Similarities and Differences Between the Light and Heavy Chain Ig Variable Region Gene Repertoires in Chronic Lymphocytic Leukemia

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Analyses of Ig VDJH rearrangements expressed by B-CLL cells have provided insights into the antigen receptor repertoire of B-CLL cells and the maturation stages of B-lymphocytes that give rise to this disease. However, less information is available about the L chain V gene segments utilized by B-CLL cells and to what extent their characteristics resemble those of the H chain. We analyzed the VL and JL gene segments of 206 B-CLL patients, paying particular attention to frequency of use and association, mutation status, and LCDR3 characteristics. Approximately 40% of B-CLL cases express Vκ genes that differ significantly from their germline counterparts. Certain genes were virtually always mutated and others virtually never. In addition, preferential pairing of specific VL and JL segments was found. These findings are reminiscent of the expressed VH repertoire in B-CLL. However unlike the Vκ repertoire, Vλ use was not significantly different than that of normal B-lymphocytes. In addition, Vκ genes that lie more upstream on the germline locus were less frequently mutated than those at the 3’ end of the locus; this was not the case for Vλ genes and is not for VH genes. These similarities and differences between the IgH and IgL V gene repertoires expressed in B-CLL suggest some novel features while also reinforcing concepts derived from studies of the IgH repertoire.

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INTRODUCTION

Immunoglobulin (Ig) variable (V) domains are the components of the B-cell antigen receptor (BCR) that interact with antigen. Understanding the gene segments that encode these domains can provide indirect information about the structure of the BCR. In addition, somatic changes that occur in these genes can suggest clues regarding the maturational history of a B lymphocyte.

These principles have been of special value in understanding the biology of the B lymphocytes that become leukemic in B-cell chronic lymphocytic leukemia (B-CLL). For example, analyses of VHDJH rearrangements expressed by these clones (1–3) has led to the recognition that B-CLL cases segregate into subgroups based on the presence or absence of mutations in VH genes, i.e., mutated and unmutated B-CLL, respectively (4,5). This division has considerable prognostic significance (6,7). Patients in the Ig VH mutated subgroup have a relatively benign clinical course. These individuals can live for many years after diagnosis (10–25 years), usually do not require therapy, and often die with the disease, not because of it. In contrast, patients in the unmutated subgroup follow a much more aggressive clinical course; these people have a median survival of less than 8 years, despite extensive therapeutic efforts which may quell but do not cure the disease. B-CLL remains incurable.

Furthermore, these studies suggest that the leukemic cells from both the mutated and unmutated subgroups are anti-
gen-experienced B-lymphocytes. Observations on the surface profiles (8) and the gene expression profiles (9,10) of the leukemic cells corroborate this notion. Finally, biased use of V$, \mu$, D, and J, gene segments suggest that the antigenic epitopes responsible for this activated and antigen-experienced/memory state are limited in nature. An alternative, not mutually exclusive, explanation, is that the normal B-cells from which B-CLL cells derive are markedly restricted in their antigen-binding repertoire, either genetically or due to prior antigen selection (1,3,16,17).

Thus, considerable basic as well as clinical information has been gleaned from studying the rearranged VHDJH segments available about the gene segments that make up the V domains of the Ig L chains of B-CLL cells and the extent to which the characteristics of these segments resemble those of the H chain (20). Therefore, it is not resolved if conclusions about the immunobiology of B-CLL cells that were derived from H chain data are recapitulated by the rearranged V,JL segments. In this study, we analyzed the DNA sequences of VL,JL rearrangements of 206 patients with B-CLL; 179 of these patients had expansions of CD5+/CD19+ B cells and 27 B-CLL; 179 of these patients had expansions of CD5+/CD19+ B cells and 27 patients displayed expansions of CD5+/CD19+ B cells expressing smIgA+. DNA sequences for some of these cases have been described (5,16,23). PBMC, obtained from heparinized venous blood by density gradient centrifugation (Ficoll-Paque; Pharmacia LKB Biotechnology, Piscataway, NJ), were used immediately or cryopreserved with a programmable cell-freezing machine (CryoMed, Mt. Clemens, MI) prior to being thawed and analyzed. cDNA prepared from samples were screened for expression of a dominant V$\kappa$ family (representing that of the B-CLL clone) by standard PCR. In this study we included only B-CLL cells that exhibited allelic exclusion.

