High Frequency of Somatic Mutations in the V<sub>H</sub> Genes Expressed in Prolymphocytic Leukemia

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Prolymphocytic leukemia (PLL) is a chronic lymphoproliferative disorder, characterized by prominent splenomegaly, prolymphocytes accounting for more than 55% of circulating lymphocytes, and short-term survival. To better characterize the nature of the cellular origin in this disease, we analyzed Ig heavy chain variable region (V<sub>H</sub>) genes in eleven cases of de novo PLL. Leukemic cells expressed a skewed repertoire characterized by predominant use of the V3 family members (73%), with preferential use of the V3-23 gene (50% of the V<sub>H</sub>3 genes). All sequences from expressed V<sub>H</sub> genes diverged from their putative germline counterpart, and in eight cases the divergence was greater than 5%. In seven cases, which expressed the V3-23 gene and V<sub>H</sub>4 family members, nucleotide substitutions could be confidently attributed to somatic mutations. The type and distribution of these mutations clearly indicated that in three cases the cells had been subjected to an antigen selection process. Taken together, these results suggest that B-PLL cells display a skewed repertoire of Ig V<sub>H</sub> regions and probably represent, at least in some instances, expansion of postgerminatal center cells that have undergone antigen driven selection.

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with the following monoclonal antibodies: CD19(B4), CD20(B1), CD10(I5), CD23(B6) (Coultronix, Margency, France); CD5(OK23), anti-Kappa, anti-Lambda, anti-IgM (Dako, Trappes, France); anti-IgD and anti-IgA (Pasteur Diagnostic, Marnes-la Coquette, France); anti-IgG (Eurobio, Les Ulis, France); FMC7 (Immunotech, Marseille, France).

Amplification of tumor cell variable region heavy chain genes. High molecular weight DNA was extracted from leukemic cells using standard procedures. Amplification of rearranged V_H genes was performed in a two-step procedure. In the first step, 1 μg of genomic DNA was amplified with a 5' consensus primer designed to anneal to framework (FR) I of most V_H genes except members of the V_H2 family: AGGTGCAACGTSWGSAGTCGG, a V_H2 family-specific primer: CAGGTCAAGX5TGGAGTCTGG, and an external 3' consensus JH primer: ACCTGAGGAGACGTGAC-CRKKGT. In the second step 2.5 μL (5%) of this first polymerase chain reaction (PCR) reaction was reamplified in six independent reactions using internal FR1 V_H family-specific primers: V_H1: TGCGATCTCCTGAGGTYTTCCTC; V_H2: GAAGATCTCACA-XCTGACCTGCACKTC; V_H3: GAAGATCTCTCTCGAGGCC-TCTGG; V_H4: GAAGATCTCCTCACCCTGCRYTG; V_H5: CCAGATCTCCTGAAAGGTTCTGG; V_H6: GAAGATCTCTCACA-XCTCACCCTGCGG; V_H7: CTGGACCTGCACKTC; v_H3: GAAGATCTCTCCAESCCG; v_H4: TGGGTCACCITGARGGCTGG; v_H5: GAAGATCTCAC~GAGAGACCGTGC; v_H6: GAAGATCTCCTCCACCCTGCACGCGG; v_H7: CTGGACCTGCACKTC.

Cloning and sequencing of PCR products. PCR products from the FR2 and LH amplifications were cloned directly into the pCR-Script vector (Stratagene). Clones harboring restriction analysis, were sequenced using the dideoxy chain termination method with T7 DNA Polymerase (Stratagene, La Jolla, CA). In one case, no PCR product could be obtained using this procedure and amplification was, therefore, performed using a T7 DNA Polymerase (Pharmacia, Uppsala, Sweden) computer Group, Inc (Madison, WI) software package and the Genebank (Los Alamos, NM) and EMBL (Heidelberg, Germany) databases.

RESULTS

Patients and PLL cells. Eleven patients with clinical and morphological features of de novo PLL were included in this study. The main phenotypic characteristics are listed in Table 1. Lymphocytosis ranged from 9 × 10^9/L to 720 × 10^9/L. The proportion of prolymphocytes varied from 59% to 98%. Three cases expressed IgM only, five cases both IgM and IgD, one case IgG, and one case IgA. The Ig heavy chain (IgH) isotype was not determined in one case. As expected, high-density membrane slg, as estimated by flow cytometry, was found in most cases (9 of 10). All cases tested expressed CD19 and FMC7. CD23 was detected in only two cases, and CD5 was present in six cases (55%). In all cases, two Ig heavy chain gene rearrangements were detected by Southern blot analysis (data not shown).

