Strikingly homologous immunoglobulin gene rearrangements and poor outcome in V_{H}3-21–using chronic lymphocytic leukemia patients independent of geographic origin and mutational status

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We recently reported that Swedish V_{H}3-21–using chronic lymphocytic leukemia (CLL) patients showed restricted immunoglobulin gene features and poor prognosis despite V_{H} mutation status. To investigate this further, we analyzed the V_{H} and V_{L} gene rearrangements in 90 V_{H}3-21\(^{+}\) patients from Sweden, Germany, Italy, United States, Finland, and Australia and correlated these data with survival and other prognostic markers. Sixty-three percent exhibited mutated V_{H} genes and 37% unmutated V_{H} genes. Fifty (56%) patients displayed a short and homologous heavy-chain CDR3, many of these with the amino acid motif DANGMDV. Also, a highly biased V_{H}2-14 use was evident in 72% of patients with a restricted light-chain CDR3, QVWDS(S/G)SDHPWV. Combined restricted heavy- and light-chain CDR3s were found in patients from all included countries. Although V_{H}3-21\(^{+}\) CLLs have a remarkably predominant \(\lambda\) expression, analyses of kappa deleting element indicated a conserved light-chain rearrangement order. The overall survival was poor in the V_{H}3-21\(^{+}\) cohort (median survival, 88 months), with no significant difference in relation to mutation status or CDR3 homology. High ZAP-70 and CD38 expression was found in both mutated and unmutated V_{H}3-21\(^{+}\) cases as well as a slight increase of 11q– aberrations. In summary, highly restricted B-cell receptors and worse outcome characterize V_{H}3-21\(^{+}\) CLLs independent of geographic origin and mutation status. (Blood. 2006;107: 2889-2894)

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Introduction

In chronic lymphocytic leukemia (CLL), preferential use of individual V_{H} genes has been revealed, for instance, the V_{H}1-69, V_{H}3-07, and V_{H}4-34 genes.\(^{1-5}\) V_{H}1-69 gene rearrangements were shown early on to display characteristic features in CLL, such as unmutated V_{H} genes, a long heavy-chain complementary determining region 3 (HCDR3), and preferential use of certain diversity (D) and joining (J_{H}) genes compared with normal B cells.\(^{1,3-6,8}\) Recently, several CLL subsets using certain V_{H} genes have been characterized with homologous HCDR3s, which also showed restricted variable light-chain (V_{L}) gene use.\(^{5,9,10}\) These findings have led to the speculation that antigen selection could play a role in the pathogenesis of CLL,\(^{5,10,11}\) where foreign antigens or autoantigens may stimulate proliferation of B cells with specific B-cell–receptor (BCR) features, increasing the risk for malignant transformation.\(^{12}\) We recently reported preferential use of the V_{H}3-21 gene in CLL.\(^{13}\) Of interest, many of the V_{H}3-21\(^{+}\) CLLs (both \(\lambda\)g unmutated and mutated) showed rearrangements with short (7 codons) and homologous HCDR3s and had a predominant \(\lambda\) expression with biased V_{H}2-14 gene use.\(^{13,14}\) These data indicate a common antigenic epitope being recognized by the individual V_{H}3-21\(^{+}\) tumors. Furthermore, V_{H}3-21–using patients had short overall survival, similar to patients with unmutated V_{H} genes, despite the fact that two thirds of this group had mutated V_{H} genes.\(^{14}\) We therefore suggested that the V_{H}3-21\(^{+}\) CLL patient group should be considered as a separate entity, independent of mutation status, a finding that was further supported by the distinct gene-expression profile recently demonstrated in V_{H}3-21\(^{+}\) CLL compared with unmutated and mutated CLL using other V_{H} genes.\(^{15}\) Furthermore, a recent study of V_{H}3-21\(^{+}\) CLLs from several Mediterranean

