Prehistory of chronic lymphocytic leukemia: clues from the B-cell receptor

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Received: August 28, 2023.
Accepted: September 11, 2023.
Early view: September 21, 2023.

https://doi.org/10.3324/haematol.2023.283799

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In this issue of *Haematologica*, Quinten *et al.* provide new insights into the early steps of the development of chronic lymphocytic leukemia (CLL).[1] Following its initial description in 2002, monoclonal B-cell lymphocytosis (MBL) has become a well-recognized entity defined by the presence of a circulating clonal B-cell population below $5x10^9$ cells/L, in the absence of clinical symptoms or cytopenias.[2] Based on their immunophenotypic profile, MBL can be divided into three subtypes: (i) CLL-type MBL, which accounts for the vast majority of cases; (ii) atypical CLL-type MBL; and (iii) non-CLL MBL.[3] In addition, depending on the number of circulating clonal B cells, two categories of MBL are recognized: high-count MBL (HC-MBL) having $\geq 0.5x10^9$ cells/L, and low-count MBL (LC-MBL) with $<0.5x10^9$ cells/L. A variable frequency (3.5% to 12%) of MBL among healthy individuals has been reported, depending on the sensitivity of the detection technique. Using highly sensitive eight-color flow cytometry, a recent large-scale study from the Mayo Clinic identified MBL in 17% of more than 10,000 individuals, with most cases (95%) being LC-MBL.[4] The prevalence increases with age, and is higher in males and in individuals from families in which two or more relatives have CLL.[5]

HC-MBL cases share similarities with CLL, both in terms of genetic abnormalities and immunoglobulin heavy chain variable (IGHV) region gene repertoire. It is considered to be a precursor state of CLL,[6] with the rate of progression from HC-MBL to CLL requiring treatment varying from 1% to 5% per year. The relationship between LC-MBL and CLL is less clear. Compared to HC-MBL, LC-MBL display a lower frequency of the genomic aberrations usually seen in CLL, and predominantly those of the ultra-stable type. They also have a different immunoglobulin (IG) gene repertoire, raising the possibility that they represent an immune senescence phenomenon rather than a pre-leukemic state.[7] However, for cases of LC-MBL occurring in relatives from CLL families, the annual rate of progression to CLL has been estimated to be 1.1%, indicating that a least a fraction of LC-MBL are precursors of CLL.[5] CLL ontogeny may in fact be initiated in a much earlier progenitor, as shown by the identification of acquired CLL in the patients' hematopoietic stem cells, and transplantation experiments of patients' hematopoietic stem cells into immunodeficient mice.[8,9]

The driving forces contributing to the emergence and growth of a CLL population from normal progenitors are still unknown but clearly include genetic as well as microenvironment factors. For the latter, antigen stimulation through the B-cell receptor (BCR) plays a central role and several auto-antigens as well as microbial antigens have been identified as targets of CLL BCR. About 10 years ago, the group of Hassan Jumaa reported the striking finding that the CLL BCR itself could serve as a target, with the third complementary determining region of the IGHV domain of one BCR recognizing epitopes in the framework regions of another BCR.[10] Using a sophisticated *in vitro* model in which a murine triple knock-out pre-B cell line was transfected with patient-derived BCR IG genes, they showed that such interactions resulted in "antigen-independent" cell-autonomous signaling evidenced by calcium flux, and moreover that this phenomenon was specific to CLL BCR.

In the present study from the same group, Quinten *et al.* have now applied this technique to MBL BCR in order to address the issue of the early stages of CLL ontogeny.[1] They analyzed a cohort of 191 siblings of subjects with non-familial CLL and performed functional and genetic analyses in paired CLL-MBL siblings. Using six-color flow cytometry, they detected 34 (17.8%) MBL cases, most of them (94%) being LC-MBL. They obtained IG heavy and light chain sequences for 17 of them, with two cases being biclonal, a finding not unusual in MBL. A third of the monoclonal cases (5/15) had characteristic features of CLL BCR stereotypy (e.g., quasi-identical IG sequences), including two cases belonging to the aggressive subset #2 group, a frequency

