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Immunologic aspects of monoclonal B cell lymphocytosis

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Abstract

Monoclonal B cell lymphocytosis (MBL) is a preclinical hematologic condition wherein small numbers of clonal B cells can be detected in the blood of otherwise healthy individuals. Most MBL have a surface immunophenotype nearly identical to that of chronic lymphocytic leukemia (CLL), though other phenotypes can also be identified. MBL has been shown to be a precursor state for CLL, but most MBL clones are quite small and apparently have minimal potential to progress of CLL or other B cell lymphoproliferative disorder (B-LPD). The investigation of MBL as a precursor state for CLL will likely lead to important insights into mechanisms of disease pathogenesis. The review will cover clinical and translational aspects of MBL, with a particular emphasis on the prevalence of MBL; the relationship between MBL, CLL, and other B-LPDs; and the capacity of MBL to modulate the normal B and T cell compartments.

Keywords

Monoclonal B Lymphocytosis; Chronic Lymphocytic Leukemia; Non-Hodgkin's Lymphoma; B Cell Receptor; B Cell Development

Introduction

Chronic lymphocytic leukemia (CLL) is the most prevalent hematologic malignancy in the United States, with approximately 15,000 new cases per year and 95,000 affected individuals [1]. Although CLL is treatable, it remains incurable. Despite years of thoughtful and detailed investigation, much remains unknown regarding the etiology of CLL (reviewed by Chiorazzi [2]). In 2002, it was first observed that small numbers of CLL-phenotype B cells could be identified in the blood of otherwise healthy individuals with normal blood counts [3]. This phenomenon was termed monoclonal B cell lymphocytosis (MBL). Since these first reports, MBL has been shown to be a precursor state for CLL [4, 5]. While MBL is a precursor of CLL, the overwhelming majority of MBL apparently do not progress to CLL. The identification of a precursor state for CLL has created an opportunity to investigate the immunologic and molecular characteristics of pre-emergent CLL. Through

careful study of this *forme fruste* of CLL, it is anticipated that critical aspects of the etiology of CLL will become clear.

MBL Prevalence

MBL was first identified through a program of systematic screening of 910 individuals over age 40 years with normal blood counts presenting for outpatient medical care to non-hematology clinics. In this cohort, the prevalence of MBL was 3.5%, a population prevalence at least 100-fold more common than CLL. Similar to CLL, the frequency of MBL increased with age. The MBL cells showed a typical CLL immunophenotype: CD5⁺, CD19⁺, CD20^{dim}, CD23⁺, CD79b^{dim}, and were clonal as determined by restricted expression of surface immunoglobulin light chains [3]. A subsequent report by this same group in Leeds, UK observed a four-fold higher in prevalence of MBL (13.5%) among unaffected family members ascertained from CLL kindreds, suggesting that MBL may also be a marker for inherited predisposition to CLL [6]. These first reports identified four parameters as the principal determinants of the prevalence of MBL: (1) the presence or absence of an absolute lymphocytosis, (2) the age of the population under investigation, (3) the detection sensitivity of the flow cytometry assay employed, and (4) the presence or absence of a family history of CLL.

As flow cytometry methods became increasingly sensitive, incrementally smaller MBL clones became detectable. In the initial population screening report by Rawstron *et al* that identified MBL in 3.5% of individuals screened, a four color flow cytometry assay was utilized, and 2.0×10^5 events were collected [3]. This method yields an approximate detection sensitivity of 1 MBL (CLL phenotype) cell per 1×10^5 events. Using a very similar methodology (4 color flow, 2.0×10^5 events), Ghia and co-workers found the prevalence of MBL to be 5.5% in a cohort of 500 primary care patients in Italy [7]. A subsequent study by the same group in Italy that employed a more sensitive flow method (5 colors, 5.0×10^5 events) found a slightly higher rate of MBL of 7.4% among 1,725 individuals participating in a population screening program. Conversely, a study performed in the United States that screened 1,926 individuals as part of a population screening program that utilized a less sensitive 2 color flow cytometry assay found a MBL prevalence of only 0.6% [8]. Most recently, a study evaluating 608 primary care patients in Spain using an 8 color flow assay and collecting 5.0×10^6 events found the prevalence of MBL to be 12% [9]. These apparently divergent prevalence values are easily reconciled when assay sensitivity is considered. For example, the assay used in the Spanish study had a detection sensitivity at least one order of magnitude greater than that employed in the early UK and Italian studies, and approximately 2/3 of the MBL clones detected in the Spanish study were smaller than the maximum detection sensitivity of the flow cytometry assay employed in the earlier studies. Recently, all of these groups, including our group at Duke University, have pooled our screening data and show very similar MBL prevalence at a similar level of detection sensitivity [10, 11].