V_H gene usage in PLL. Fourteen different V_H sequences were amplified by DNA PCR from the 11 patients studied. Deduced amino acid sequences are shown in Fig 1. Eleven of these sequences represented productive rearrangements (one for each patient), while nonproductive rearrangements were also obtained in three cases (PLL-1b, PLL-2b, and PLL-6b). These nonfunctional IgH genes were the results of out of frame rearrangements (PLL-1b, PLL-6b) or had stop codons in their CDR3 sequences (PLL-2b). Identification of the V_H genes is shown in Table 2. Of the productive rearrangements, a V_H3 gene was found eight times and a V_H4 gene three times. The V3-23 (V_H26) gene was used with a surprisingly high incidence, as it constituted half of these eight V_H3 genes and 36% of all productive rearrangements. The other four productive V_H3 genes consisted of V3-74, V3-72, V3-48, and V3-7. The three other functional genes were members of the V_H4 family and consisted of V4-34 (V_H4.21) in two cases and V4-59 (V_H4.11) in one case. One V_H1 gene (V1-2), one V_H2 gene (VII-5), and one V_H3 gene (V3-13) were used in the nonproductive rearrangements.

Presence of mutations in V_H genes. Sequences were compared with those of the germline V_H genes available in current databases (see Materials and Methods and Fig 1). Most sequences obtained diverged substantially from their closest germline counterparts, except for the nonproductive V_H3 gene (PLL-2b; Table 2). The percentage homology varied from 86.7% to 99.6%, with only two sequences showing more than 98% homology. This divergence could be due to somatic mutation, polymorphism or to previously unidentified germline sequences. This latter explanation is unlikely since most, if not all V_H germline genes have been identified. Polymorphism is well recognized in the case of the V_H3 genes, which represent the largest V_H family. Many "mutations" detected in the PLL V_H3 gene sequences might therefore correspond, in fact, to polymorphic variations. However, it should be noted that divergence from the germline sequence also extended to the D and JH gene segments (Fig 2) in some cases (PLL-5, 6a, 7, 9). Formal delineation of somatic mutations from polymorphic variations requires the isolation of the germline V_H gene counterpart from nonleukemic cells. Because this material was not available in most of our PLL cases, the following approach was undertaken. (1) We restricted our analysis to the seven cases expressing either the V3-23 or the V_H4 family genes, for which polymorphism has been studied extensively. In the case of the V3-23 gene, polymorphisms have been shown to result from either deletion/insertion of gene copies, or from sequence differences at endonuclease restriction sites in the gene's flanking regions, but not in the coding region itself. Members of the V_H4 family are known to display very little polymorphism. (2) We performed a computer analysis with over 1,500 Ig gene sequences deposited in databases (Genbank, EMBL, VBase). The original sequence and possi-


Table 1. Biological Features and Immunophenotype of 11 Cases of PLL

<table>
<thead>
<tr>
<th>Patients</th>
<th>Lymphocytes (x 10^6/L)</th>
<th>%PL</th>
<th>sig</th>
<th>CD19</th>
<th>CD20/CD19</th>
<th>CD23</th>
<th>FMC7</th>
</tr>
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<tbody>
<tr>
<td>PLL-1</td>
<td>18</td>
<td>59</td>
<td>IgMDx (S)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>PLL-2</td>
<td>720</td>
<td>98</td>
<td>IgGx (L)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>PLL-3</td>
<td>115</td>
<td>95</td>
<td>IgMDx (S)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>PLL-4</td>
<td>21</td>
<td>66</td>
<td>IgMx (S)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>PLL-5</td>
<td>11</td>
<td>78</td>
<td>IgMDx (S)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>PLL-6</td>
<td>9</td>
<td>70</td>
<td>IgAx (S)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>PLL-7</td>
<td>22</td>
<td>65</td>
<td>IgAx* (S)</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>PLL-8</td>
<td>100</td>
<td>76</td>
<td>IgMDx (S)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>PLL-9</td>
<td>600</td>
<td>96</td>
<td>IgMx (ND)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PLL-10</td>
<td>83</td>
<td>75</td>
<td>IgMx (S)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PLL-11</td>
<td>85</td>
<td>65</td>
<td>IgMDx (S)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Abbreviations: PL, prolymphocytes; S, strong intensity; L, low intensity; ND, not determined.