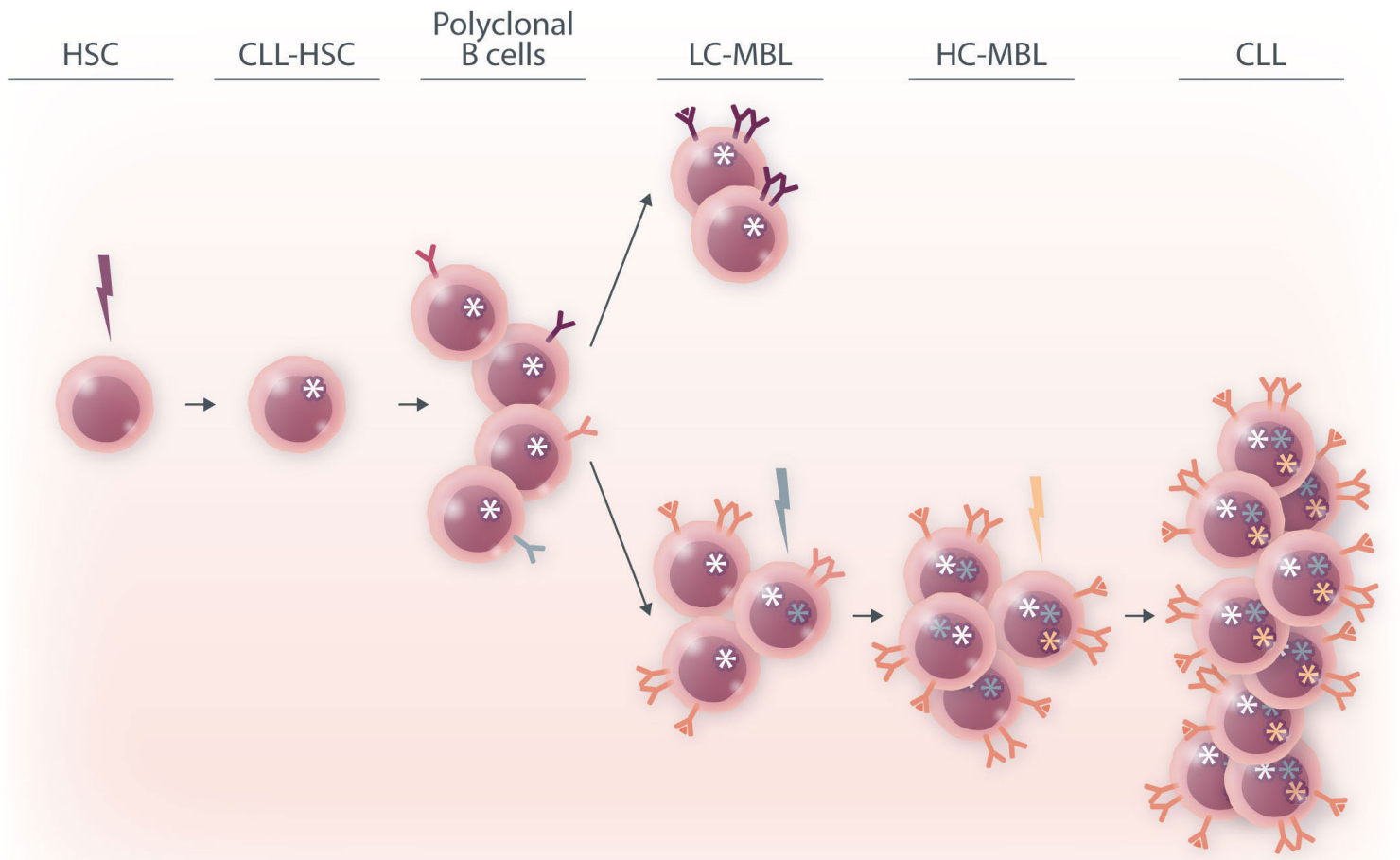

Figure 1. A model of the hypothesized chronic lymphocytic leukemia ontogeny. The initial hit may occur in a hematopoietic stem cell which promotes the production of polyclonal B cells bearing the founder mutation. B-cell receptor (BCR) stimulation by autonomous signaling and possibly by self or foreign antigens leads to oligoclonal low-count monoclonal B-cell lymphocytosis. Further BCR stimulation, as well as the acquisition of genetic lesions, drive gradual selection and clonal expansion of high-count monoclonal B-cell lymphocytosis, which ultimately progresses to overt chronic lymphocytic leukemia. HSC: hematopoietic stem cell; CLL: chronic lymphocytic leukemia; LC-MBL: low-count monoclonal B-cell lymphocytosis; HC-MBL: high-count monoclonal B-cell lymphocytosis.

higher than those previously reported.[7] BCR IG from 11 MBL sorted cells were transfected in triple knock-out pre-B cells, resulting in autonomous signaling for all of them. Surprisingly, when comparing CLL and MBL sibling pairs, autonomous signaling was significantly weaker with BCR *IG* from cases of MBL than those from patients with CLL.

To address the question of a potential genetic susceptibility, the authors tested a panel of 24 risk loci. For both CLL and MBL siblings they found a CLL polygenic score higher than the score for the reference population, indicating a genetic susceptibility to the initial expansion of CLL-type clones, but not to their progression from MBL to CLL. They also searched for CLL-associated genetic lesions, and found similar prevalences of both copy number variants and single nucleotide variants in paired CLL and MBL cases, but with a lower variant allele fraction in MBL.

Altogether, this study demonstrates that MBL, and more specifically LC-MBL, are already equipped with a BCR capable of autonomous signaling, thus allowing emergence of clonal expansions. The fact that these clones also bear CLL driver genomic alterations, albeit at a subclonal level, supports the hypothesis of a continuum between LC-MBL and CLL, with progressive clonal evolution due to stepwise acquisition of genomic lesions (Figure 1).

The article by Quinten *et al.* sheds further light onto the issue of CLL ontogenesis, but also raises some questions. In particular, the finding of lower BCR signaling strength in MBL compared to CLL is puzzling. In leukemic cells, this could be explained by the fact that BCR signaling may be modulated by genetic, epigenetic and/or microenvironmental factors. Such explanations would not, however, fit with the *in vitro* system used by the authors, in which BCR expressed by the triple knock-out cells originated from cloned synthetic DNA fragments. Longitudinal studies to see whether the signal strength increases along with the MBL clone size would be particularly interesting. Finally, as some predisposing factors may contribute to B-cell clone emergence in siblings of CLL patients, it remains to be shown whether the same results would be obtained for MBL in individuals with no family history of CLL.

Disclosures

FD has received honoraria from AstraZeneca and Janssen. These relationships do not raise a conflict of interest regarding the published work.

Acknowledgments

I thank Luk Cox for his helpful expertise in preparing the illustration.

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