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 V_HDJ_H 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 V_L and J_L 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 V_L genes that differ significantly from their germline counterparts. Certain genes were virtually always mutated and others virtually never. In addition, preferential pairing of specific V_L and J_L segments was found. These findings are reminiscent of the expressed VH repertoire in B-CLL. However unlike the V_H repertoire, V_L gene 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 V_H 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 V_HDJ_H 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 V_H genes, i.e., mutated and unmutated B-CLL, respectively (4,5). This division has considerable prognostic significance (6,7). Patients in the Ig V_H 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-

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gen-experienced B-lymphocytes. Observations on the surface phenotypes (8) and the gene expression profiles (9,10) of the leukemic cells corroborate this notion. Finally, biased use of V_H, D, and J_H gene segments (5,7,11–13) and selective combinatorial associations (14–19) 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 V_HDJ_H segments that code for the V domains of the Ig H chain in these leukemic cells. In contrast, less information is currently 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_LJ_L segments. In this study, we analyzed the V_L and J_L gene segments expressed by a large cohort of B-CLL patients, focusing on the frequency of use and association, mutation status, and characteristics of the 3rd complementarity determining region (CDR3) that is critical for antigen-binding (21,22). These analyses reveal similarities as well as some differences in these features between the V region H and L chain repertoires expressed in B-CLL.

METHODS

B-CLL Samples

We analyzed the DNA sequences of V_LJ_L 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 $smIgG^+$ or $smIgA^+$. 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_L 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 (Biotecx 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_LJ_L Gene Sequencing and Analysis

V_LJ_L 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_L 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 $R = n \times CDR\ Rf$ (or $FR\ Rf$) $\times\ CDRrel$ (or $FRrel$) where n is the total number of observed mutations, *Rf* is the R frequency inherent to the CDR or FR, and *CDRrel* 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_L isotypes (κ vs. λ), C_H isotypes (IgM^+ vs. non-IgM^+), V_H mutation status, and other categorical variables using the Fisher's Exact Test. The standard goodness-of-fit test was used to determine whether specific Vκ-Jκ and Vλ-Jλ pairings were more frequently encountered than others. The Mann-Whitney test was used to compare LCDR3 length and charge between specific group comparisons.

RESULTS

κ and λ L Chain Use in B-CLL Cells

The distribution of κ and λ chain use in normal, polyclonal B-cell populations is ~2:1 (27). Therefore, we analyzed the V_L 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 Vκ gene and in 32.0% (66/206) a Vλ gene. This distribution was similar for IgM^+ (66.5% $κ^+$ and 33.5% $λ^+$) and non-

Table 1. Vκ and Vλ Gene Family Use by the IgM+/- and non-IgM+/- B-CLL Groups and by Normal IgM+/- B Lymphocytes

	All B-CLL Cases	IgM+ B-CLL Cases	Non-IgM+ B-CLL Cases	Normal IgM+ B Cells[A]	Normal IgM+ CD5+/- B Cells[A]	Normal IgM+ CD5- B Cells[A]
Vκ Family						
κI	52.1% (73/140)	51.3% (61/119)	57.1%(12/21)	36.2% (58/160)	33.8% (26/77)	38.6% (32/83)
κII	16.4% (23/140)	16.8% (20/119)	14.3%(3/21)	28.1% (45/160)	26.0% (20/77)	30.1% (25/83)
κIII	25.7% (36/140)	25.2% (30/119)	28.6%(6/21)	28.1% (45/160)	32.5% (25/77)	24.1% (20/83)
κIV	5.0% (7/140)	5.9% (7/119)	—	6.2% (10/160)	6.5% (5/77)	6.0% (5/83)
κV	—	—	—	1.2% (2/160)	1.3% (1/77)	1.2% (1/83)
κVI	0.7% (1/140)	0.8 (1/119)	—	—	—	—
Vλ Family						
λI	21.2% (14/66)	20% (12/60)	33.3% (2/6)	23.1% (69/299)		
λII	19.7% (13/66)	16.7% (10/60)	50.0% (3/6)	24.1% (72/299)		
λIII	42.4% (28/66)	45.0% (27/60)	16.7% (1/6)	34.5% (103/299)		
λIV	—	—	—	4.7% (14/299)		
λV	1.5% (1/66)	1.7% (1/60)	—	3.7% (11/299)		
λVI	—	—	—	2.0% (6/299)		
λVII	6.1% (4/66)	6.7% (4/60)	—	3.0% (9/299)		
λVIII	3.0% (2/66)	3.3% (2/60)	—	1.7% (5/299)		
λIX	—	—	—	1.0% (3/299)		
λX	6.1% (4/66)	6.7% (4/60)	—	2.3% (7/299)		

[A]Data derived from references 28 and 29.
[B]Data derived from references 30–32.

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.