MBL is more common among the unaffected first degree relatives of CLL patients, suggesting that MBL may be a marker of inherited predisposition for CLL [6, 12, 13]. As part of our ongoing research within the Genetic Epidemiology of CLL Consortium, a

collaboration of researchers from seven institutions with the overall aim of investigating the genetic basis of CLL through the collection of CLL families, we recently reported the prevalence of MBL to be 17% in a cohort of 505 unaffected relatives of CLL patients [14]. The high prevalence in this report is likely due to ascertainment, because only first degree relatives over 40 years of age from families with two documented cases of CLL (high-risk CLL) were included. Age remains an important factor among MBL ascertained from CLL kindreds; the prevalence of MBL increased with age and was over 60% among individuals over 90 years of age [14]. Matos *et al* recently reported the prevalence of MBL to be 6% among a relatively small cohort of 113 unaffected first degree relatives of sporadic (non-familial CLL) over age 40 [14a]. Interestingly, the rate of MBL among relatives over age 60 was 15% suggesting that MBL prevalence in “sporadic” and “familial” MBL is comparable and may have shared genetic determinants. Emerging data supports this hypothesis; a case-control single nucleotide polymorphism (SNP) association study of 10 SNPs associated with inherited risk for CLL showed that 9 of 10 SNPs were also associated with risk of development of MBL, and 6 of these associations were statistically significant [15]. This finding suggests that inherited risk to CLL may be conferred by inherited risk to MBL.

Types of MBL

While the initial reports of MBL focused on the identification of CLL phenotype cells, other apparently less common phenotypes were subsequently identified. Ghia *et al* described three different forms of MBL in the report of 500 outpatients over age 65 in Italy [7] (Table 1). (I) “CLL-like” MBL was the most common, with 22 cases observed. Three cases showed an (II) “atypical CLL-like” phenotype: CD5⁺, CD10⁻, CD19⁺, CD20^{high}, CD23⁺, CD79b^{dim}, IgM^{dim}. This phenotype does not have a clear correlate either among normal B cells or in B cell lymphoproliferative disorders. It is most consistent with CLL with an atypical immunophenotype or mantle cell lymphoma (MCL). Because CD23 was expressed in all cases, pre-emergent MCL was considered less likely. The third form of MBL described was (III) “CD5^{neg}” or “non-CLL like” MBL, observed in 7 cases with a phenotype showing: CD5⁻, CD10⁻, CD19⁺, CD20⁺, CD23⁻, FMC7⁺, CD79b⁺. Because these cells fall within in a normal B cell compartment on a CD5 by CD19 or CD5 by CD20 flow cytometry plot, skewing of the ratio of surface immunoglobulin $\kappa : \lambda$ of $> 3:1$ or $< 0.3 : 1$ was required for identification. Therefore, unlike CLL-like or atypical MBL (both of which can be detected at very low levels), CD5⁻ MBL clones must comprise a significant proportion of the normal B cell compartment to be detected. This immunophenotype is not specific for any single B-LPD; it is potentially consistent with marginal zone lymphoma (MZL), lymphoplasmacytic lymphoma (LPL), and hairy cell leukemia (HCL). Familial clustering of CLL, LPL, and HCL has been described, so further characterization of this likely heterogeneous group of MBLs is of interest [16].

The relative proportion of these MBL subtypes in different populations remains an area of active investigation. In our recent report of familial-CLL associated MBL, 86 MBL were identified among 505 study participants. Of these, the majority (74 of 86, 86%) were “CLL-phenotype”, 7 were “Atypical-CLL phenotype” (7 of 86, 8%), and 5 were “Non-CLL phenotype” (5 of 86, 6%) [14] It is possible that the proportion of CLL-like MBL is enriched among individuals with a family history of CLL. The relative proportion of different MBL

subtypes may well be different in population studies or among individuals with a family history of NHL rather than CLL.