**Preparation of RNA and cDNA Synthesis**

Total RNA was isolated from fresh or cryopreserved PBMC using Ultraspec RNA (Biotec Laboratories, Houston, TX) according to the manufacturer’s instructions. One µg of RNA was reverse transcribed using 200U M-MLV reverse transcriptase (GIBCO BRL, Life Technologies, Grand Island, NY), 1U of RNase inhibitor (Eppendorf, Hamburg, Germany), as previously described (5).

**Ig V,JL Gene Sequencing and Analysis**

V,JL sequences were determined as reported previously (16). Sequences were compared with the V BASE sequence directory (24) using MacVector software, version 7.0 (Accelrys, San Diego, CA), to GenBank, and to the international ImmunoGeneTics information system® http://imgt.cines.fr (Initiator and coordinator: Marie-Paule Lefranc, Montpellier, France; ref. (25)). In those instances where > 1% mutation was found in an expressed V$\kappa$ gene, the algorithm of Chang and Casali (26) was employed to determine the extent to which “antigen-selection” of the replacement (R) mutations had occurred, taking into account the inherent susceptibility of CDR to R mutations. The expected number of R mutations in CDR and FR was calculated using the formula \( R = n \times CDRrel \) (or \( FRrel \)) \( \times CDRel \) (or \( FRrel \)) where \( n \) is the total number of observed mutations, \( Rf \) is the R frequency inherent to the CDR or FR, and \( CDRel \) and \( FRrel \) are the relative sizes of these segments. A binomial probability model was used to evaluate whether the excess of R mutations in CDR or the scarcity in FR was due to chance (26).

**Analyses of LCDR3 Rearrangements**

LCDR3 length was determined by counting the number of amino acids (aa) immediately following the conserved cystine (C) at position 88 at the end of FR3 to the aa preceding position 98 at the beginning of FR4 (a conserved phenylalanine (F) in all JL segments). LCDR3 charge, as defined by an estimated pI, was determined using the MacVector software program (version 7.0).

**Statistical Analyses**

Analyses focused primarily on descriptive statistics (summaries using means, medians, standard deviations, proportions). Additional analyses examined associations between study groups (B-CLL vs. normal subjects) and \( V\kappa \) isotypes (\( \kappa \) vs. \( \lambda \)), C\( 1\) isotypes (IgM+ vs. non-IgM+), \( V\kappa \) mutation status, and other categorical variables using the Fisher’s Exact Test. The standard goodness-of-fit test was used to determine whether specific \( \kappa \)-\( \kappa \) and \( \lambda \)-\( \lambda \) pairings were more frequently encountered than others. The Mann-Whitney test was used to compare LCDR3 length and charge between specific group comparisons.

**RESULTS**

\( \kappa \) and \( \lambda \) Chain Use in B-CLL Cells

The distribution of \( \kappa \) and \( \lambda \) chain use in normal, polyclonal B-cell populations is \( \sim \)2:1 (27). Therefore, we analyzed the \( V\kappa \) gene sequences expressed by 206 B-CLL clones (179 IgM+ and 25 IgG+ and 2 IgA+) to determine if this was the case in the B cells transformed in this disease (Supplemental Tables S1, S2). In 67.9% (140/206) of the cases, the leukemic cells expressed a \( \kappa \) gene and in 32.0% (66/206) a \( \lambda \) gene. This distribution was similar for IgM+ (66.5% \( \kappa \) and 33.5% \( \lambda \)) and non-

**METHODS**

**B-CLL Samples**

We analyzed the DNA sequences of \( V\kappa J\kappa \) rearrangements of 206 patients with B-CLL; 179 of these patients had expansions of IgM+/CD5+/CD19+ B cells and 27 patients displayed expansions of CD5+/CD19+ B cells expressing smIgA+.
IgM+ (77.8% κ and 22.2% λ) cases. Thus, the ratio of κ and λ chains in B-CLL resembles that of normal B lymphocytes.

**Vκ Gene Family Use**

Among the 140 κ-expressing samples, Vκ genes were derived from families I, II, III, IV, and VI (Table 1) in the following order of frequency: VκI (52.1%) > VκIII (25.7%) > VκII (16.4%) > VκIV (5.0%) > VκVI (0.7%). The order of Vκ family distribution and their relative frequencies were unchanged when the cases were divided into subgroups based on Cλ isotype. In addition, there was no significant difference in the distribution and frequency of Vκ family use in IgM+ B-CLL cases compared with the reported repertoires of normal IgM+ CD5+ and IgM+ CD5− B cells (Table 1) (28, 29). A comparison between non-IgM+ B-CLL cases and normal non-IgM+ B cells could not be performed because data on the latter 2 control populations are lacking in the literature.

