

New Insights into the Phenotype and Cell Derivation of B Cell Chronic Lymphocytic Leukemia

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Abstract For many decades, B cell chronic lymphocytic leukemia (B-CLL) stood out as a B cell-derived malignancy that was difficult to position within the framework of the available B cell differentiation scheme: First, the histology as well as the immunophenotype did not quite resemble that of any normal lymphocyte; second, in contrast to almost all other B cell tumor subtypes, the immunoglobulin variable region (IgV) genes of B-CLL cases could be either unmutated or somatically mutated; third, the genomic lesions observed in B-CLL were markedly distinct from those of the other major B cell malignancies, which typically exhibit balanced chromosome translocations. Recent advances in the characterization of both B-CLL and normal B cell subpopulations by phenotypic analysis, global gene expression profiling, as well as extensive IgV gene repertoire analyses have shed new light on the phenotype and the cell derivation of B-CLL and provided novel hypotheses concerning its pathogenesis. Here we summarize recent work relevant to these issues and conclude that B-CLL may be derived from a cell that can be referred to as a marginal zone B cell. Moreover, we propose that the lack of chromosomal translocations in B-CLL may be related to their derivation from marginal zone B cells, since somatic hypermutation and Ig class switch, the processes that generate chromosome translocations in most germinal center (GC)-derived malignancies, are no longer active in marginal zone B cells. Also, we discuss similarities and differences between B-CLL and hairy cell leukemia (HCL) and suggest that also HCL may be derived from a post-GC memory or marginal zone B cell.

Abbreviations

B-CLL	B cell chronic lymphocytic leukemia
Ig	Immunoglobulin
IgV	Immunoglobulin variable
GC	Germinal center
MZB	Marginal zone B cell
MM	Multiple myeloma
HCL	Hairy cell leukemia

1**Introduction**

B-CLL is characterized by the monoclonal expansion of mature, resting B lymphocytes that are present in the peripheral blood, bone marrow, and lymphoid organs, and by an indolent disease course that ultimately becomes lethal (Caligaris-Cappio and Hamblin 1999). In contrast to most other B cell malignancies, which typically show reciprocal balanced chromosome translocations, no specific genetic alteration has yet been associated with this disease (Döhner et al. 1999). The low proliferative rate of B-CLL cells and their prolonged life span suggests that a critical alteration might be a defect in apoptosis (Caligaris-Cappio and Hamblin 1999).

B-CLL has been the focus of intense investigations over many decades, and many studies revealed a number of surprising and unexpected findings in the light of previously obtained knowledge on healthy and transformed B lymphocytes. First, although based on the expression of the CD5 antigen on the tumor cells B-CLL had initially been thought to be derived from—namely the small subset of CD5-positive B lymphocytes (Dighiero et al. 1996)—the immunophenotype of the B-CLL tumor cells (CD5⁺, CD23⁺, CD27⁺, and low levels of surface Ig expression) is clearly distinct from that of any known normal B cell (Kipps 1998; Caligaris-Cappio and Hamblin 1999). Second, the finding that B-CLL cases can express either somatically mutated or unmutated IgV genes (Schroeder and Dighiero 1994; Oscier et al. 1997; Fais et al. 1998)—and that those subgroups have a different prognosis, since the IgV gene-unmutated B-CLL cases show a more aggressive clinical course (Damle et al. 1999; Hamblin et al. 1999)—was surprising since the tumor cells exhibit a largely homogeneous phenotype. A proposed correlation between the expression of the CD38 cell surface marker on B-CLL cells and clinical course has been controversial (Damle et al. 1999; Hamblin et al. 2000; Jelinek et al. 2001; Hamblin et al. 2002), in part due to the fact that CD38 expression levels are heterogeneous within the same tumor case and may also change over

the disease course (Hamblin et al. 2002). Third, IgV gene repertoire analyses early on suggested that B-CLL express a restricted set of IgV gene segments (Chiorazzi and Ferrarini 2003), a circumstance that invokes a possible role for antigen in the pathogenesis of B-CLL. This hypothesis found support in the high replacement to silent nucleotide exchange (R/S) ratios in IgV genes of somatically mutated B-CLL cases, which are indicative of antigenic selection (Fais et al. 1998). Finally, B-CLL exhibit various genetic lesions, mostly chromosomal deletions, many of which are specific and recurrent (Döhner et al. 1999). Most of those genomic alterations are not unique to B-CLL as they are frequently observed in other non-hematological tumors. There does not appear to be a strong correlation between a particular genetic lesion in B-CLL and either the level of IgV gene hypermutation or clinical course. Therefore, the level of IgV gene hypermutation appears as the best prognostic marker in B-CLL.