The term MBL is now used to describe all types of clonal B cell populations detectable in blood. However, since most MBL are CLL-phenotype, there is the potential for confusion when comparing data series depending on whether all subtypes are included. Therefore, efforts have been made regarding consensus nomenclature for these different groups [17, 18]. As initially described by Ghia *et al*, three groups are defined: (I) “CLL-phenotype”, in which an immunophenotype showing CD5⁺, CD19⁺, CD20^{dim}, CD23⁺, and sIg^{dim} is required; (II) “Atypical CLL-phenotype”, in which a CD5⁺, CD19⁺ population is present with bright expression of either CD20 or sIg, or dim expression of CD23 is present; and (III) “Non-CLL phenotype”, in which the immunophenotype is CD5⁻, CD19⁺, CD20⁺, sIg^{high}, and skewing of the $\kappa : \lambda$ ratio is required. For the remainder of this review, only “CLL-phenotype” MBL will be considered, and as such, hereafter the term MBL will be used synonymously with this subtype.

The Relationship between MBL and CLL

Over the past 30 years, the diagnostic criteria for CLL have changed significantly. The availability of automated blood counters, the identification of CD5 positivity as a hallmark feature of CLL, and the recent widespread application of flow cytometry all contributed to the improved diagnostic specificity and sensitivity for CLL. This ultimately led to establishment of a minimum value required for a diagnosis of CLL in the NCI-96 guidelines of an absolute lymphocyte count (ALC) of $5.0 \times 10^9 / L$ [19]. This diagnostic criterion was derived largely from detection sensitivity rather than clinical outcome, and it was subsequently appreciated that this diagnostic value did not correlate well with future need for CLL-directed therapy. Therefore, in the recent International Workshop on CLL (iwCLL) 2008 revision of the NCI-96 guidelines, the diagnostic threshold for CLL was changed to a B-cell lymphocyte count (B-ALC, rather than ALC) of $5.0 \times 10^9 / L$ [20]. This value correlates better with relevant clinical outcomes, such as need for CLL-directed therapy.

In the iwCLL 2008 guidelines, MBL was specifically defined as the presence of a CLL phenotype population with $< 5.0 \times 10^9 B$ cells / L in blood in the absence of any other disease-related symptoms or clinical findings [20]. One intriguing aspect of this shift in diagnostic criteria was that some individuals previously diagnosed as stage 0 CLL became reclassified as MBL. Shanafelt *et al* performed an analysis of 459 patients presenting to the Mayo clinic with MBL or Rai stage 0 CLL. Although patients classified as MBL under the current schema were less likely to require CLL-directed therapy than patients with Rai 0 CLL, the authors determined that a B-ALC of $11.0 \times 10^9 / L$ provided the best cut-off for both need for future CLL-directed therapy and overall survival [21]. Confirmatory independent datasets will likely lead to further refinement of the minimum B-ALC and improved diagnostic ability to distinguish CLL and MBL.

While the Shanafelt *et al* study studied a retrospective cohort of patients who were previously considered Rai 0 CLL under the NCI-96 formulation, a central question was whether MBL would progress to CLL, and if so at what rate. By combining a group of MBL

identified through population screening of 1,520 individuals and a series of MBL identified through diagnostic evaluation of 2,228 patients with an absolute lymphocytosis, Rawstron *et al* identified a cohort of 387 MBL that could be followed prospectively for evolution to CLL and for need of CLL-directed therapy. Longitudinal analysis with a median follow-up of 6.7 years of these individuals with MBL showed that among 185 participants with an absolute lymphocytosis, CLL developed in 28 (15%), and CLL-directed chemotherapy was required in 13 (7%). The annualized cumulative risk of MBL progression to need for CLL directed therapy was 1.1% per year. There were only 4 CLL-attributable deaths in the entire cohort during the period of follow-up. Similarly, in a retrospective analysis of 302 patients newly identified MBL (predominantly patients considered Rai 0 under the NCI-96 formulation) presenting to the Mayo clinic, Shanafelt *et al* observed that 1.4% per year (7 % total) of the study subjects with MBL required CLL-directed therapy within 5 years of diagnosis [22]. The B-ALC was the most important determinant of risk for future need for CLL-directed therapy in both the Rawstron *et al* and Shanafelt *et al* studies, with individuals with a B-ALC $< 1.9 \times 10^9 / L$ at very low risk for progression to CLL [4, 22].