Vκ genes expressed in our B-CLL cohort derived from the Vκ families in the following frequencies (Table 1): VκIII (42.4%) > VκI (20.9%) > VκII (19.7%) > VκⅢ and VκX (each 6.1%) > VκⅧ (3.0%) > VκⅤ (1.5%). These values were not significantly different from those reported in normal IgM+ B cells (30–32).

When the non-IgM+ and IgM+ cases were analyzed separately (Table 1), the Cλ isotype-switched group exhibited a preponderance of VκII genes (50% versus 16.7%) and a lower fraction of VκIII genes (16.7% versus 45.9%). Although substantial, these comparisons were not significantly different, possibly due to the number of non-IgM+ B λ+ cases studied.

**Use of Specific Vκ and Vλ Genes**

IgV use among normal B lymphocytes is not stochastic; rather it is skewed by genetic and environmental pressures (33–36). The distribution of individual Vκ genes of IgM+ B-CLL cases resembled that reported in normal CD5+ and CD5− IgM+ B cells (data not shown) (28, 29). A similar comparison of specific Vλ gene expression was not possible because of the lack of data reported for normal control subsets.

IGKV1-39 was the most frequently encountered Vκ gene (Table 2) representing 17.9% of all the κ-expressing B-CLL cases and 16.8% (20/119) of the IgM+ and 23.8% (5/21) of the non-IgM+ cases. When considering only Vκ1-expressing cases, IGKV1-39 use represented 34.2%. IGKV3-20, 1-33, 1-5, 3-15, and 2-28/2-30 were the next most commonly expressed genes in our cases (Table 2). Of interest, although the use of IGKV1-39, 3-20, 2-28 and 2-30 was similar among IgM+ and non-IgM+ B-CLL cases, the expression of IGKV1-33, 1-5, and 3-15 differed. IGKV1-33 and 2-28 were not identified among the non-IgM-expressing cases and IGKV2-30 was enriched in this group (14.3% vs. 4.2%).

IGLV3-21 was the most frequently encountered Vλ gene (21.2%, 14/66 of all cases and 23.3%, 14/60 of IgM+ cases). Of note, IGLV3-21 was not found in the Cλ isotype-switched group. IGLV3-1, 1-44, and 2-14 were the next most frequently used Vλ genes (Table 2). These frequencies were similar to those of the normal IgM+ B cell repertoire (data not shown) (30–32).

**Number and Location of VL Gene Mutations**

When mature B cells encounter antigen, they can undergo the somatic hyper-
mutation process that alters the structure of Ig H and L V region genes and possibly the protein structure of the BCR [reviewed in (37)]. This is especially frequent if the antigen bound elicits T-cell help (38). Thus the presence of IgV mutations indicates antigenic experience as well as implies maturational pathway. We analyzed the number, type, and location of somatic changes in expressed V\textsubscript{L} J\textsubscript{L} of our B-CLL cohort. A difference of 2% or more from the most similar germline gene was taken as the cut-off point to define a sequence as mutated (4). Approximately 42% (87/206) of B-CLL V\textsubscript{L} genes differed from the most similar germline gene by 2% or more (Table 3). C\textsubscript{H} isotype-switched cases were more often mutated (63%) than IgM+ cases (39.1%; 83.3% of the latter differed by 2% from the most similar germline counterpart. Many more isotype-switched V\textsubscript{L}-expressing cases were mutated (83.3%) than IgM+ V\textsubscript{L}-expressing cases (35.0%; Table 3; \(P = 0.02\)). When V\text{κ}- and V\text{λ}-expressing cases were analyzed separately, 43.6% of the former and 39.4% of the latter differed by \(\geq 2\%\) from the germline counterpart. Many more isotype-switched V\text{κ}-expressing cases were mutated (83.3%) than IgM+ V\text{κ}-expressing cases (35.0%; Table 3; \(P = 0.03\)).