Taken together, the above observations pointed towards the existence of subgroups within B-CLL that may correspond to different B cell developmental stages. In fact, the B-CLL cases with somatically mutated IgV genes may originate from B cells that have undergone the GC-response of T cell-dependent immune responses in which B cells hypermutate their rearranged IgV genes to generate high-affinity antibody mutants (MacLennan 1994; Rajewsky 1996); whereas B-CLLs with unmutated IgV genes would be derived from the malignant transformation of antigen-inexperienced, naïve B cells. Although this widely suggested hypothesis (Oscier et al. 1997; Fais et al. 1998; Hamblin et al. 1999; Küppers et al. 1999) failed to explain the different clinical courses of IgV-unmutated and -mutated B-CLLs, it provided a reasonable explanation consistent with current concepts of B cell development (MacLennan 1994; Rajewsky 1996).

However, this hypothesis was challenged by recent studies on gene expression profiles and large-scale IgV gene repertoire analyses of clinically characterized B-CLL cases, as well as by new observations that emerged from studies on normal B cell subpopulations.

2

B-CLL Shows a Homogeneous Gene Expression Profile

Gene expression profiling using various gene chip “platforms” were performed by several laboratories during the last several years (Klein et al. 2001; Rosenwald et al. 2001; Stratowa et al. 2001; Dürig et al. 2003; Jelinek et al. 2003; Wang et al. 2004). The surprising theme that surfaced in these analyses was that all B-CLL cases displayed a common gene expression profile that

is independent of the level of IgV gene somatic hypermutation (Klein et al. 2001; Rosenwald et al. 2001) or the expression of CD38 (Dürig et al. 2003). This finding implies that B-CLL represents a homogeneous disease despite genotypic (IgV gene mutational status and genomic alterations) and clinical differences. Unsupervised hierarchical clustering or supervised analysis of phenotypically or genotypically defined subgroups in the various studies generally yielded only a small number of gene expression differences (see Sect. 3). Overall, these observations did not support the hypothesis introduced above that B-CLL reflect distinct B cell developmental stages, as, for instance, is the case for diffuse large B cell lymphoma (DLBCL) (Alizadeh et al. 2000; Shipp et al. 2002). These observations, on the other hand, are consistent with the notion that all B-CLL derive from a common cellular precursor that has been subjected to a common pathogenetic mechanism (see Sect. 4).

B-CLL gene expression profiles have been compared against (1) age-matched, peripheral blood B cells (Jelinek et al. 2003), (2) normal B lymphocytes of different developmental stages, and (3) malignant B cells of other tumor types (Klein et al. 2001; Rosenwald et al. 2001). The B-CLL gene expression signatures identified in these studies were considerably overlapping despite using different microarray platforms (Klein et al. 2001; Rosenwald et al. 2001). A further analysis by Wang et al. compared the B-CLL microarray data from the previous studies with their own expression data using a different gene chip platform (Wang et al. 2004). Their results indicate that differences between the genes in the signatures are due to the variation in the representation of genes on the microarrays, rather than to methodological or technical differences attributed to the isolation procedures or the various platforms. Similarly, a work that compared B-CLL microarray data from different laboratories using the same platform (i.e., Affymetrix oligonucleotide arrays) found the data to be highly consistent (Jelinek et al. 2003). Thus, the B-CLL signature appears to be very strong and truly different from other lymphoma subtypes, and indeed, by now a large number of genes/gene products have been verified in independent panels by polymerase chain reaction (PCR) and Northern and Western blot analyses (Rosenwald et al. 2001; Jelinek et al. 2003; and our own unpublished data). Therefore, many of the B-CLL-specific genes identified by the gene expression profiling, including the protein tyrosine kinase ZAP-70 (see Sect. [PackageErrorxmlreadUnknown crossref type seeheading](#)) and the guanosine diphosphate (GDP) exchange factor EPAC (exchange protein activated by cyclic AMP) (Klein et al. 2001; Tiwari et al. 2004), are being studied and/or used for diagnostic purposes. Another observation from the B-CLL-specific signature was that the mRNAs encoding cell cycle-associated genes were downregulated compared to all normal and transformed B cells analyzed, even below levels of normal resting B cells (Klein