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countries showed rearrangements identical to the ones previously reported by us, although they had a lower frequency of V<sub>H</sub>3-21–using patients (∼3%).<sup>16</sup> In this study, V<sub>H</sub>3-21<sup>+</sup> patients with a homologous HCDR3 had a worse prognosis compared with patients with a nonhomologous HCDR3.<sup>16</sup>

In the present study, we investigated whether V<sub>H</sub>3-21<sup>+</sup> CLLs have similar molecular characteristics of the V<sub>H</sub>/V<sub>L</sub> gene rearrangements, independent of geographic origin, in an extended joint material of V<sub>H</sub>3-21<sup>+</sup> CLLs (90 cases) from Sweden, Germany, Italy, United States, Finland, and Australia. We also analyzed the prognostic impact of V<sub>H</sub>3-21 gene use and its relation to other prognostic markers (eg, CD38 and ZAP-70 expression and genomic aberrations). Since most V<sub>H</sub>3-21<sup>+</sup> cases display a strikingly biased V<sub>λ</sub> gene use, we investigated the rearrangement order of the light-chain loci and assessed the kappa deleting element (KDE) rearrangement status in λ-expressing V<sub>H</sub>3-21<sup>+</sup> CLLs.

**Patients, materials, and methods**

**Patients and materials**

Tumor samples from 90 CLL patients with V<sub>H</sub>3-21 gene use were obtained from larger CLL cohorts partly included in previous studies<sup>13,14,17</sup> from the University hospitals of Uppsala (n = 17), Umeå (n = 7), Linköping (n = 7), Huddinge (n = 1), Sweden; Tampere (n = 3), Finland; Ulm (n = 37), Germany; Modena (n = 9), Italy; North Shore, Manhasset (n = 7), NY; and Sydney (n = 2), Australia. All V<sub>H</sub>3-21<sup>+</sup>-using CLL patients from the respective cohorts were included. The tumor material was obtained mainly from peripheral blood and bone marrow, but also from lymph node and spleen in a few cases. The CLL diagnosis was based on morphologic and immunophenotypic features according to the World Health Organization (WHO) classification.<sup>18</sup>

**CD38 and ZAP-70 expression**

CD38 expression was assessed by 3-color flow cytometry as described earlier.<sup>17,19,20</sup> ZAP-70 expression was measured by 4-color flow cytometry using CD38 and ZAP-70 expression.

**Cytogenetic analysis**

Fluorescence in situ hybridization (FISH) analysis of the following aberrations was performed as described previously.<sup>22</sup> 11q<sup>−</sup>, +12, 13q<sup>−</sup>, and 17p<sup>−</sup>.

**Analysis of V<sub>H</sub> and V<sub>λ</sub> gene rearrangements**

The polymerase chain reaction (PCR) amplification of the V<sub>H</sub> and V<sub>λ</sub> gene rearrangements was performed with either genomic DNA or cDNA using family-specific V<sub>H</sub> (either framework region 1 [FR1] or V<sub>H</sub> leader primers), V<sub>λ</sub>, V<sub>λ</sub> primers together with consensus J primers as previously described.<sup>2,11,14,17</sup> In 9 cases, V<sub>H</sub> gene rearrangements were amplified with consensus FR2 and J<sub>λ</sub> primers.<sup>23</sup> The PCR conditions for the V<sub>H</sub> family PCR amplification were as earlier outlined.<sup>2,11,14,17,24</sup> The V<sub>λ</sub> family PCR analysis was either carried out as reported earlier<sup>11,25</sup> or in a 50-μL reaction containing 200 ng DNA, 0.2 mM of each dNTP, 2.0 mM MgCl<sub>2</sub>, 2.5 U Platinum Taq (Invitrogen, Paisley, United Kingdom), 0.125 μM of each primer, and 1 × PCR Rxn buffer (Invitrogen). The V<sub>H</sub>J<sub>λ</sub> gene rearrangements were amplified with an initial 2-minute denaturation at 95°C followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 61°C, and 30 seconds at 72°C, ending with a 5-minute elongation at 72°C. The V<sub>λ</sub> family PCR was performed at 95°C for 2 minutes, 65°C for 1 minute, and 72°C for 1 minute followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 61°C, and 45 seconds at 72°C, ending with 72°C for 5 minutes.