The V\text{κ} families differed in mutation frequency with a distribution of V\text{κ}IV \(>\) V\text{κ}I \(>\) V\text{κ}III \(>\) V\text{κ}II (Tables S1,S2). In addition, certain V\text{κ} genes displayed more mutations than others (Table 4). IGKV1-5 and 3-20 exhibited significant levels of mutation (80%, 8/10 cases and 63.6%, 12/19 cases, respectively). In contrast, other genes were rarely mutated. For example, in every instance (8/8) IGKV2-28 was \(> 98\%\) similar to the germline sequence; all of these cases expressed IgM. Likewise, IGKV1-33, which was also found only among IgM+ cases, and IGKV1-39 were very similar to their germline counterparts (9/10, 90%, and 21/25, 84%, respectively), even though 42.5% (31/73) of the V\text{κ}-expressing cases were mutated. Of V\text{λ}-expressing cases, 85.7% (12/14) of those using IGLV3-21 were minimally divergent from the germline sequence (Tables 4,S1,S2); all these cases were IgM+.

Finally, most of the V\text{κ} genes that remained unmutated were positioned considerably upstream on the V\text{κ} locus (Figure S1). Specifically, 46.5% (27/58) of the

### Table 2. Distribution of the Most Frequently Encountered V\text{L} Genes among the IgM+ and non-IgM+ B-CLL Groups

| Specific V\text{L} Gene | V\text{L} Family | All B-CLL Cases | IgM+ Cases | non-IgM+ Cases |
|-------------------------|-----------------|----------------|------------|---------------|
| IGKV1-05                | L12             | 7.1% (10/140)  | 5.9% (7/119) | 14.3% (3/21)  |
| IGKV1-33                | O18/O8          | 7.1% (10/140)  | 8.4% (10/119) | 0% (0/21)    |
| IGKV1-39                | O12/O2          | 17.9% (25/140) | 16.8% (20/119) | 23.8% (5/21) |
| IGKV2-28                | A19/A3          | 5.7% (8/140)   | 6.7% (8/119)  | 0% (0/21)    |
| IGKV2-30                | A17             | 5.7% (8/140)   | 4.2% (5/119)  | 14.3% (3/21) |
| IGKV3-11                | L6              | 5.0% (7/140)   | 4.2% (5/119)  | 9.5% (2/21)  |
| IGKV3-15                | L2              | 7.1% (10/140)  | 7.6% (9/119)  | 4.8% (1/21)  |
| IGKV3-20                | A27             | 13.6% (19/140) | 13.4% (16/119) | 14.3% (3/21) |

### Table 3. Percentages of B-CLL Cases with Differences \(\geq 2\%\) from Most Similar Germline Genes

| V\text{L} Family | All Cases | IgM+/κ Cases | non-IgM+/κ Cases |
|-------------------|-----------|--------------|-----------------|
| \(\kappa + \lambda\) | 42.2% (87/206) | 39.1% (70/179)\(^\text{A}\) | 63.0% (17/27)\(^\text{A}\) |
| \(\kappa\) | 43.6% (61/140) | 41.2% (49/119)\(^\text{A}\) | 57.1% (12/21)\(^\text{A}\) |
| \(\lambda\) | 39.4% (26/66) | 35.0% (21/60)\(^\text{B}\) | 83.3% (5/6)\(^\text{B}\) |

\(^\text{A}\) indicates statistical significance (\(P = 0.02\)).
\(^\text{B}\) indicates statistical significance (\(P = 0.03\)).

### Table 4. Differences in Mutation Frequencies between Certain V\text{L} Genes

| Specific V\text{L} Gene | V\text{L} Family | \(< 2\%\) Difference from Germline | \(\geq 2\%\) Difference from Germline |
|-------------------------|-----------------|---------------------------------|---------------------------------|
| IGKV2-28                | A19/A3          | 100.0% (8/8)                    | 0.0% (0/8)                      |
| IGKV1-33                | O18/O8          | 90.0% (9/10)                    | 10.0% (1/10)                    |
| IGKV1-39                | O12/O2          | 84.0% (21/25)                   | 16.0% (4/25)                    |
| IGKV1-5                 | L12             | 20.0% (2/10)                    | 80.0% (8/10)                    |
| IGKV3-20                | A27             | 36.8% (7/19)                    | 63.6% (12/19)                   |
| IGLV3-21                | 3h              | 85.7% (12/14)                   | 14.3% (2/14)                    |
Types of VL Mutations

BCRs that have been selected by antigen often display a higher frequency of R mutations in CDRs and/or a lower frequency of such mutations in FRs (39,40). Based on these considerations, ~50% of both κ-expressing and λ-expressing cases demonstrated evidence of antigen selection (Tables S1,S2).

