Infrequent “chronic lymphocytic leukemia-specific” immunoglobulin stereotypes in aged individuals with or without low-count monoclonal B-cell lymphocytosis

Chronic lymphocytic leukemia (CLL) is a chronic, incurable malignancy of antigen-experienced B cells, mainly affecting the aged population. Immunogenetic analysis in CLL revealed the existence of subsets of patients expressing stereotyped B-cell receptor immunoglobulins (BcR IG), which represent homogeneous CLL variants with distinct biological and clinical characteristics. Little is known regarding the presence of “CLL-specific”, stereotyped BcR IG within the repertoire of healthy individuals. Low-throughput studies led to the identification of cases with stereotyped BcR IG, followed by next-generation sequencing studies that found CLL stereotypes in normal B-cell populations, albeit at very low frequencies.

This issue is more pertinent to monoclonal B-cell lymphocytosis (MBL), characterized by clonal “CLL-like” B-cell expansions detected in 3-12% of the population. “CLL-like” MBL is categorized into high-count (HC-MBL) and low-count (LC-MBL) forms, based on a cutoff of 0.5x10⁹ cells/L. Previously, we showed that HC-MBL was immunogenetically similar to CLL, whereas LC-MBL was characterized by low frequency and different characteristics of stereotyped BcR IG. However, this evidence derived from low-throughput data and was, thus, inherently limited with regards to reflecting the complexity of the BcR IG repertoire.

Here, using a high-throughput methodology we analyzed the composition of the BcR IG repertoire expressed by: (i) clonal and normal B cells from individuals with “CLL-like” LC-MBL (n=23), as well as (ii) naive and memory B cells from age-matched, healthy individuals (n=6) (Online Supplementary Table S1).

Blood samples (5 mL) were obtained from all individuals and cell-sorted within 24 hours. IGHV-IGHD-IGHJ gene rearrangements were amplified by polymerase chain reaction, sequenced on the MiSeq platform and bioinformatically processed (Online Supplementary Methods).

Overall, we identified 592,023 unique BcR IG clonotypes. Of these, 238,075 (40.2%) were expanded (>1 read) and distributed across sample categories as follows: (i) individuals with LC-MBL: 9,014 clonotypes in MBL cell samples (mean: 751/sample); 29,587 clonotypes in peripheral blood mononuclear cell (PBMC) samples (mean: 2,690/sample); and 88,366 clonotypes in normal B-cell samples (mean: 11,046/sample); (ii) healthy individuals: 67,358 clonotypes in naïve B-cell samples (mean: 11,226/sample); and 43,750 clonotypes in memory B-cell samples (mean: 8,750/sample) (Online Supplementary Table S2). The presence of stronger biases in the normal B-cell compartment of individuals with LC-MBL compared to that of healthy donors is in line with the hypothesis of an impaired pre-germinal center B-cell production in LC-MBL, which would lead to a limited B-cell repertoire.

Clonality assessment focused on abundant clonotypes (individual frequency >0.92%; Online Supplementary Methods). In total, 222 abundant clonotypes were identi-
fied (Online Supplementary Table S3), of which 216 (97.3%) belonged to either the MBL (70/222, 31.5%) or the PBMC samples (146/222, 65.8%) from LC-MBL. The average number of abundant clonotypes per sample was 6.4 for the MBL cell samples, 9.8 for the PBMC samples and ≤1 for all sample categories of normal B cells.

With regards to clonality, the majority of samples (9/12, 75%) displayed a monoclonal pattern characterized by the presence of a single abundant clonotype dominating the repertoire (frequency range, 42.5-97.9%). The remaining three samples (MBL-5, MBL-10 and MBL-12) were characterized by an oligoclonal pattern. The median LC-MBL clone size was large (6.5 cells/μL), especially when compared to that in populations of Asian origin (median: 0.12 cells/μL), perhaps linked to the low prevalence of oligoclonality in our cohort. The assessment of the relation between the size of the MBL clone and the level of clonality (Spearman rho correlation coefficient) showed a positive trend, in line with previous studies showing a lower frequency of oligoclonality along the spectrum from LC-MBL to HC-MBL and, eventually, CLL. However, results were not statistically significant (P=0.18), perhaps because of the small size of the cohort. No correlations were found between clone size and age, sex or cytogenetic aberrations.

All but one PBMC sample from LC-MBL carried abundant clonotypes (10/11, 90.9%), yet a relatively high-frequency clonotype (individual frequency of >6%) was identified in 3/11 samples (27.3%). Finally, normal B-cell samples were clearly polyclonal without significant expansions; only four abundant clonotypes were identified in two samples, but with very low frequencies (range, 1.1-1.8%). Figure 1 illustrates the clonality patterns in all sample groups. As expected, LC-MBL cell populations displayed high levels of clonality, thus clearly differing from the polyclonal normal B-cell populations. Such differences in clonality patterns among LC-MBL cell samples may reflect different timepoints in the process of clonal evolution. In other words, LC-MBL could initially involve a polyclonal B-cell population that at some point acquires the CLL phenotype due to sustained antigenic stimulation. The specific immunogenetic characteristics of the BcR IG along with the progressive acquisition of genetic lesions, e.g., cytogenetic alterations, by individual MBL clones could drive their expansion, thus leading to oligoclonal and, eventually, monoclonal LC-MBL cell populations, as proposed previously.