The sequencing of the V<sub>H</sub> gene rearrangements was performed as described previously.<sup>11,14,17</sup> The V<sub>λ</sub> PCR products were direct sequenced using the Big Dye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems, Foster City, CA) or cloning was performed, as described earlier.<sup>14</sup> For the V<sub>H</sub> gene rearrangement, a minimum of 5 colonies was subsequently sequenced from each cloned sample. All V<sub>λ</sub> sequences were analyzed using an automated DNA sequencer (ABI 377 or ABI 3700; Applied Biosystems).

The obtained V<sub>H</sub>DC<sub>λ</sub> and V<sub>λ</sub>J<sub>λ</sub> sequences were submitted to 3 different Ig databases (GenBank, IMGT, and V-Base) and aligned to the closest germ-line sequence as detailed previously.<sup>26</sup> V<sub>H</sub> and V<sub>λ</sub> sequences with less than 98% homology to the germ-line sequence were considered mutated. At least 7 consecutive nucleotides aligned to the most homologous germ-line sequence were required to identify the D gene segment. All in-frame sequences from the V<sub>H</sub> and V<sub>λ</sub> gene rearrangements were converted into amino acid sequences. The HCDR3 length was calculated between codons 95 to 102 and the light-chain CDR3 (LCDR3) between codons 89 to 97, as described by Kabat.<sup>27</sup> Alignments of the HCDR3s and LCDR3s were performed by using Clustal X version 1.83 multiple sequence alignment software (UBC Bioinformatics Centre, Vancouver, BC, Canada) for Windows. High CDR3 homology was defined as 1 or fewer amino acid deviations from the most frequently occurring CDR3 sequence, whereas sequences with less homology (eg, 2-3 deviations from the most homologous sequence) were referred to as CDR3s with moderate homology. The V<sub>λ</sub> genes were named according to the nomenclature used in the GenBank database in order to avoid confusion between the V<sub>H</sub> and V<sub>λ</sub> genes since V<sub>H</sub>,2-14 (GenBank) is named V<sub>H</sub>3-21 using the IMGT nomenclature.

**Analysis of KDE rearrangements**

Analysis of the KDE rearrangements was performed on 42 samples with known λ expression using primers as previously described.<sup>28</sup> Amplification of germ-line KDE was carried out using conditions identical to those of the V<sub>H</sub>J<sub>λ</sub> PCR, as described in the previous section. The same conditions were also applied for amplification of the rearrangement between the KDE and the recombination signal sequence (RSS) in the I<sub>κ</sub>-C<sub>κ</sub> intron region (Figure S1, available on the Blood website; see the Supplemental Materials link at the top of the online article), with the exception of a lower MgCl<sub>2</sub> concentration (1.5 mM) and a shorter elongation step (30 seconds). Primers detecting KDE-V<sub>λ</sub> gene rearrangements were included in the V<sub>H</sub>-PCR for the λ-expressing cases.

**Statistical analyses**

Overall survival was defined as the time from diagnosis until last follow-up or death. Kaplan-Meier survival analysis and log-rank test were carried out to investigate survival differences between subsets. All statistical calculations were performed with Statistica 6.0 (Stat Soft, Tulsa, OK).