Among the IgM+ mutated cases (Table S1), 40.1% (20/49) exhibited either a significantly increased frequency of R mutations in CDR (n = 1) or more often a significantly decreased frequency of R mutations in FR (n = 12) and 9 cases displayed mutation patterns in both the CDR and FR that were consistent with antigen selection. Of the IgM+ mutated cases (Table S1), 47.6% (10/21) demonstrated evidence for antigen selection. In 6 cases, there were fewer R mutations in FR and in 2 instances more R mutations in CDR than predicted. In 2 cases, both criteria for selection were found.

Approximately 83% of the κ-expressing and 50% of λ-expressing isotype-switched cases demonstrated similar evidence for antigen selection (Table S2). Although none of the cases showed a significantly increased frequency of R mutations in CDR, 8 exhibited a significant decrease in R mutations in the FR and 3 displayed significant changes in CDR and FR.

Allelic Polymorphisms

To ensure that the differences observed were primarily the effect of somatic changes and did not reflect known polymorphic variants, Vκ genes encountered in our analyses were compared with the list of polymorphisms available in the IMGT and GenBank databases. In every instance, the identified differences were consistent with somatic mutations. Furthermore, the alleles most commonly used in B-CLL were the same as those identified in our normal subject Vκ database. Thus, allele IGKV1-39*01 was used in 100% of the B-CLL cases and in 91.7% of normal controls. Similarly, the frequency of use of alleles IGKV1-5*03, 3-20*01, and 3-11*01 were identical in all B-CLL and normal B-cells.

Jκ Gene Use

Jκ family use among the entire B-CLL cohort, the IgM+ cases, and the non-IgM+ cases are listed in Table 5. The frequency of Jκ family use was the same in each group: Jκ1 > Jκ2 > Jκ4 > Jκ3 > Jκ5. For Jκ, the order of frequency in the entire B-CLL cohort and the IgM-expressing cases was Jκ3 > Jκ2/3 > Jκ1. Of note, all the cases expressing IgG or IgA used the Jκ2/3 or Jκ3 gene segment.

Vκ-Jκ Joining

An analysis of the frequency of joining of individual Vκ genes with Jκ genes in IgM+ B-CLL (Table S1) revealed a preferential combination of the IGKV1-39 and Jκ2 (B-CLL 7.6%; (9/119) versus normal 1.9%; (3/160); P = 0.03), and IGLV3-21 and Jκ3 (B-CLL 13.6%; (9/60) versus normal: 5.9%; (8/135); P = 0.05).

LCSR3 Characteristics

The antigen-binding pocket of the BCR is a composite of both the H and L rearrangements (41). Although HCDR 1 and 2 and LCDR 1 and 2 are important contributors, the H and L CDR3s have the greatest impact on the structure of the binding site for most antigens (21,22). In B-CLL, the HCDR3 of the BCR often displays unique features that differ between the Ig Vκ mutated and unmutated subgroups. Therefore, we carefully examined the LCSR3 of the κ- and λ-expressing cases in regards to length, amino acid composition, and charge.

A. κ-expressing B-CLL cells. The average length of LCSR3 for Vk+ B-CLL cells was 9.4 ± 0.8 aa (n = 140; Tables S1,S2). This value was similar in IgM+ (9.4 ± 0.9 aa, n = 119) and non-IgM+ (9.5 ± 0.7 aa, n = 21) cases and among cases expressing different Vk gene families (Vk1: 9.5 ± 0.8, Vk2: 9.5 ± 0.8, Vk3: 9.4 ± 0.9, Vk4: 9.1 ± 0.4). The average LCSR3 length was also not different from the average LCSR3 length (9.1 ± 1aa, n = 160) of normal subjects (28,29).
However, many κ cases (n = 47) had extended Vκ lengths (> 9.0 aa) suggesting that N-addition occurred at the time of V-J segment rearrangement (Tables S1,S2). Indeed, 39.5% of the IgMκ and 38.1% of the non-IgMκ cases exhibited at least 1 additional amino acid (3 nucleotides) in LCDR3, suggesting that TdT was active in these B cells at the time of V-J recombination.