et al. 2001). This finding is in accordance with the low proliferative capacity of the B-CLL tumor cells, although this type of analysis cannot exclude that a small fraction of cells may be proliferating and may not be abundant enough to confer a proliferative signature to the overall population (Chiorazzi and Ferrarini 2003).

Finally, the comparative analysis of the gene expression profiles of B-CLL with normal B cells (Klein et al. 2001; Rosenwald et al. 2001; Jelinek et al. 2003) yielded a large amount of new information to dissect the B-CLL phenotype. For instance, consistent with the known long-lived and apoptosis-resistant phenotype of B-CLL, pro-apoptotic genes were found to be downregulated and anti-apoptotic genes upregulated in the tumor cells. The upregulation of mRNA encoding several cytokine and chemokine receptors suggests that the tumor cells may react to certain stimuli abnormally compared to their normal counterparts. Clearly, the gene expression data obtained so far will prompt future studies on B-CLL and should eventually yield insights into its pathogenesis, and perhaps also identify suitable therapeutic targets.

3 B-CLL Subtypes

Perhaps the main objective of the initial microarray analyses was to identify genes whose expression is associated with a certain B-CLL subtype, defined through patient survival and disease staging (Stratowa et al. 2001), IgV mutational status (Klein et al. 2001; Rosenwald et al. 2001), or CD38-expression (Dürig et al. 2003). These approaches were meant to provide insights into the pathogenesis and derivation of the various subtypes, and the comparison of the IgV gene-mutated and -unmutated B-CLL cases was expected to provide clues about the derivation and the distinct clinical course of the subgroups. Although, as already pointed out in the previous section, IgV gene-mutated and -unmutated B-CLL cases do not separate in unsupervised hierarchical cluster analyses and exhibit a generally homogeneous gene expression profile (Klein et al. 2001; Rosenwald et al. 2001), supervised analysis of the two subgroups *did* identify a small set of genes that could be used to predict the IgV gene mutational status of B-CLL cases in independent panels (Klein et al. 2001; Rosenwald et al. 2001). This shows that although the gene expression differences between IgV-mutated and -unmutated B-CLLs are subtle, they reflect a consistent phenotypic difference between the subgroups.

Several studies investigated otherwise phenotypically (Dürig et al. 2003) or clinically (Stratowa et al. 2001) defined subtypes of B-CLL, or found evidence for subgroups among the analyzed B-CLL cases (Dürig et al. 2003; Jelinek

et al. 2003). Thus, Dürig et al. aimed at identifying differences in the gene expression profile of CD38⁺ and CD38⁻ B-CLL cases and found only very few differences in gene expression (Dürig et al. 2003). The same study identified two subgroups of B-CLLs by unsupervised clustering within the same panel; one of those subgroups reportedly comprises patients with a more favorable clinical course with longer progression-free survival and reduced chemotherapy requirements (Dürig et al. 2003). Stratowa et al. report the identification of a set of genes whose expression levels correlated with patient survival and/or clinical staging (Stratowa et al. 2001). A third group, Jelinek et al., identified a small set of genes that may distinguish between low-risk (Rai stage 0) and high-risk (Rai stage 4) patients (Jelinek et al. 2003). We would like to note, however, that although all of these studies may identify a trend, the significance of the gene expression differences within the B-CLL panels reported in the above works remains uncertain. This is because the respective sets of genes discriminating potential B-CLL subtypes were not used to generate a classifier that can predict subtypes and were not validated in independent panels for their ability to classify. As an additional complication, in two of the quoted studies (Stratowa et al. 2001; Dürig et al. 2003), B-CLL cells were not purified. In our own experience, it was absolutely necessary to purify the tumor cells in order to identify gene expression differences among the IgV-mutated and -unmutated B-CLLs by supervised analysis. The specific signatures established on purified cells could then be used to interrogate and successfully classify unpurified samples (Klein et al. 2001; Rosenwald et al. 2001). We believe that cellular contamination of the peripheral blood samples drawn from B-CLL patients, even if highly enriched for tumor cells, has a profound impact on the analysis of unpurified tumor cells, because B-CLL cells seem to contain low levels of mRNA.