These translational outcomes studies of MBL showed that the most important determinant of MBL progression was the B lymphocyte count at diagnosis. The majority of MBL cases with an absolute lymphocytosis at identification are found through a clinical referral for diagnostic evaluation of an abnormal complete blood count. However, analysis of population-based screening for MBL suggests that 95% of all MBL have a CLL-phenotype cell count $< 56 \times 10^6$ cells / L and 75% have an MBL cell count $< 3 \times 10^6$ cells / L. Thus MBL with lymphocytosis is very rare in the general population [23]. The observation of a small MBL clone ($< 100 \times 10^6$ cells / L) observed in conjunction with otherwise normal B-ALC has been termed “low count MBL” by Dagklis *et al* [9, 24]. The majority of individuals followed in the Rawstron *et al* and Shanafelt *et al* clinical studies were “high count MBL” (*i.e.*, an absolute B cell lymphocytosis was present), so the biology and natural history of small MBL clones remains unclear. The excess risk of progression to need for CLL-directed therapy for individuals with low count MBL is currently believed to be negligible.

Another important clinical question regarding MBL is whether CLL is always preceded by an MBL phase. To investigate this question, Landgren *et al* studied 45 participants from the Prostate, Lung, Colon, and Ovarian (PLCO) Cancer Screening Trial who developed CLL after enrolling in this prospective observational cohort [5]. These 45 CLL cases were identified from the 77,469 participants in this nationwide study. All 45 of these study subjects had cryopreserved blood samples that were archived for the PLCO study prior to their diagnosis of CLL, and these samples predated the diagnosis of CLL by up to 6.4 years. Using 6 color flow cytometry screening and reverse transcriptase PCR, MBL was identified in 44 or 45 CLL (98%) cases, and the MBL clone was detectable by both PCR and flow cytometry in 91% of cases. This strongly supports the hypothesis that MBL precedes essentially all cases of CLL [5].

Biologic Insights from the B Cell Receptor

Investigation of the B cell receptor (BCR) has yielded insights that are central to the current understanding of CLL (reviewed by Caligaris-Cappio [25]). First, despite the tremendous potential breadth of the immunoglobulin gene repertoire, patients with CLL use a highly restricted set of BCRs [26, 27]. Furthermore, stereotypy, the observation of nearly identical complementarity determining region 3 (CDR3) sequences between CLL cases, is observed among a significant minority of CLL cases. These observations led to the hypothesis that tonic stimulation by stereotyped auto- or alloantigens drives expansion of the malignant clone in CLL [25, 28, 29]. Second, patients with mutated immunoglobulin heavy chain (IGVH) genes follow a more indolent disease course than those with unmutated IGVH [30]. It was initially suggested that CLL may be two distinct entities: one deriving from naïve (IGVH unmutated) B cells, and the other from post-germinal center memory (IGVH mutated) B cells. However, gene expression profiling studies in CLL show that both IGVH mutated and unmutated CLL are derived from memory B cells [31, 32]. The origin of IGVH unmutated CLL remains controversial. Chiorazzi and others have proposed that unmutated CLL arise from B cells that mature through a T cell independent pathway [2]. Third, BCR-mediated signaling is possibly the key determinant of CLL biology. CLL is characterized by enhanced activation of BCR downstream intracellular signaling pathways, and the capacity of CLL cells to upregulate BCR-mediated signaling correlates with the mutation status of the IGVH [33]. Given the significance of the BCR in CLL biology, investigation of the BCR in MBL is of significant interest.

To determine the IGVH usage and mutation status in MBL, we purified single MBL cells by FACS sorting from a series of individuals with MBL ascertained from familial CLL kindreds [12]. The immunoglobulin heavy and light chain sequences were obtained from genomic DNA after whole genome amplification and immunoglobulin gene-specific nested PCR. This detailed characterization was performed on six “low count” MBL cases, and several interesting features were noted. The IGVH genes used were typically mutated and included families commonly observed in CLL, with V_H families 3–07, 3–15, and 4–34 observed in multiple individuals. When observed in CLL, these V_H families are associated with a favorable prognosis. In addition, 4 of the 6 MBL cases were oligoclonal, rather than truly monoclonal, with 2 to 4 apparently unrelated MBL clones present in the same individual.