The average charge of LCDR3, as determined by the estimated pI value, for the κ-expressing samples was 6.5 ± 1.9 (Tables S1,S2), a value similar to those for IgMκ (6.4 ± 1.9) and non-IgMκ (6.9 ± 1.9) cases and cases expressing different Vκ gene families. It is also similar to the average LCDR3 pI (6.4 ± 1.9) of healthy individuals (calculated from refs. 28,29).

B. λ-expressing B-CLL cases. The average length of LCDR3 for λ cases was 10.9 ± 1.0 aa (n = 66; Tables S1,S2). This value was similar for the IgMλ (10.9 ± 1.0 aa, n = 60) and non-IgMλ (11.0 ± 0.6, n = 6) cases and among the cases in different Vλ gene families. The value did not differ from the average λ LCDR3 length (10.4 ± 1.0 aa, n = 299) of normal subjects (30–32). Nucleotide insertions were observed in 11.7% of the IgMλ and 16.7% of the non-IgMλ clones.

The average estimated pI of LCDR3 for the λ-expressing samples was 4.6 ± 1.8 (Tables S1,S2). This value was not significantly different from the IgMλ (4.4 ± 1.3, n = 60) or non-IgMλ (6.9 ± 3.5, n = 6) cases (P = 0.06) and it was very similar to the average LCDR3 pI (4.6 ± 1.7) of normal individuals (calculated from refs. 30–32). However, when we analyzed LCDR3 pI values among the cases expressing different Vκ gene families, we did identify significant differences: Vκ1: 3.7 ± 1.0, Vκ2: 6.1 ± 1.7, and Vκ3: 4.2 ± 1.8 (P = 0.001). Pairwise comparisons show that Vκ1 differed significantly from Vκ2 (P = 0.001) and Vκ2 differed significantly from Vκ3 (P = 0.001).

Pairing of the Most Commonly Encountered Vκ and Vλ Genes with Specific IgVH Genes

Because, as mentioned above, both Vκ1Jκ1 and Vλ1Jλ1 rearrangements contribute to antigen binding, we searched for selective associations of certain Vλ with Vκ gene families. In fact, we found 2 examples of such associations. IGKV1-39 paired mainly with Vκ1 (10/20) and Vκ3 (6/20) family members in the IgMλ B-CLL cases; in IgGλ cases, IGKV1-39 paired almost exclusively with IGHV4-39 gene (4/5). In addition, a preferential pairing of IGVL3-21 gene with Vκ3 gene family members (11/14) was identified.

Please note that supplementary information is available on the Molecular Medicine website (www.molmed.org).

DISCUSSION

In this study, we analyzed the expression of Vλ and Jλ segments in a cohort of 206 B-CLL patients, paying particular attention to [1] frequencies of utilization and association of these segments, [2] frequency, level, and location of somatic mutations, and [3] characteristics of LCDR3. We found similarities and some differences between the L chain and H chain V region repertoires of B-CLL cells (Table 6).

Gene Use

The most striking difference between the IgL and IgH repertoires is the lack of bias in Vλ and Jλ gene use. The IgH repertoire in B-CLL is characterized by a use of IGHV1-69 (5,7,12,42) and alleles (11,13) that differs from B cells of normal individuals. In contrast, our data indicate that expression of IgL κ and λ families and genes and Jλ segments mirrors that encountered most frequently with Jκ1 gene family-related differences exist for HCDR3 charge (Vκ3 > Vκ4 > Vκ1) and length (for example, longer in Vκ1, in particular 1-69 and shorter in Vκ6, in particular 3-07) (5).

However, 1 similarity in gene use does exist between the IgL and IgH repertoires in B-CLL, i.e., use of certain genes solely among IgM-expressing cases. For example, 3 of the most commonly encountered Vκ genes (IGKV1-33 and 2-28 of the κ repertoire and IGLV3-21 of the λ repertoire) were not found among CH isotype-switched cases. This phenomenon is reminiscent of that seen for the IGHV1-69 gene, which is rarely encountered in IgG or IgA B-CLL cases (16).