Do the IgV gene-mutated versus -unmutated gene expression profiles provide new information about those B-CLL subtypes? It has been noted that the IgV gene-unmutated tumor cells, in contrast to the mutated B-CLLs, express higher levels of genes that are activated during *in vitro* activation of B lymphocytes, leading to the suggestion that the IgV-unmutated B-CLL cells have ongoing B cell receptor (BCR) signaling (Rosenwald et al. 2001). Perhaps more relevant, ZAP-70, a member of the Syk-ZAP-70 protein tyrosine kinase family involved in T cell activation, was found to be specifically associated with the unmutated B-CLL subgroup (Rosenwald et al. 2001). On the one hand this finding will undoubtedly fuel new studies on BCR signaling in this B-CLL subtype; on the other it has already led to the development of flow-cytometric assays for the detection of ZAP-70, whose expression level may be used as a surrogate for the level of IgV gene mutational levels, and in turn predict the clinical course (Crespo et al. 2003; Orchard et al. 2004). However, while these

analyses demonstrate a high level of concordance between IgV mutational status and ZAP-70 expression, the correlation is not absolute, suggesting that multiple determinants may be involved in the difference between the two subtypes of B-CLL.

Several studies comparing IgV gene mutation levels with IgV gene usage in B-CLL led to the conclusion that the expression of certain IgV gene segments correlates with their mutational status, and therefore also with clinical prognosis (this topic has been extensively reviewed by Chiorazzi and Ferrarini 2003). For example, certain V_H gene segments (e.g., *VH1-69*) occur generally in unmutated configurations, others (e.g., *VH3-07*) are strongly associated with IgV gene mutations (Chiorazzi and Ferrarini 2003). Contrary to those observations, a recent study showed that B-CLL cases that carry a rearranged *VH3-21* gene segment tend to have a poor overall survival independent of the level of IgV hypermutation (Tobin et al. 2003). In addition, these cases showed a restricted junctional repertoire, while the *VH3-21*-bearing heavy chain appeared to be predominantly associated with the expression of a particular λ light chain gene. Together, these observations, analogous to other V_H gene segments frequently observed in B-CLL (Chiorazzi and Ferrarini 2003), strongly imply a role for a common antigen in the development of *VH3-21*-bearing B-CLL cases. In turn, this implies that a B-CLL subtype can be defined by the expression of a particular V_H gene or V_H/V_L gene combination regardless of its IgV gene mutational level. Clearly, the results by Tobin et al. (2003) suggest a re-evaluation on the currently accepted subdivision of B-CLL cases: It may turn out that it is a particular antigen receptor that correlates with good or bad clinical prognosis, rather than the level of IgV somatic hypermutation in the rearranged V_H and V_L genes.

4

Cellular Derivation of B-CLL

B-CLL was long thought to be derived from the malignant transformation of $CD5^+$ B cells, which comprise a small subset of B cells in mice and humans. In favor of this hypothesis were observations made in mice that revealed that $CD5^+$ B cells have functional characteristics resembling B-CLL cells, such as long life, the capacity to replenish autonomously (Kantor et al. 1995), and the occasional ability to outgrow as a monoclonal population in old animals (Förster et al. 1988; LeMaout et al. 1999). Others argued, however, that $CD5$ expression was merely a consequence of certain activation requirements, a notion supported by the observation that $CD5$ is upregulated by $CD5^-$ B cells upon in vitro stimulation (Wortis et al. 1995). $CD5^+$ B cells in mouse were long

