoadenopathy or with spontaneous regression of lymph nodes that were enlarged at initial diagnosis. For all these reasons, they might represent, as proposed by the authors, a tissue equivalent of clinical MBL rather than a fully fledged lymphoma. Thus, the authors suggested[16] a new diagnosis for this entity, "involvement by CLL/SLL-like cells of uncertain significance" rather than SLL, analogous to terminology recently proposed for the *in situ* versions of follicular lymphoma and mantle cell lymphoma.[17]

Future perspectives

This report is limited in terms of number of cases and, of course, its findings need to be confirmed in larger series, but it does suggest an attractive scenario that could have two consequences. On the one hand, it may allow a better refinement of the diagnostic approach to CLL/SLL, identifying a stage of the disease with lower risk of progression (if not regression) somehow mirroring the overlapping relationship between Rai stage 0 CLL and clinical MBL. For this reason, a better term for this entity could be "nodal (or extranodal) MBL" as this would avoid referral to the uncertainty of the fate of such a phenomenon, which may create anxiety in affected individuals, as pointed out at the time when the MBL term was coined (Jerry Marti, *personal communication*).

Unfortunately, in the study by Gibson *et al.*,[16] no clinical, histological or biological features examined were able to predict progression except the detection of any lymph node of 1.5 cm or more in diameter in radiological staging studies and the largest lymph node on computed tomography scans at diagnosis being greater in progressive cases. These data do not provide a clear way to discriminate cases with different risks of progression i.e. to identify those individuals not needing long-term follow-up, as previously discussed in relation to Rai stage 0 CLL. In contrast, the high rate of identification of deep lymphoadenopathy in patients who were negative at physical examination casts doubts on the potential need to perform a radiological assessment in addition to a thorough physical examination, when clinical MBL is hypothesized, in order to exclude the presence of an underlying SLL with some degree of certainty. This, of course, deserves further studies before the clinical routine can be established.

On the other hand, the concept of "nodal MBL", as proposed by Gibson *et al.*,[16] although still to be formally proven, does provide the possibility of further consideration about the actual origin of CLL-like B cells. If clinical MBL is a precursor of CLL, the cells cannot originate *tout court* from the peripheral blood but must rather originate from lymphoid tissues. Though the bone marrow is frequently involved at levels that are independent of the actual count in the peripheral blood,[18] this could be due to a later invasion from the peripheral blood. In addition, there are several lines of evidence indicating that CLL may originate from lymph nodes in which antigenic stimulation occurs to fuel the disease.[19] Confirmation of the presence of CLL-like B-cell aggregates in morphologically unaffected lymph nodes would provide further support to this possibility. Although the issue of MBL is getting more complicated, it is becoming ever more interesting.

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