In approximately 3% of IGVH mutated CLL, clonally related subgroups of leukemic cells with progressive IGVH gene mutations can be identified [34]. We identified progressive immunoglobulin gene mutation in one of the low count MBL subjects. This VH3-07, D3-10 clone from one subject consisted of related subgroups of MBL that shared the same immunoglobulin gene rearrangements and some somatic mutations, but also had several partially shared mutations. The MBL immunoglobulin gene rearrangements could be placed into a genealogical tree in which sequentially related clones were ordered based on sequence homology with the germline sequence and subsequent contiguous clones with progressive somatic mutations (Figure 1). MBL clones arranged in the genealogical tree furthest away from the putative germline immunoglobulin sequence had evidence of antigen-driven immunoglobulin sequence changes [35]. We and others have suggested that similar clonally-

related subgroups from CLL cases represent antigen-driven refinement of the BCR [34, 36, 37]. We believe that evidence of oligoclonal diversification favors an antigen-driven process for some subjects with MBL.

When interpreted in the context of other published reports, our single cell IGVH analyses help clarify the biologic continuum of MBL and CLL. Among cases of MBL that progressed to CLL, both Rawstron *et al* and Landgren *et al* found that the IGVH sequences were typical for CLL, and approximately 80% were mutated [4, 5]. All of these cases were monoclonal (albeit using bulk sequencing techniques) and the majority of these cases had B cell lymphocytosis at the time of ascertainment. Investigations of predominantly “low count” MBL reveal interesting contrasts with larger MBL clones. Dagklis *et al* also found a preponderance of mutated IGVH sequences. However, the V_H families identified, including $V_{H4-59/61}$ and V_{H3-30} , are not commonly observed in CLL [24]. Nieto *et al*, investigating IGVH usage among 7 very small MBL clones wherein the majority of cases had $< 1.0 \times 10^6$ MBL cells / L, found both mutated and unmutated IGVH sequences with predominant usage of V_{H1} and V_{H3} family genes [9]. Both Dagklis *et al* and Nieto *et al* observed oligoclonal MBL among a minority of low count MBL cases based on the absence of skewing of the $\kappa:\lambda$ light chain ratio by flow cytometry. Taken together, the data suggest that small MBL clones are commonly oligoclonal and that they may use a different subset (and perhaps a more diversified subset) of immunoglobulin genes than CLL. Larger MBL clones are more akin to CLL, with IGVH usage similar to IGVH mutated CLL and are almost always monoclonal. Though speculative, these observed differences may reflect the diminished capacity of some BCRs for downstream signaling and, in turn, clinical progression from MBL to CLL. The characterization of BCR downstream signaling pathways in MBL is an area of ongoing effort in the laboratory.

Changes within the B Cell Compartment in MBL

Acquired immune dysfunction is one of the hallmark clinical features of CLL. Most patients with CLL have complete or near replacement of the normal B cell compartment by malignant CD5⁺ cells. With time, hypogammaglobulinemia develops in a significant proportion of patients. Infections remain a leading cause of morbidity and mortality among CLL patients.

Although most cases of CLL have few if any residual normal B cells at the time of diagnosis, most “low count” MBL have a preserved normal B cell compartment. To investigate the phenomenon of “drop out” of normal B cell subsets, Rawstron analyzed absolute MBL and normal B cell numbers from 1,081 MBL ascertained by MBL screening programs worldwide. The normal B cell count was found to range between 50 and 500×10^6 cells / L. Interestingly, most MBL showed absolute numbers that were either less than or greater than this range of values, suggesting a bimodal distribution of MBL cell numbers [23]. This observation remains unexplained. Further, it was noted that MBL with absolute CLL-phenotype cell counts $> 500 \times 10^6$ / L correlated with decreasing numbers of normal B cells, and complete loss of the normal B cell compartment was observed at MBL cell counts as low as $1,000 \times 10^6$ MBL cells / L. A hypothetical model of MBL progression with depletion of the B cell compartment is presented in Figure 2.