V_L 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 C_H 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+ CD5+ and CD5- B cells could not be performed because data on the latter 2 control populations are lacking in the literature.

Vλ genes expressed in our B-CLL cohort derived from the Vλ families in the following frequencies (Table 1): VλIII (42.4%) > VλI (20.9%) > VλII (19.7%) > VλVII and VλX (each 6.1%) > VλVIII (3.0%) > VλV (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 C_H isotype-switched group exhibited a preponderance of VλII genes (50% versus 16.7%) and a lower fraction of VλIII genes (16.7% versus 45.9%). Although substantial, these comparisons were not significantly different, possibly due to the number of non-IgM+ Vλ+ 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. IGKV 1-33 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 C_H 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-

Table 2. Distribution of the Most Frequently Encountered V_L Genes among the IgM+ and non-IgM+ B-CLL Groups

Specific V_L Gene		V_L Family	Percentage of specific genes compared with all κ genes			Percentage of specific genes compared with the percentage of κ genes within the family		
			All B-CLL Cases	IgM+ B-CLL Cases	Non-IgM+ B-CLL Cases	All B-CLL Cases	IgM+ B-CLL Cases	non-IgM+ B-CLL Cases
IGKV1-05	L12	κI	7.1% (10/140)	5.9% (7/119)	14.3% (3/21)	13.7% (10/73)	11.5% (7/61)	25.0% (3/12)
IGKV1-33	O18/O8	κI	7.1% (10/140)	8.4% (10/119)	0% (0/21)	13.7% (10/73)	16.4% (10/61)	—
IGKV1-39	O12/O2	κI	17.9% (25/140)	16.8% (20/119)	23.8% (5/21)	34.2% (25/73)	32.8% (20/61)	41.7% (5/12)
IGKV2-28	A19/A3	κII	5.7% (8/140)	6.7% (8/119)	0% (0/21)	34.8% (8/23)	40.0% (8/20)	—
IGKV2-30	A17	κII	5.7% (8/140)	4.2% (5/119)	14.3% (3/21)	34.8% (8/23)	25.0% (5/20)	100% (3/3)
IGKV3-11	L6	κIII	5.0% (7/140)	4.2% (5/119)	9.5% (2/21)	19.4% (7/36)	16.7% (5/30)	33.3% (2/6)
IGKV3-15	L2	κIII	7.1% (10/140)	7.6% (9/119)	4.8% (1/21)	27.8% (10/36)	30.0% (9/30)	16.7% (1/6)
IGKV3-20	A27	κIII	13.6% (19/140)	13.4% (16/119)	14.3% (3/21)	52.8% (19/36)	53.3% (16/30)	50.0% (3/6)
IGLV1-44	1c	λI	7.6% (5/66)	8.3% (5/60)	0% (0/6)	35.7% (5/14)	41.7% (5/12)	—
IGLV2-14	2a2	λII	7.6% (5/66)	6.7% (4/60)	16.7% (1/6)	38.5% (5/13)	40.0% (4/10)	33.3% (1/3)
IGLV3-01	3r	λIII	7.6% (5/66)	8.3% (5/60)	0% (0/6)	17.9% (5/28)	18.5% (5/27)	—
IGLV3-21	3h	λIII	21.2% (14/66)	23.3% (14/60)	0% (0/6)	50.0% (14/28)	51.9% (14/27)	—

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_LJ_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_L genes differed from the most similar germline gene by 2% or more (Table 3). C_H isotype-switched cases were more often mutated (63%) than IgM+ cases (39.1%; $P = 0.02$). When Vκ- and Vλ-expressing cases were analyzed separately, 43.6% of the former and 39.4% of the latter differed by ≥ 2% from the germline counterpart. Many more isotype-switched Vλ-expressing cases were mutated (83.3%) than IgM+ Vλ-expressing cases (35.0%; Table 3; $P = 0.03$).

The Vκ families differed in mutation frequency with a distribution of VκIV > VκI > VκIII > VκII (Tables S1,S2). In addition, certain Vκ 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 VκI-expressing cases were mutated. Of Vλ-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 Vκ genes that remained unmutated were positioned considerably upstream on the Vκ locus (Figure S1). Specifically, 46.5% (27/58) of the

Table 3. Percentages of B-CLL Cases with Differences ≥ 2% from Most Similar Germline Genes

V_L Family	All Cases	IgM+/- Cases	non-IgM+/- Cases
κ + λ	42.2% (87/206)	39.1% (70/179)[A]	63.0% (17/27)[A]
κ	43.6%% (61/140)	41.2% (49/119)	57.1% (12/21)
λ	39.4% (26/66)	35.0% (21/60)[B]	83.3% (5/6)[B]

[A]Indicates statistical significance ($P = 0.02$).
[B]Indicates statistical significance ($P = 0.03$).