known to express a restricted antibody repertoire and to carry unmutated IgV genes (Kocks and Rajewsky 1989). In the human, CD5⁺ B cells were analyzed by single-cell PCR (Brezinschek et al. 1997; Fischer et al. 1997; Geiger et al. 2000) (this approach became necessary as it avoids problems imposed by differing Ig mRNA levels among B cell subsets). These studies showed that the vast majority of CD5⁺ B cells, across all ages, express unmutated IgV genes, thus mirroring the situation in the mouse. The actual IgV gene repertoire of human CD5⁺ B cells, on the other hand, does not differ from that of CD5⁻ B cells (Brezinschek et al. 1997). When it turned out that B-CLL can also express mutated IgV genes, models that invoked CD5⁺ B cells as the precursors of B-CLL had to make the assumption that IgV-mutated B-CLL are derived from CD5⁺ B cells that in rare instances proliferate and mutate their IgV genes in the GC reaction (Fischer et al. 1997). Meanwhile, putting more weight on the emerging picture that the IgV genes in unmutated B-CLLs show evidence of antigenic selection, alternative models of B-CLL pathogenesis were proposed (Oscier et al. 1997; Fais et al. 1998). These models referred to the canonical B cell developmental scheme, which states that B cells with unmutated IgV genes are naïve B cells, whereas somatically mutated B cells represent memory B cells, with the CD5⁺ B cells being part of the naïve B cell fraction. However, an important, perhaps surprising element to resolve this controversy was provided by gene expression profile analysis, which basically ruled out the CD5⁺ B cell as the principal normal counterpart of B-CLL since the gene expression profiles of B-CLL were vastly different from those derived from cord blood CD5⁺ B cells (Klein et al. 2001; Rosenwald et al. 2001). Although it remains possible that CD5⁺ B cells of newborns are different from age-matched CD5⁺ B cells, overwhelming evidence (see below) suggested that CD5⁺ B cells may not be related to B-CLL and thus may not represent the target of transformation in B-CLL.

Conversely, the comparison of the B-CLL gene expression data to B cell subset-specific signatures revealed that the gene expression profile of B-CLL, independent of the level of IgV gene mutation, was mostly related to that derived from tonsillar CD27⁺ (memory) B cells (Klein et al. 2001). These observations led us to suggest that B-CLL may be derived from the malignant transformation of CD27⁺ memory B cells (Klein et al. 2001). Nonetheless, several observations made over the last few years suggest that this CD27⁺ fraction comprises a heterogeneous set of cells. First, and unexpected from the situation in the mouse, somatically mutated B cells in humans turned out to be surprisingly heterogeneous in their Ig isotype (Klein et al. 1998; Agematsu et al. 2000) and cell surface marker expression (Dono et al. 2000; Bar-Or et al. 2001), and also in terms of their function as suggested by *in vitro* studies (Agematsu et al. 1997; Kindler and Zubler 1997; Dono et al. 2001;

Werner-Favre et al. 2001). The apparent responsiveness of IgM-expressing CD27⁺ B cells to T-independent antigens supported the suggestion that those cells might be marginal zone B cells (Dono et al. 2001; Werner-Favre et al. 2001), and thus the equivalent of murine marginal zone B cells, which are now generally accepted to comprise a separate B cell subset (Martin and Kearney 2002). Second, studies in the mouse showed that somatic hypermutation can occur outside the GC (William et al. 2002), and independent of T cells (de Vinuesa et al. 2000), although at significantly lower rates (Toellner et al. 2002); it has been proposed that also in the human, somatic hypermutation may be GC- and T-independent (Weller et al. 2001). Third, recent work on the peripheral B cell repertoire of either patients with genetic immune defects (Weller et al. 2004) or splenectomized children (Kruetzmann et al. 2003) brought up the intriguing possibility that the subset of somatically mutated IgM-expressing B cells is generated in an antigen-independent fashion, suggesting that somatic hypermutation can generate a diversified pre-immune repertoire, as is the case in sheep (Reynaud et al. 1995). IgM⁺, IgV gene-mutated cells react to T-independent antigens and may be generated by a currently unknown extrafollicular pathway in the spleen (Kruetzmann et al. 2003; Weller et al. 2004).