Although depletion of the normal B cell compartment was clearly correlated with increasing MBL cell numbers, this effect was relatively variable, and the determinants of contraction of the normal B cell compartment with increasing MBL clone size remain under study. To further investigate this phenomenon, Hauswirth et al recently reported preliminary investigation of normal B cell subsets in MBL [23a]. They observed that all B cell subsets, including immature, naïve, memory B-lymphocytes and plasmablasts, were significantly reduced in MBL when compared to controls. A statistically significant decrease in all subsets was noted when MBL cells comprised > 1% of the entire B cell compartment. They concluded that this observation suggests a direct suppressive effect of the MBL clone on normal B cell lymphopoiesis. In preliminary investigations in our laboratory, we have observed inversion of the ratio of naïve to memory B cells among a minority of MBL cases, potentially suggesting impaired production of new naïve B cells as an initial etiology of depletion of normal B cell subsets (Lanasa *et al*, unpublished).

Changes within the T Cell Compartment in MBL

In addition to B cell and humoral immune abnormalities, CLL patients also have qualitative and quantitative T cell abnormalities. Most malignancies are associated with decreased numbers of circulating T cells. However in chronic lymphocytic leukemia (CLL), T cell numbers are elevated 2 to 4 times normal [38, 39]. Rather than promoting an anti-tumor response, this increased population of T cells may contribute to a tumor microenvironment that promotes progression of the malignant clone [40]. Direct contact between CLL cells and T cells causes downregulation of cellular pathways important for T cell cytotoxicity [41, 42]. CLL cells secrete immunomodulatory cytokines such as IL-6 and IL-10 that shift the helper T cell response from a Th1 response to an anergic Th2 response [41, 43, 44]. Suppressive regulatory T cells are also increased in patients with CLL [45, 46]. The T cell repertoire is significantly contracted in CLL, with presence of oligoclonal and monoclonal subsets [47–49]. Taken together, this global impairment in T cell function may be an important cause of tumor progression, increased susceptibility to infection, and secondary malignancies in patients with CLL.

As previously stated, it has been proposed that IGVH unmutated CLL arise from B cells that mature through a T cell independent pathway, because CD4⁺ T cell help is required for somatic mutation and isotype switching in the germinal center reaction [2, 50, 51]. Therefore, IGVH mutated and unmutated CLL may have differing capacities for T cell interaction and immune modulation. A recent report by Palmer *et al* showed that increased numbers of T and “natural killer” (NK) cells relative to the number of CLL lymphocytes in patients with newly diagnosed CLL is associated with improved prognosis [52]. Further, they showed that increased T and NK cell : CLL lymphocyte ratios correlated with a mutated IGVH. This finding supports the hypothesis that IGVH mutated and unmutated CLL induce different T cell responses and that these T and NK cell responses precede clinical CLL.

To evaluate if similar T cell abnormalities are observed in MBL as in CLL, we enumerated T cells from a series of 20 untreated patients with CLL (10 IGVH mutated and 10 unmutated) and 8 MBL subjects [53]. The CD4⁺ count was similar in IGVH unmutated

CLL, IGVH mutated CLL, and MBL subjects. The CD8⁺ count was higher in IGVH unmutated compared to IGVH mutated CLL patients (891 vs. 593×10^6 cells / L, *n.s.*), and was significantly higher in IGVH unmutated than MBL subjects (891 vs. 273×10^6 cells / L, $p < 0.05$). Similarly, the ratio of CD4⁺: CD8⁺ T cells was significantly lower in IGVH unmutated CLL compared to IGVH mutated CLL or MBL (1.6 vs. 2.6 , $p < 0.05$, and 1.6 vs. 4.5 , $p < 0.01$, respectively).