* Heavy chain isotype not determined.

Fig 1. Deduced amino acid sequences of the heavy chain variable regions of 11 cases of PLL (productive and nonproductive rearrangements). The upper sequences correspond to the closest germline V_H genes; *, stop codon; **, out of frame D to JH rearrangement; the portion of the CDR3 corresponding to the JH segment is not shown in these cases. The corresponding nucleotide sequences have been contributed to the EMBL database.
sequence. (3) To further confirm the identity of the corresponding germline \( V_n \) gene, we analyzed the leader (LH) intron sequence in the selected seven cases. These sequences are known to be specific for individual \( V \) genes and less somatically mutated than the coding regions.\(^{26}\) Using LH family-specific primers for PCR, we isolated clones carrying CDR3 sequences identical to those previously obtained with FR1 or FR2 primers. PLL-2a, 4, 6a, and 11 had intron sequence strictly identical to that of their corresponding germline gene, while PLL-3 and 9 had one nucleotide mismatch and PLL-5 two nucleotide mismatches (data not shown).

Taken together, these results indicate that in these seven PLL cases, the putative germline \( V_n \) gene counterpart had been correctly identified, and that most of the observed nucleotide substitutions could be attributed to somatic mutations with confidence. Of note, two of the three nonproductive genes (PLL-1b and 2b) were very close to their germline counterpart (98.4% and 99.6% homology, respectively), while one (PLL-6b) diverged substantially (95.6% homology).

**Distribution pattern of mutations.** The role of antigenic selection can be deduced from the distribution of somatic mutations in \( V \) regions. Considering the respective length of CDRs and FRs, a random distribution of mutations should give a ratio of CDR to FR mutations of less than 0.3.\(^{27}\) When considering only the seven cases for which nucleotide substitutions could be attributed to somatic mutations with most certainty and excluding nucleotide substitutions possibly contributed by polymorphism, all had a CDR:FR ratio above 0.3, ranging from 0.6 to 2, indicating a nonrandom distribution of mutations (Table 2). Furthermore, when analyzing the type of nucleotide substitutions, a random pattern of mutations gives a ratio of replacement (R) mutations to silent (S) mutations of 2.9 or less.\(^{28}\) In contrast, in cases undergoing antigenic selection, the R:S ratio is higher than 2.9 in the CDRs, whereas it is below this value in the FRs. As can be seen in Table 2, five cases (PLL-3, 4, 5, 6a, and 11) had an R:S ratio equal to or above 3 in their CDRs, while applying a binomial model,\(^{29,30}\) the R:S ratio values in the CDRs reached significance \((P < .05)\) for only three cases (PLL-5, 6a, and 11) and was just above the threshold for one case (PLL-4) (Table 3). These results strongly suggest that in these cases, the leukemic cell progenitors had been subjected to an antigenic selection process. There were no clearcut differences in the frequency of mutations between cases expressing slgM, slgM and slgD, slgD, slgG, or slgA. Similarly, a roughly equivalent number of mutations was found in CD5\(^+\) and CD5\(^-\) cases.

**Intracloidal diversity.** In each case studied, four to six sequences were obtained from independent PCR. Single point mutations were occasionally detected within different clones for the same patient, but with a low frequency (1 of 1,200). To determine whether this was due to intracloidal diversification or to nucleotide misincorporation by the \( Pfu \) polymerase, we performed an identical amplification and analysis of the \( V_n \) genes in two cases of ALL which had previously been sequenced in our laboratory.\(^{30}\) Analysis of more than 6,000 base pairs showed only one nucleotide difference. It is, therefore, possible that the rare nucleotide differences observed in our cases of PLL might correspond to intracloidal diversity.