Table 4. Differences in Mutation Frequencies between Certain V_L Genes

Specific V_L Gene		V_L Family	< 2% Difference from Germline	≥ 2% Difference from Germline
IGKV2-28	A19/A3	κII	100.0% (8/8)	0.0% (0/8)
IGKV1-33	O18/8	κI	90.0% (9/10)	10.0% (1/10)
IGKV1-39	O12/2	κI	84.0% (21/25)	16.0% (4/25)
IGKV1-5	L12	κI	20.0% (2/10)	80.0% (8/10)
IGKV3-20	A27	κIII	36.8% (7/19)	63.6% (12/19)
IGLV3-21	3h	λIII	85.7% (12/14)	14.3% (2/14)

Vκ genes located at the 3′ end of the locus (within the interval from IGKV4-1 to 3-20) were unmutated, whereas 71.7% (43/60) of the Vκ genes 5′ of IGVK3-20 (starting at 6-21 and considering also the duplicated portion of the locus) were unmutated. In contrast, unmutated and mutated Vλ genes were distributed uniformly along the Vλ locus.

Table 5. J_L Use among B-CLL cases

	All Cases	IgM^+ Cases	non-IgM^+ Cases
Jκ Family			
Jκ1	33.6% (47/140)	33.6% (40/119)	33.3% (7/21)
Jκ2	32.9% (46/140)	33.6% (40/119)	28.6% (6/21)
Jκ3	10.0% (14/140)	8.4% (10/119)	9.5% (2/21)
Jκ4	18.6% (26/140)	16.8% (20/119)	23.8% (5/21)
Jκ5	6.4% (9/140)	6.7% (8/119)	4.8% (1/21)
Jλ Family[A]			
Jλ1	18.2% (12/66)	20.0% (12/60)	—
Jλ2/3	27.3% (18/66)	25.0% (15/60)	50% (3/6)
Jλ3	54.5% (36/66)	55.0% (33/60)	50% (3/6)

[A]Jλ3 indicates the use of the Jλ3*02 allele, Jλ2/3 indicates the use of gene Jλ3*01 or Jλ2*01 because they are identical.

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 $IgM\kappa^+$ 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 mutations patterns in both the CDR and FR that were consistent with antigen selection. Of the $IgM\lambda^+$ 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, V_L 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 V_L 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.

J_L Gene Use

J_L 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 Jλ, the order of frequency in the entire B-CLL cohort and the IgM-expressing cases was Jλ3 > Jλ2/3 > Jλ1. Of note, all the cases expressing IgG or IgA used the Jλ2/3 or Jλ3 gene segment.

V_L-J_L Joining

An analysis of the frequency of joining of V_L gene families with J_L genes indicated that there was no preferential association, either for κ ($P = 0.28$) or λ ($P = 0.8$). However, when we compared the distribution of V_L-J_L pairing in IgM^+ B-CLL with that observed in normal subjects (28–32), we did notice that Vλ2 genes paired more frequently with the Jλ3 segment in B-CLL compared with normal individuals (11.7% (7/60) versus 1.5% (4/135), $P = 0.04$).

Interestingly, an analysis of the frequency of joining of individual V_L genes with J_L 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 Jλ3 (B-CLL 13.6% (9/60) versus normal: 5.9% (8/135); $P = 0.05$).

LCDR3 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 V_H mutated and unmutated subgroups. Therefore, we carefully examined the LCDR3 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 LCDR3 for $V\kappa^+$ 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 Vκ gene families (Vκ1: 9.5 ± 0.8, Vκ2: 9.5 ± 0.8, Vκ3: 9.4 ± 0.9, Vκ4: 9.1 ± 0.4). The average LCDR3 length was also not different from the average LCDR3 length (9.1 ± 1aa, n = 160) of normal subjects (28,29).

However, many κ$^+$ cases (n = 47) had extended V_L 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 IgM$^+$ (6.4 ± 1.9) and non-IgM$^+$ (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 IgM$^+$ (10.9 ± 1.0 aa, n = 60) and non-IgM$^+$ (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 IgM$^+$ (4.4 ± 1.3, n = 60) or non-IgM$^+$ (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 Vλ gene families, we did identify significant differences: Vλ1: 3.7 ± 1.0, Vλ2: 6.1 ± 1.7, and Vλ3: 4.2 ± 1.8 (*P* = 0.001). Pairwise comparisons show that Vλ1 differed significantly from Vλ2 (*P* = 0.001) and Vλ2 differed significantly from Vλ3 (*P* = 0.001).