To estimate the CD4⁺ and CD8⁺ T cell repertoire diversity, we coupled PCR amplification of the CDR3 length polymorphism of 23 TCRV β families (“Spectratyping”) with a novel, quantitative method for analysis of TCR repertoires by using SpA [54]. The calculated mean divergence coefficients (a calculated unitless value to quantify the degree of “skewness” from a pseudo-Gaussian distribution) for the T cell Spectratypes were compared between groups. Combined analysis of all CLL patients showed the mean CD8⁺ TCR Spectratype divergence was 0.32, significantly higher than the mean CD4⁺ divergence (0.07, 2 sided t-test, $p < 10^{-6}$). Oligoclonal and monoclonal V β families were frequently observed among CD8⁺ cells, but rarely observed among CD4⁺ cells. Next, we evaluated whether MBL is associated with a restricted TCR repertoire. The mean CD8⁺ and CD4⁺ divergence among MBL subjects was similar to that observed in CLL patients (mean CD8⁺ divergence: 0.35 for MBL vs. 0.32 for all CLL; mean CD4⁺ divergence: 0.06 for MBL vs. 0.07 for all CLL). We performed a multivariate correlation to identify variables associated with restriction of the CD8⁺ repertoire in MBL. Patient characteristics, the absolute number of different lymphocyte subsets, and “MBL characteristics” (ZAP70 status, CD38 status, and the “MBL percentage,” defined as the number of MBL cells divided by the total number of CD19⁺ B cells) were considered. A correlation between the absolute number of CD4⁺ cells and the diversity of the CD8⁺ repertoire was observed: higher numbers of CD4⁺ cells correlated with more diverse CD8⁺ repertoire ($\rho = -0.82$, $p < 0.05$). Loss of specific CD4⁺ subsets may cause contraction of the CD8⁺ repertoire; alternatively some CLL and MBL may have the capacity to modulate both the CD4⁺ and CD8⁺ subsets as independent effects [53].

Summary and Future Directions

In summary, ongoing research of MBL by our group and others is yielding important insights into the pathogenesis of CLL. These investigations reveal MBL and CLL to be part of a continuum in which small MBL clones apparently share greater similarity to the normal B cell compartment (as determined by absolute B cell numbers, IGVH usage, and clonality studies) than to MBL with lymphocytosis, which in turn, is more similar to CLL. The immunologic and cellular mechanisms that allow “low count” MBL to expand beyond the size of the normal B cell compartment may be centrally important in CLL leukemogenesis. Areas of ongoing investigation include: (a) MBL as a marker of inherited risk for CLL and the role of genetics in MBL development; (b) the clinical outcomes of individuals with low count MBL; (c) the biologic characteristics of MBL as a determinant of risk for the development of CLL; and (d) host characteristics that are either permissive for or protective against progression of MBL to CLL. These investigations should help clarify the process of CLL leukemogenesis and may identify novel therapeutic targets in CLL.

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Figure 1. Antigen-driven intraclonal diversification in an MBL subject

Agenealogical analysis of related MBL oligoclonal subgroups based on an analysis of single cells and analysis of the rearranged immunoglobulin heavy chain from each MBL cell subgroup (adapted from reference 12). Letters A – M represent individual subgroups of MBL cells with a related immunoglobulin heavy chain gene sequence containing 1 to 10 single cell clones per subgroup. GL represents a hypothetical MBL clone expressing the germline VH3–07 sequence. H1 – H3 represent hypothetical transitional MBL clones that were not observed in the analysis.

Figure 2. Hypothetical model of MBL progression

The B cell lymphocyte count is shown on the vertical axis. Since the duration of an MBL phase prior to CLL is quite variable, we use the phrase “biologic progression” on the horizontal axis as a surrogate for time-to-progression. As the MBL clone expands (dotted line), the absolute B cell count (solid line) initially remains normal during the “low count” MBL phase. Once the MBL clone size reaches normal B cell count, the number of residual normal B cells (long dashed line) begins to decline. Once the size of the MBL clone reaches approximately $1,900 \times 10^6/L$, MBL cells comprise most or all of B cell compartment, and the subject is at high risk of progressive lymphocytosis. Once the B cell count exceeds $5,000 \times 10^6/L$, the diagnostic criteria for CLL are met. On the right, the triangle designates that the prevalence of “low count” MBL is far greater than either CLL or MBL with an absolute lymphocytosis.

Table 1

Subtypes of MBL

MBL Type	Immunophenotype						Relative proportion in a population cohort (%) [7]	Relative proportion in a familial CLL cohort (%) [14]
	CD5	CD10	CD19	CD20	CD23	sig		
CLL Phenotype	+	neg	+	dim	+	dim	69	86
Atypical CLL Phenotype	+	neg	+	+	+/-dim	+	9	8
Non-CLL Phenotype	neg	neg	+	+	neg	+	22	6