**D and JH gene usage.** Analysis of JH genes (Fig 2) showed that four cases used a JH3 gene segment, four a JH4 gene segment, only one a JH5 gene segment, and four a JH6 gene segment. In one case (PLL-1a), the corresponding JH gene could not be identified because of extensive removal of its \( S^r \) region during the D to JH recombination. No preferential usage of D segment could be observed. As noted in a previous section, one or two point mutations were present in the D and JH segments for some patients.

**DISCUSSION**

Analysis of Ig genes variable regions in B-cell tumors has provided important information on these cells, both in terms of their development and function. The analysis of somatic mutations and antigenic selection has provided insights into the clonal diversity and selection pressures acting on these cells. The role of random somatic mutation and antigen-driven selection in generating diversity and specificity in the B-cell repertoire has been extensively studied. The results presented here support the notion that somatic hypermutation and antigen-driven selection play important roles in the development of PLL. The distribution of mutations, the ratio of CDR to FR mutations, and the variability of JH usage suggest that these cases were subject to antigenic selection. The high frequency of nucleotide substitutions, particularly in the CDRs, indicates a high level of somatic hypermutation, which is consistent with the postulated role of PLL in the immune response.

**Table 2. Characteristics of PLL \( V_n \) Region Sequences**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Family</th>
<th>GL Counterpart</th>
<th>Homology</th>
<th>Mutations*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLL-1a</td>
<td>V(_n)3</td>
<td>V3-74</td>
<td>92.4</td>
<td></td>
</tr>
<tr>
<td>PLL-1b</td>
<td>V(_n)2</td>
<td>V3-80</td>
<td>92.4</td>
<td></td>
</tr>
<tr>
<td>PLL-2a</td>
<td>V(_n)4</td>
<td>V3-74</td>
<td>95.6</td>
<td></td>
</tr>
<tr>
<td>PLL-2b</td>
<td>V(_n)3</td>
<td>V3-12</td>
<td>99.6</td>
<td></td>
</tr>
<tr>
<td>PLL-3</td>
<td>V(_n)4</td>
<td>V4-59</td>
<td>97.3</td>
<td></td>
</tr>
<tr>
<td>PLL-4</td>
<td>V(_n)4</td>
<td>V3-23</td>
<td>98.2</td>
<td></td>
</tr>
<tr>
<td>PLL-5</td>
<td>V(_n)3</td>
<td>V3-23</td>
<td>89.1</td>
<td></td>
</tr>
<tr>
<td>PLL-6a</td>
<td>V(_n)3</td>
<td>V3-23</td>
<td>94.9</td>
<td></td>
</tr>
<tr>
<td>PLL-6b</td>
<td>V(_n)1</td>
<td>V1-2</td>
<td>95.6</td>
<td></td>
</tr>
<tr>
<td>PLL-7</td>
<td>V(_n)3</td>
<td>V3-72</td>
<td>86.7</td>
<td></td>
</tr>
<tr>
<td>PLL-8</td>
<td>V(_n)3</td>
<td>V3-48</td>
<td>94.9</td>
<td></td>
</tr>
<tr>
<td>PLL-9</td>
<td>V(_n)3</td>
<td>V3-23</td>
<td>97.6</td>
<td></td>
</tr>
<tr>
<td>PLL-10</td>
<td>V(_n)3</td>
<td>V3-7</td>
<td>94.1</td>
<td></td>
</tr>
<tr>
<td>PLL-11</td>
<td>V(_n)4</td>
<td>V4-34</td>
<td>95.9</td>
<td></td>
</tr>
</tbody>
</table>

When all mutations induced replacements (S = 0), results are expressed as > number of replacements observed.

Abbreviations: GL, germline; ORF, open reading frame throughout the VH-DJH region.

* Nucleotide substitutions corresponding to possible polymorphism were not taken into account.
of repertoire usage and the presence of somatic mutations. This has allowed the comparison of tumor cells and their normal counterpart within the B-cell development pathway and evaluation of the stage of clonal expansion. Several such studies have been performed in CLL. In CD5+ CLL, developmentally restricted Ig genes, which are often associated with antiidiotype cross-reactivity, are preferentially used. At least 50% of these CLL express germline VH genes, suggesting that they represent expansion of pregerminal center B cells. However, the rare CLL cases expressing IgG isotypes tend to use more frequently mutated VH genes, with evidence for antigen selection in some cases.