The IGHV gene repertoire was characterized by significant restrictions in all sample categories (Online Supplementary Table S4). The application of a 5% frequency cutoff led to the identification of seven frequent IGHV genes in the MBL cell category, which collectively accounted for 38.1% of the total repertoire. With regard to normal B cells from individuals with and without LC-MBL, restrictions were less pronounced with four and three frequent IGHV genes, respectively. Analysis at the individual IGHV gene level revealed statistically significant differences in the expression of two frequent genes (IGHV3-23, IGHV3-30) and ten low-frequency IGHV genes (analysis of variance, P<0.05) (Online Supplementary Table S5, Figure 2). These differences concerned mostly the category of normal B cells from healthy individuals who, overall, displayed a distinct IGHV repertoire compared to that of individuals with LC-MBL. Biases in the IGHD and IGJH gene repertoires were evident in all sample categories (Online Supplementary Tables S6 and S7). Overall, the existence of distinct biases in the IGHV gene repertoire, especially between CD5+ CLL-like cells from individuals with LC-MBL and normal (mostly CD5-) B cells, point towards different selection processes and
functions of these B-cell subpopulations. In order to identify BcR IG stereotypes, we applied our purpose-built algorithm on all abundant BcR IG clonotypes (frequency of >0.92%) from the present cohort along with 30,221 CLL clonotypes from the IMGT/CLL-DB (http://www.imgt.org/CLLDBInterface/query). According to our findings, 43/222 (19.4%) abundant clonotypes were assigned to 42 distinct clusters (Online Supplementary Table S8).

The vast majority of abundant clonotypes with stereotyped VH CDR3 (42/43, 97.7%) were found in samples from individuals with LC-MBL (12 in MBL cell samples and 30 in PBMC samples). Virtually all abundant stereotyped LC-MBL clonotypes (47/48, 97.9%) were assigned to minor clusters with a mean size of four clonotypes (range, 2-22 clonotypes). Only a single abundant clonotype was assigned to a large CLL subset, namely subset #148B (CLUSTER-4-0003), which contained 150 clonotypes in the current analysis. Hence, “CLL-specific” stereotyped clonotypes were observed in most samples, yet these were scant and, for the most part, exhibited low frequency. In the case of subset #148B, which was the only well-documented CLL subset from the present analysis, its biological and clinical characteristics were “compatible” with LC-MBL: a high frequency of del(13q), low frequency of CD38 positivity, young age at diagnosis and a long time to first treatment (9.2 years).

The complete absence of “CLL-specific” BcR IG stereotypes belonging to major CLL stereotyped subsets prompted us to search for such sequences among all expanded BcR IG clonotypes (>1 sequence), irrespective of individual clonotype frequency. We identified 142 of 238,075 (0.0006%) clonotypes belonging to 14 major CLL subsets (Online Supplementary Table S9) in 27/42 samples (64.3%) from all sample categories, although at very low frequencies (range, 0.0002-0.28%). Most “CLL-specific” BcR IG stereotypes were found in naive B-cell samples (average: 8.5); in contrast, MBL and PBMC samples from LC-MBL had the fewest (averages: 1.3 and 1.1, respectively).

Interestingly, the distribution of “CLL-specific” BcR IG stereotypes differed from that reported in CLL (Figure 2) as shown by the low incidence of subsets #1 and #2, the largest in CLL, and the absence of rearrangements similar to those of CLL subsets #8, #31, #59 and #99, all associated with aggressive disease. Furthermore, stereotyped clonotypes typical of CLL subset #4’ were significantly (P<0.05) biased to naive B-cell populations. This BcR IG stereotype is distinctive for utilizing the IGHV4-34 gene, notable for its germline-encoded autoreactive potential.

This gene is frequent in naive B cells but suppressed in classical memory B cells and, instead, enriched in autoimmune repertoires and certain lymphoproliferations, prompting an argument for stringent censoring of IGHV4-34 B cells in healthy individuals. This appears to be supported by the present study as well, in which we found that LC-MBL is devoid of CLL stereotypes related to aggressive disease and that the subset #4 stereotype is confined to the naive B-cell repertoire.

In conclusion, in the first next-generation sequencing immunoprofiling study of LC-MBL we report differences from age-matched individuals without MBL. Critically, the very low incidence of “CLL-specific” stereotyped BcR IG in LC-MBL (as well as in elderly individuals without LC-MBL) further attests to the unique immunogenetic signature of CLL while also highlighting the role of microenvironmental triggering, mediated through the BcR, as a major driver even before the onset of CLL. This could also explain the low incidence of CLL in the East, since not only the genetic background but also environmental triggers could differ between Asian and Caucasian populations.

Andreas Agathanggelides,1,2 Chrysí Gáthagáldou,1,3 Lydia Scárfo,1 Theo doros Móysiádis,1,2 Alessandra Rovida,1 Maria Gounari,1 Fotis Psomopoulou,2 Pamela Ranghetti,1 Alex Galanis,1 Frederic Davi,2 Kostas Stannatopoulos,1,2 Anastasia Chatzidimitriou2 and Paolo Ghia3,4 also on behalf of the Euroclonoma NGS Working Group

1Strategic Research Program on CLL and B-Cell Neoplasia Unit, Division of Experimental Oncology, University Vita-Salute San Raffaele and IRCCS Ospedale San Raffaele, Milan, Italy; 2Institute of Applied Biosciences, Center for Research and Technology Hellas, Thessaloniki, Greece; 3Department of Molecular Biology and Genetics (MBG), Democritus University of Thrace, Alexandroupolis, Greece; 4Department of Molecular Medicine and Surgery, Karolinska Institute, Stockholm, Sweden and Assistance Publique - Hôpitaux de Paris (AP-HP), Hôpital Pitié-Salpêtrière, Department of Biological Hematology, Sorbonne Université, UMR_S 1138, Centre de Recherche des Cordeliers, Paris, France

AG and PG contributed equally as co-senior authors.

Correspondence: ANASTASIA CHATZIDIMITRIOU - achatzidimitou@certh.gr

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