Pairing of the Most Commonly Encountered Vκ and Vλ Genes with Specific IgV$_H$ Genes

Because, as mentioned above, both V_HDJ_H and V_LJ_L rearrangements contribute to antigen binding, we searched for selective associations of certain V_L with V_H genes. In fact, we found 2 examples of such associations. IGKV1-39 paired mainly with V_H1 (10/20) and V_H3 (6/20) family members in the IgM$^+$ B-CLL cases; in IgG$^+$ cases, IGKV1-39 paired almost exclusively with IGHV4-39 gene (4/5). In addition, a preferential pairing of IGVL3-21 gene with V_H3 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 V_L and J_L 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 V_L and J_L gene use. The IgH repertoire in B-CLL is characterized by a use of V_H, D, and J_H genes (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 J_L segments mirrors that reported for the normal adult human B cell repertoire. However, Stamatopoulos recently reported skewed representation of individual IgL κ and λ genes in B-CLL (20). The reason for this discrepancy is unclear but may relate to the normal control populations used for comparison. Only, 1 clear difference between the IgL repertoire in B-CLL and normal B cells appears to exist, i.e., exclusive use of Jλ2 among λ$^+$ C_H isotype-switched cases.

As might be expected from the similarity in V_L and J_L gene use, the LCDR3s of both the κ and λ rearrangements were similar to their counterparts in the normal B-cell repertoire. Again, this is different from the IgH repertoire, which differs in length, amino acid composition, and charge (Tables S1,S2) (5,11,13) from that of normal circulating B cells. Of note, however, certain Vλ families (Vλ2 > Vλ3 > Vλ1) do differ significantly in LCDR3 pI values. In this latter regard, the IgL and IgH B-CLL repertoires are similar because V_H family-related differences exist for HCDR3 charge (V_H3 > V_H4 > V_H1) and length (for example, longer in V_H1 genes, in particular 1-69 and shorter in V_H3, 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 V_L 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 IgL repertoire does differ from that of normal B cells in gene family pairing. We found that Vλ2 and Jλ3 genes paired more frequently in B-CLL than in normal subjects (Tables S1,S2). At the specific gene level, in IgM$^+$ cases, IGKV1-39 associated preferentially with Jκ2 and IGLV3-21 paired often with Jλ3. However, among C_H isotype-switched cases, IGKV1-39 associated most frequently with Jκ1. Coordinate association of V and J genes also occurs in IgH repertoire, where V_H1-69-expressing B-CLL cells often use J_H6 and 3-07-expressing cells often use J_H4 (5,11). Our data differ somewhat from those reported recently (20), mainly by a lower percentage of IGKV4-1 and IGLV2-8 genes in our cohort.

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 VλII 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 – Jλ3 families Vκ1-39 – Jκ2 Vλ3-21 – Jλ3	VH1-69 - D3-3 - JH6 VH4-39 - D6-13 - JH5
Presence of mutation	All cases: 42.2% IgM$^+$ cases: 39.1% Isotype-switched cases: 63%	All cases: 56.6% IgM$^+$ cases: 51.6% Isotype-switched cases: 73.7%
Difference in mutation frequency between specific genes	Yes, for example, Vκ1-5 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 difference in distribution for Vλ genes	No consistent distribution of mutated genes along locus
Evidence for antigen selection[A]	Mutated κ$^+$ cases: 49.2% Mutated λ$^+$ cases: 50.0%	Mutated cases: 75.5%
CDR3 length	No differences in κ or λ	Differences between most used VH genes
CDR3 charge	Differences between λ cases	Differences between VH families

Somatic Mutation

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 V_L 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 V_L sequences was significantly higher among C_H isotype-switched cases (63%) than IgM$^+$ cases (36%; P = 0.0165); this finding resembles that of the V_H repertoire in B-CLL (1-3). Of note, virtually all Vλ$^+$ cases that expressed a switched C_H 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 IGKV3-20 (~89%)). It should be noted that Stamatopoulos et al found that IGKV1-5 and 3-20 were similarly represented among mutated and unmutated B-CLL (20). The differential accumulation of V_L mutations in gene families and individual genes resembles the V_H repertoire in which certain families (for example, V_H1) and certain genes (for example, V_H1-69 and 4-39) rarely contain somatic mutations, whereas others (for example, V_H3 family and the V_H3-07 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 V_H genes in B-CLL (5,7,12,42).

Finally, the definition of a somatically-mutated sequence employed here, and in prior studies of the V_H repertoire in B-CLL, used a ≥ 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 ≥ 1% difference to assign this designation, the numbers of "mutated" sequences change minimally and insignificantly (≥ 1% = 47.4% mutated sequences vs. ≥ 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 Vκ (IGKV1-39) and Vλ (IGLV3-21) genes. In both instances, pairing appeared to be non-random. IGKV1-39 paired preferentially with V_H1 and V_H3 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 ~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 superantigen drives some B-CLL cells and their precursor B-lymphocytes. Nevertheless, the association of certain specific V, (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 Vκ genes lie upstream on the Vκ 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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