Based on the above observations, it appears that mutated IgV genes and CD27-expression can be found in distinct B cell subpopulations and not only in post-GC memory B cells reacting to T-dependent antigens. (Of note, while unmutated IgV genes have been observed among CD27⁺ cells, the reverse is almost never the case; CD27-negative B cells, which are generally IgM⁺IgD⁺, seem to be truly “naïve” in their Ig status.) Thus, it can be suggested that the CD27⁺ population comprises “classical” memory B cells generated in the GC-reaction, antigen-experienced cells reacting to T-independent antigens, and perhaps a somatically mutated subset that may have been generated in a both antigen- and T-independent fashion. Because all of these cells can reside in the marginal zone of the peripheral lymphoid organs, one may collectively refer to them as marginal zone B cells. CD27⁺ B cells of all isotype combinations (class switched, IgM-only, IgM⁺IgD⁺CD27⁺) have indeed been shown to respond efficiently to various activation stimuli in vitro, while CD27⁻ cells are less responsive or unresponsive (Agematsu et al. 1997; Kindler and Zubler 1997; Werner-Favre et al. 2001). Taken together, these observations suggest that while marginal zone B cells may represent developmentally distinct subsets, they may have the same function: to efficiently react to exogenous antigens and quickly differentiate into antibody-forming plasma cells.

The above-discussed features make marginal zone B cells, derived from either T-dependent or T-independent developmental stages, strong candidates for a normal counterpart of B-CLL. A large fraction of B-CLL expresses

somatically mutated IgV genes, and CD27 is commonly expressed on the tumor cells (van Oers et al. 1993). While B-CLL cases are mainly IgM⁺IgD⁺, they can also be IgM only and class switched (Chiorazzi and Ferrarini 2003). The restricted IgV gene repertoire in both unmutated and mutated cases, as well as the evidence of antigenic selection in the mutated cases by R/S-values, suggests that antigen-stimulation plays a role at some point in B-CLL pathogenesis (discussed by Chiorazzi and Ferrarini 2003). Taken together, this evidence suggests that B-CLL predominantly originates through malignant transformation of marginal zone B cells (Fig. 1).

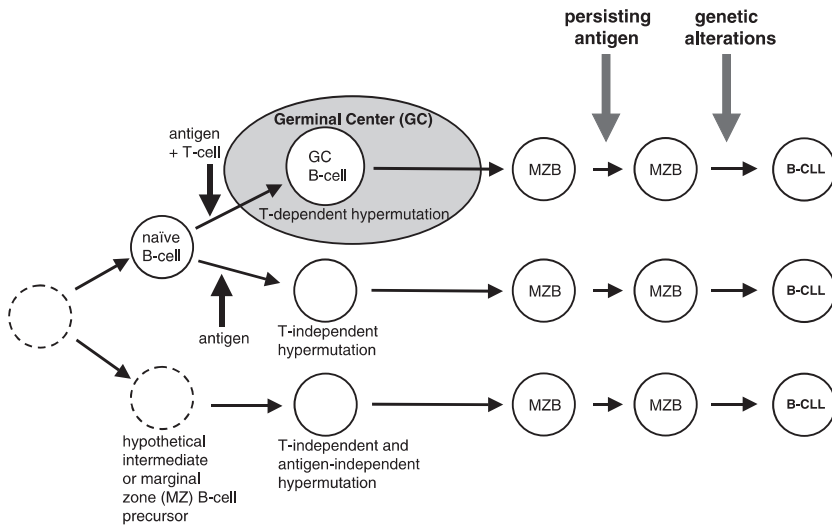


Fig. 1 A model for the cellular derivation of B-CLL. Antigen-inexperienced, naïve B cells may be driven into either a T-dependent or T-independent immune response (*top two branches*). A separate pathway (*bottom branch*) has been proposed recently in which somatically mutated B cells are generated in an antigen-independent fashion, suggesting that somatic hypermutation can generate a diversified pre-immune repertoire (see text). Upon completion of either the T-dependent GC response or the T-independent response(s), which may take place in a currently unknown extrafollicular pathway, the cells differentiate into marginal zone B cells (MZBs). MZBs may be continuously activated through persisting antigen and acquire genetic alterations that eventually lead to B-CLL development. *MZB*, marginal zone B cell; *GC*, germinal center