Little is known about VH repertoire in PLL, in part, because of the relative rarity of the disease. Previous clinical, morphological and immunological studies have shown that it represents a separate entity. However, it may bear some relationship with CLL, because the latter can transform into true PLL.

In this study, we have analyzed Ig heavy chain gene sequences in 11 cases of PLL. VH expression was characterized by a distinct overrepresentation of the largest VH3 family (73% of the productive rearrangements), and a clear under-representation of VH1 (7%), the second largest family. Similar findings have been reported in CD5+ CLL. There was an obvious skewed use of individual VH3 genes, because one gene, V3-23, accounted for 50% of all productive IgH genes. Similarly, the V4-34 gene represented two of the three VH4 family genes expressed. Taken together, these two genes accounted for more than half of the PLL repertoire. They have been identified in auto antibodies with anti-DNA activity and specificity for the erythroid I/i antigen (V3-23) and (V4-34). They are
Fig 3. Nucleotide sequence comparisons of the VH gene V3-23 with its polymorphic varied and our PLL cases. Regions corresponding

to the CDR1 and CDR2 are underlined. Identical nucleotides are shown as dots. Polymorphic variants are aligned above the original sequence.

PLL-3 FR 5 2 3 2
PLL-2a FR 8 2 3
PLL-9 FR 4 1 2 2
PLL-5 FR 7 2 2 4
PLL-6a FR 3 2 1
PLL-4 FR 2 3 2
PLL-6a FR 2 3 2
PLL-9 FR 2 3 2
PLL-11 FR 2 3 2

% Homology

A95 GL16 A73 VE26 A77 V3 -23 PLL-4 PLL-5 PLL-6a PLL-9

A95 GL16 A73 VE26 A77 V3 -23 PLL-4 PLL-5 PLL-6a PLL-9

Table 3. Distribution of Somatic Mutations in PLL VH Genes

<table>
<thead>
<tr>
<th>Patient</th>
<th>Location</th>
<th>Expected</th>
<th>Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLL-2a</td>
<td>FR</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>PLL-3</td>
<td>FR</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>PLL-4</td>
<td>FR</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>PLL-5</td>
<td>FR</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>PLL-6a</td>
<td>FR</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>PLL-9</td>
<td>FR</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>PLL-11</td>
<td>FR</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>CDR</td>
<td>2</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

P is the probability that the number of observed replacement mutations in the CDR would occur by chance alone. It was calculated using a binomial distribution model. In the case of PLL-9, no R mutation occurred in the CDR and, therefore, P refers to the occurrence of R mutation in the FR.

overrepresented in the fetal and adult normal repertoires, as well as in lymphoid tumors such as CLL. The reasons for the overexpression of these genes remain unclear. Several explanations have been proposed, including the occurrence of several genomic copies, particular regulatory regions and selection through certain antigen binding or idiotypic determinants. In the case of the V3-23 gene, the fact that the same degree of overexpression has been observed in both productive and nonproductive rearrangements of normal B cells favors an intrinsic genetic mechanism. Analysis of JH gene usage showed not only predominant use of the JH4 and JH6 genes, but also of the JH3 gene, a feature reminiscent of the fetal repertoire. Apart from two nonproductive rearrangements, all PLL sequences analyzed here diverged substantially (>2%) from their putative germline counterparts. Restricting our analysis to the cases where polymorphism could be ruled out with certainty, ie, the V3-23 and VH4 genes, this divergence could be attributed to somatic mutations. This conclusion is further strengthened by the detection of point mutations in the corresponding D and JH gene segments for some cases. In three and possibly four instances, there was clear evidence that
these mutations appeared to have been antigen selected, with an accumulation of R over S mutations in the CDRs. The very low rate of intraclonal heterogeneity indicates that the mutational machinery was still minimally active. A similar low rate of somatic diversification has been reported in cases of CLL,\textsuperscript{41,42} a disease classically known for its absence of intraclonal diversity, and is much lower than that observed in FL.\textsuperscript{12,14} Overall these results show that the clonal expansion in PLL occurred in a progenitor cell that had already encountered an antigen. This pattern resembles that observed in some cases of IgG\textsuperscript{+} CLL,\textsuperscript{30} of Burkitt's lymphoma\textsuperscript{30} (BL), and of monoclonal gammopathy of undetermined significance.\textsuperscript{44} It contrasts with FL where the tumor cells can still undergo important clonal evolution due to numerous ongoing somatic mutations.\textsuperscript{12,14}