Interestingly, the B-CLL Igκ repertoire does differ from that of normal B cells in gene family pairing. We found that Vκ2 and Jκ3 genes paired more frequently in B-CLL than in normal subjects (Tables S1,S2). At the specific gene level, in IgMκ cases, IGKV1-39 associated preferentially with Jκ2 and IGLV3-21 paired often with Jκ3. However, among Cκ1 isotype-switched cases, IGKV1-39 associated most frequently with Jκ1. Coordinate association of V and J genes also occurs in IgH repertoire, where Vκ1-69 expressing B-CLL cells often use Jκ1 and 3-07-expressing cells often use Jκ4 (5,11). Our data differ somewhat from those reported recently (20), mainly by a lower percentage of IGLV4-1 and IGLV2-8 genes in our cohort.
Another feature shared by the V genes of the H and L chain repertoires is the presence of somatic mutations, which in some instances is limited to specific genes. In approximately 42% of our B-CLL cases, the expressed VL genes differed by 2.0% or more from the most similar germline counterpart (Table 3); the level and extent of gene difference was the same for Vκ- and Vλ-expressing cases. These percentages agree with those recently reported by Stamatopoulos and co-workers (20). In addition, the frequency of mutated Vλ sequences was significantly higher among Cμ iso-type-switched cases (63%) than IgM+ cases (36%; P = 0.0165); this finding resembles that of the VH repertoire in B-CLL (1-3). Of note, virtually all Vκ+ cases that expressed a switched Cμ isotype were mutated (83.3%, P = 0.01; Table 4).

The Vκ families differed in the occurrence of somatic mutations, with VκIII and VκI frequently exhibiting gene diversification and VκII rarely doing so (Table S1). In addition, certain VL genes expressed few (<2%) or no mutations (Tables 5, S1, S2); this was especially notable for cases using IGKV2-28 (100%), IGLV3-21 (90%) and IGKV1-39 (73.3%). In contrast, other genes were frequently mutated (IGKV1-5 (75%) and IGLK3-20 (~89%)). It should be noted that Stamatopoulos et al found that IGK1-5 and 3-20 were similarly represented among mutated and unmutated B-CLL (20). The differential accumulation of Vλ mutations in gene families and individual genes resembles the VH repertoire in which certain families (for example, VH1-69) and certain genes (for example, VH1-69 and 4-39) rarely contain somatic mutations, whereas others (for example, VH1-3 family and the VH3-07 gene) almost always do (4, 5, 42).

The IgL and IgH gene repertoires also were similar in regards to the type and location of nucleotide differences (Tables S1, S2). Of the 87 sequences with at least 2% difference from the germline, 49.4% (43/87) exhibited evidence for antigen selection. Of these 43 cases, selection was suggested most often by a preservation of FR structure (65.2%, 28/43), followed by a preservation of FR with a change in CDR structure (27.9%, 12/43), and then solely a change in CDR structure (7.0%, 3/43). This is consistent with the pattern of R mutations in mutated VH genes in B-CLL (4, 5, 42).

### Table 6. Similarities and Differences between L Chain and H Chain IgV Gene Repertoires in B-CLL

| Light chains | Heavy chains |
|--------------|--------------|
| V family use repertoire | Similar to normal B cell repertoire, with possible exception of VκII and VλII family use | Different from normal B cell |
| V segment use, Overall repertoire | Similar to normal B cell repertoire | Different from normal B cell |
| V segment use, between M-CLL and U-CLL repertoire | Differences exist for both Vκ and Vλ | Different from normal B cell |
| J segment use | No preferential use of J segments | Preferential associations of VH1-JH6 and VH3-JH4 |
| Evidence for N insertion | Yes | Yes |
| Frequently observed V-(D)-J associations | Vλ2 – Jκ3 families | VH1-69 – D3-3 - JH6 |
| | Vκ1-39 – Jκ2 | VH4-39 – D6-13 - JH5 |
| | Vλ3-21 – Jκ3 | |
| Presence of mutation | All cases: 42.2% | All cases: 56.6% |
| | IgM+ cases: 39.1% | IgM+ cases: 51.6% |
| | Isotype-switched cases: 63% | Isotype-switched cases: 73.7% |
| Difference in mutation frequency between specific genes | Yes, for example, Vκ-1-5 and 3-20 - mutated Vκ2-28 and Vλ3-21 - unmutated | Yes, for example, VH1-69 and 4-39 - unmutated VH3-07 and 4-34 - mutated |
| Location of mutated gene on germline locus | More 3′ Vκ genes mutated than 5′ Vκ genes | No consistent distribution of mutated genes along locus |
| Evidence for antigen selection | Mutated κ+ cases: 49.2% | Mutated cases: 75.5% |
| | Mutated λ+ cases: 50.0% | |
| CDR3 length | No differences in κ or λ | Differences between most used VH genes |
| CDR3 charge | Differences between λ cases | Differences between VH families |
Finally, the definition of a somatically-mutated sequence employed here, and in prior studies of the \( V_H \) repertoire in B-CLL, used a \( \geq 2\% \) difference from the most similar germline gene as an arbitrary cutoff to define “mutation.” This cutoff was originally selected to account for unknown polymorphisms in the human \( IgV_H \) locus (4). However, if one uses a \( \geq 1\% \) difference to assign this designation, the numbers of “mutated” sequences change minimally and insignificantly (\( \geq 1\% = 47.4\% \) mutated sequences vs. \( \geq 2\% = 40.7\% \) mutated sequences). This is consistent with our finding that known polymorphisms of specific \( V_L \) genes do not account appreciably for the differences reported here or for the differences in B-CLL \( V_H \) gene sequences reported by others (43).