5

B-CLL Pathogenesis

The observation that B-CLL is more related to memory or marginal zone B cells than to any other known normal B cell subset suggests that the process leading to the clonal expansion may initiate in these cells. This notion is supported by the particular cytogenetic profile of B-CLL in relation to other B cell malignancies that are thought to originate from the malignant transformation of a GC-derived B cell. The GC-derived group of tumors, comprising e.g., DLBCL, follicular lymphoma (FL), Burkitt lymphoma (BL), and multiple myeloma (MM), are characterized by extensively mutated IgV genes and by the occurrence of specific reciprocal balanced chromosome translocations. Many of these translocations involve Ig loci, and the location of the breakpoints within the Ig loci suggest that the genomic alterations can be the result of mistakes in the recombination of the V, D, and J gene segments, Ig class switching, or IgV somatic hypermutation (reviewed by Küppers and Dalla-Favera 2001). The ongoing somatic hypermutation observed in FL and subsets of BL and DLBCL is suggestive of the tumor cells originating from a bona fide GC B cell. For the post-GC tumor MM, the malignant plasma cell equivalent, the discovery of pre-switch B cells clonally related to the MM cells suggested that the precursor cell preceding the MM clone might be a GC B cell (Corradini et al. 1993; Taylor et al. 2002). In contrast, despite showing IgV somatic mutations in a fraction of cases, B-CLL do not typically show reciprocal or other specific chromosome translocations (Döhner et al. 1999), although some exceptions have been reported (Döhner et al. 1999; Buhmann et al. 2002). Instead, the major genomic alterations are deletions and amplifications. This observation, together with the marginal zone B cell-like phenotype (see Sect. 4, above), suggests that B-CLL may lack chromosome translocations because the mechanisms involved in these aberrations, somatic hypermutation and Ig class switch, are no longer active in these cells (Fig. 2).

As to what may be the transforming mechanisms leading to B-CLL development, this is an open question and out of the scope of this review. Briefly, B-CLL is characterized by only a few common chromosome abnormalities including an association with 13q14 deletions that is present in around 50% of cases, depending on the panel studied (Corcoran et al. 1998; Döhner et al. 1999; Mabuchi et al. 2001; Migliazza et al. 2001). These deletions are thought to reflect the inactivation of an as-yet-unknown tumor-suppressor gene. Generally, the homogeneous gene expression profile of B-CLL suggests that its pathogenesis is associated with a largely common mechanism of transformation.