Of the seven cases of PLL expressing clearly mutated genes, three expressed surface IgM and IgD (PLL-3, 5, and 11), two IgM (PLL-4 and 9), one IgG (PLL-2), and one IgA (PLL-6). There is now good evidence that both normal and tumor IgM\textsuperscript{+} B cells can be subjected to somatic mutations.\textsuperscript{41,43-47} More intriguing is the finding of mutations in the IgM\textsuperscript{+} IgD\textsuperscript{+} cells, which correspond to a more immature population. Indeed, these cells, which represent the major B-cell subset in the peripheral blood, express Ig light chain V\textsubscript{K} genes with no or little mutations.\textsuperscript{47} In keeping with this, a recent study on tonsillar B cells has shown that somatic mutations tend to appear when B cells lose their slgD.\textsuperscript{58} Similarly, we have shown in a previous study\textsuperscript{32} that IgM\textsuperscript{+} IgD\textsuperscript{+} CLL express V\textsubscript{H} genes with a lower mutation rate than IgM\textsuperscript{+} CLL (1.9 % vs 5.4%). There were, however, some exceptions as one IgM\textsuperscript{+} IgD\textsuperscript{+} case had a 4.6% rate of mutation. IgM\textsuperscript{+} IgD\textsuperscript{+} tumor cells with somatic mutations (up to 5%) have also been described in splenic lymphomas with villous lymphocytes\textsuperscript{46} and BL.\textsuperscript{45} All of these cases may therefore represent transformation of cells belonging to a rare normal B-cell population, in which the process of somatic mutations operates at an immature stage of development. Alternatively, the transformation event may result in aberrant IgD expression or in alteration of the isotype-switch process.

CD5\textsuperscript{+} B lymphocytes are postulated to constitute a separate B-cell lineage that predominates in early ontogeny, expresses a germline repertoire of Ig genes, and is mainly committed to secreting natural antibodies.\textsuperscript{49} Analyses of individual cells from human germinal centers have shown that the majority of B cells from the mantle zone, which express CD5, display unmutated Ig genes, whereas germinal center B cells Ig genes are extensively mutated.\textsuperscript{50} Studies of CD5\textsuperscript{+} malignancies, namely CLL\textsuperscript{50} and MZL,\textsuperscript{51} are consistent with this pattern. CD5\textsuperscript{+} CLL can, however, display mutated V\textsubscript{H} genes, and this seems to be related with slg isotype expression since the mutation rate was found to be higher in IgM\textsuperscript{+} and particularly IgG\textsuperscript{+} CLL than in IgM\textsuperscript{+} IgD\textsuperscript{+} CLL.\textsuperscript{51,52} About half of the PLL studied here did not express CD5 and the mutation rate in these cases was not significantly different from the CD5\textsuperscript{+} cases. Our data, therefore, confirm that CD5 expression is not restricted to pregerminatal center cells.

A recent study including six cases of PLL failed to detect any mutations in the V\textsubscript{K} sequences of these tumors.\textsuperscript{51} The reasons for this discrepancy with our results are not clear, although several cases have been reported in which the light chain V genes remained germline or minimally mutated, whereas the V\textsubscript{H} genes were mutated. These include several CLL,\textsuperscript{64,65} a case of mixed cryoglobulinemia,\textsuperscript{66} cells expressing autoantibodies to the acetylcholine receptor,\textsuperscript{67} and a murine secondary immune response to influenza virus hemagglutinin.\textsuperscript{68} It is, therefore, possible that in some situations the mutational machinery affects heavy and light chain genes independently, or that certain light chain genes are protected against this process. Alternatively, PLL might be an heterogeneous disease with regard to the presence of somatic mutations.

Overall our results show that PLL cells, at least in some cases, resemble postgerminatal center cells that have been selected by antigens. Whether some unidentified antigen plays a direct role in the development of the tumor remains to be elucidated.

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