**Pairing of \( V_H \) and \( V_L \) Segments in the BCRs of B-CLL Cells**

Pairing of specific \( IgV_H \) genes with \( V_L \) genes was studied for the most frequently encountered \( V_k \) (IGKV1-39) and \( V_\lambda \) (IGLV3-21) genes. In both instances, pairing appeared to be non-random. IGKV1-39 paired preferentially with \( V_{H,1} \) and \( V_{L,3} \) family members in IgM+ cases, although no preferential coupling with a specific \( V_H \) gene in these families was observed. Conversely, in IgG+ cases IGKV1-39 gene almost always paired with IGHV4-39 gene. These cases represent a B-CLL subgroup with almost identical BCR structures that involve the entire VHDJH and VLJL rearrangements, including H and L CDR3s with unique amino acids at the V-(D)-J junctions (16). Similarly, IGLV3-21 gene (represented only in IgM+ cases) was paired mainly with VH3 gene family members (11/14 cases) and often with IGHV3-21 (4/10). Indeed, these B-CLL cases represent another subgroup of B-CLL with remarkably similar BCR structures (15). A comprehensive analysis of \( IgV_H \) and \( V_L \) pairing in a large number of B-CLL cases is being prepared (Ghiotto et al., manuscript in preparation).

**Concluding Remarks**

What do these studies of the \( IgV_L \) repertoire add to the knowledge already gleaned from studies of the \( IgH \) repertoire? The presence of significant levels of somatic mutations in the \( V_L \) genes of \( \approx 40\% \) of patients confirms the conclusion drawn from \( V_H \) analyses that many B-CLL cases derive from mature B-lymphocytes that have experienced antigenic stimulation at some point in their development. However, the \( V_L \) data do not shed additional light on the manner in which the B-CLL cell precursors accumulated these mutations. Certainly, reactivity with a foreign antigen that elicited a classical T-cell-dependent, germinal center-mediated somatic hypermutation process may have occurred. Alternatively, a T-cell-independent process initiated by non-protein antigens, such as those expressed on the surface of certain microbes, could be responsible for the observed \( V_L \) gene changes.

The \( V_L \) mutation data also support the contention that B-CLL cells derive from cells selected by specific antigens. A clear bias for selection against R mutations in FR exists, because less than 50% of the “antigen selected” \( V_L \) sequences involved an amino acid replacement in LCDR1 or LCDR2 (Tables S1,S2). This type of structural conservation is consistent with the need for B cells to retain an intact BCR, a principle that has been illustrated clearly in animal systems (44). Furthermore, the greater tendency to preserve FR structure, rather than altering CDR amino acid composition, can be viewed as favoring securing antigen outside of the classical binding pocket, implying that superantigens drives some B-CLL cells and their precursor B-lymphocytes. Nevertheless, the association of certain specific \( V_L \) (D), and J segments (5,7,11–13) and their selective combination in assembling H and L chain rearrangements (14–19) supports binding of specific antigens in those cases.

Finally, because more cases using unmutated \( V_k \) genes lie upstream on the \( V_k \) locus (Figure S1), receptor editing may have taken place in these cells (45,46). Because unmutated B-CLL cases are enriched in poly/autoreactivity (47), receptor reconfiguration in these cases may not have been effective in eliminating low-affinity autoreactivity (48), due to the use of certain germline \( V_L \) (49) and \( V_H \) (50,51) genes and rearranged HCDR3 segments (52). This phenomenon has been observed in transgenic murine models (53,54). Additional studies that compare \( IgV \) mutation status with antigen binding will be necessary to confirm this possibility.

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