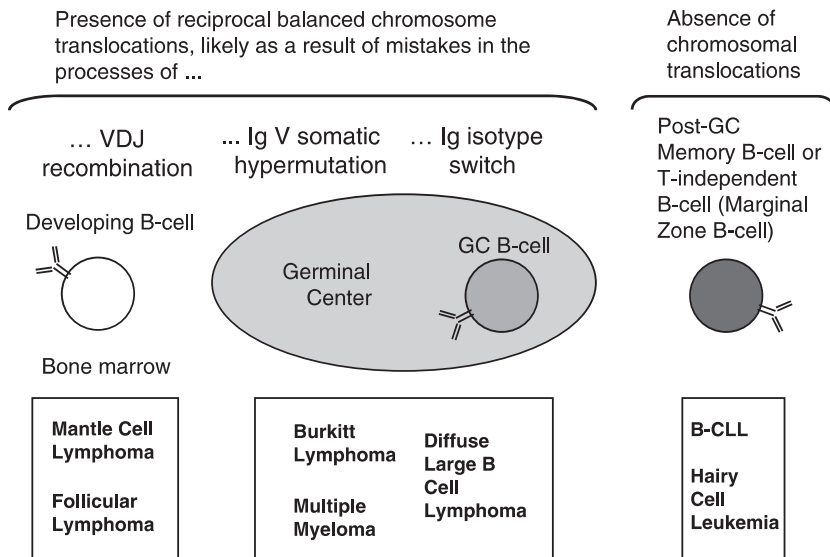


Fig. 2 Genetic aberrations and Ig-modifying processes. The major types of non-Hodgkin lymphoma, including mantle cell lymphoma, follicular lymphoma, Burkitt lymphoma, diffuse large B cell lymphoma, and multiple myeloma, are characterized by the presence of reciprocal balanced chromosome translocations that are likely a result of mistakes during VDJ recombination, IgV somatic hypermutation, or Ig isotype switch. On the other hand, B-CLL and hairy cell leukemia (HCL) lack such translocations, suggesting that the mechanisms involved in these aberrations are no longer active in the B-CLL and HCL precursor cells

A number of recent large-scale IgV gene repertoire analyses strongly imply the role for antigen in the development of B-CLL. Not only are particular IgV_H gene segments over-represented in antibodies expressed by B-CLLs (Chiorazzi and Ferrarini 2003), but there is also strong evidence for selection of particular IgV_H and light chain combinations (Ghiotto et al. 2004; Kolar and Capra 2004), and subgroups of B-CLL show similar CDRIII regions of light and/or heavy chains (Widhopf et al. 2004). These observations may suggest that the binding of specific antigen receptors to particular foreign antigens or autoantigens provides a continuous stimulatory signal through the BCR that keeps the B-CLL precursor in cell cycle for long periods of time and eventually allows for the acquisition of genomic aberrations.

Finally, we would like to introduce the possibility that B-CLL and hairy cell leukemia have a similar cell of origin. We have recently reported that HCL, whose phenotype is markedly distinct from B-CLL and any other lymphoma

subtype (Harris et al. 1994), also resembles in its gene expression profile the CD27⁺ B cells (HCL also expresses CD27) (Basso et al. 2004). As is the case for B-CLL, HCL lack chromosomal translocations (Haglund et al. 1994; Sambani et al. 2001). The fraction of cases expressing somatically mutated IgV genes, however, is higher in HCL compared to CLL (Maloum et al. 1998; Forconi et al. 2001). Perhaps B-CLL and HCL are commonly derived from the malignant transformation of a similar cellular precursor, a CD27⁺ marginal zone or a memory B cell (Fig. 1). The different transformation processes acting on the precursor cells would determine their distinct phenotype and clinical presentation. Alternatively, the two tumor entities could be derived from the malignant transformation of distinct subsets of CD27⁺ B cells (see previous section). In fact, a possible relationship between HCL and a particular tonsillar, somatically mutated B cell subpopulation (phab V-3⁺, CD27⁺, CD11c⁺, CD23⁻) has been noted (van Der Vuurst De Vries and Logtenberg 1999; Basso et al. 2004). Nonetheless, it might turn out that a subset of B cell malignancies including B-CLL and HCL is derived from a “group” of cells that was specifically generated for one purpose in immunity: to quickly establish an antibody-mediated immune response to foreign antigen.

6

Summary and Conclusions

Independent of their IgV gene mutational status, B-CLL cases resemble in their gene expression profile CD27⁺ B cells, which represent a heterogeneous cell population with predominantly somatically mutated IgV genes, and mostly localize in the marginal zone or marginal zone equivalents. Clearly, the precise origin of B-CLL within this heterogeneous group of cells remains to be established. The identification of a subgroup of B-CLL cases with a particular V_H/V_L combination showing a generally unfavorable clinical course independent of the IgV mutational status suggests that the separation of B-CLL cases into IgV-unmutated and -mutated subgroups, and their association with good and bad prognosis, might have to be revised to include a critical role for the antigen. The absence in B-CLL of balanced reciprocal chromosome translocations that have been associated with mistakes in VDJ recombination, Ig class switching and somatic hypermutation suggest that the multistep transformation leading to B-CLL begins in a cell where these mechanisms are no longer active, consistent with a memory/marginal zone derivation. While global gene expression profile analysis has provided new insights into the phenotype and cell derivation of B-CLL, several gene products identified in those analyses might turn out to be targets for improved diagnosis and

therapy. Unfortunately, the genetic alterations that are associated with the pathogenesis of this disease are still obscure.

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