**Results**

**Characterization of V<sub>H</sub>3-21<sup>+</sup> gene rearrangements**

Of 90 V<sub>H</sub>3-21<sup>+</sup> CLL cases, all but 2 (cases 36G and 898) had V<sub>H</sub>3-21<sup>+</sup> rearrangements that were in-frame and potentially functional. In 57 (63%) cases, more than 2% somatic hypermutation was detected within the V<sub>H</sub> gene, with a median mutation rate of 3.1% (range, 2.1%-10%), whereas 33 (37%) cases showed unmethylated V<sub>H</sub> genes. The D gene segment was recognized in 31 of 90
VH3-21+ rearrangements; the most common D genes were D3-3 and D3-10, detected in 5 rearrangements each. In the remaining 59 VH3-21+ rearrangements, it was not possible to assign the D gene; the reason for this remains unknown but could hypothetically be due to extensive deletion of nucleotides during VDJ recombination. The most used JH gene was JH6, which was amplified in 60 rearrangements (67%), followed by JH4 (16 rearrangements, 18%). All VH3-21 sequencing data are shown in Table S1.

At the amino acid level, the functional VH3-21+ rearrangements displayed HCDR3s with a median length of 7 codons (range, 5-25 codons). Fifty (56%) of these, including cases from all countries, showed an HCDR3 with an unidentified D gene together with a JH6 gene. Fifteen patients had an identical amino acid sequence expressing the Asp-Ala-Asn-Gly-Met-Asp-Val (DANGMDV) motif, and the HCDR3 was highly homologous (1 amino acid difference) in another 21 of the patients. In a further 14 patients, moderate homology of the HCDR3 was shown, corresponding to 2 to 3 deviations from the DANGMDV sequence. Of the VH3-21+ cases with high/moderate HCDR3 homology, 74% (n = 37) had a mutated VH gene with a mutation frequency ranging between 2.2% and 10% (median, 3.4%). The HCDR3 sequences for all functional VH3-21 gene rearrangements are displayed in Figure 1.

A summary of the characteristics of the VH3-21+ rearrangements from the different countries is shown in Table 1.

V<sub>L</sub> gene rearrangements

Of 90 VH3-21+ cases, at least 1 V<sub>L</sub> gene rearrangement was demonstrated in each case, and in total 89 V<sub>L</sub> (26 functional and 63 nonfunctional) and 88 V<sub>H</sub> (78 functional and 10 nonfunctional) rearrangements were amplified and sequenced. Sixty-eight cases showed VL rearrangements and 77 VH gene rearrangements (55 cases with both VH and VL gene rearrangements). All VL gene sequencing data are shown in Table S1.

**Functionality.** At least one functional V<sub>L</sub> gene rearrangement was demonstrated in 82 of 90 cases. In the 10 cases with no functional V<sub>L</sub> rearrangements, all showed functional VH rearrangements, and in the 57 cases with a functional VH rearrangement, a functional VL rearrangement was detected in 52 of these. In the remaining 23 cases without known light-chain expression, 16 cases had a functional VH rearrangement, 1 case had a functional VH rearrangement, 4 cases showed both functional VH and VL rearrangements, and 2 cases had no functional rearrangement amplified.

**V<sub>L</sub> gene mutation status.** The majority of the V<sub>L</sub> genes were unmutated, and only 8 of 89 rearrangements harbored somatic hypermutation, with a median mutation rate of 2.9% (range, 2.3%-5.8%). Somatically mutated V<sub>L</sub> genes were detected in 20 of 88 rearrangements, with a mutation frequency of a median of 3.4% (range, 2.4%-8.1%).

**V<sub>L</sub>/J<sub>H</sub> gene use.** The most frequently rearranged V<sub>L</sub> genes were V<sub>L</sub>B3 (n = 16) and V<sub>L</sub>A27 (n = 12). J<sub>H</sub>4 and J<sub>H</sub>1 were the most commonly used J<sub>H</sub> genes, detected in 31 and 19 rearrangements, respectively. The most frequently rearranged V<sub>L</sub> gene was V<sub>L</sub>2-14, which was shown in 65 (72%) cases, followed by V<sub>L</sub>2-1 detected in 4 (4%) cases. The J<sub>H</sub>3 gene was found in 68 (76%) patients and the J<sub>H</sub>1 and J<sub>H</sub>2 genes in 10 and 6 patients, respectively.

The combination of the V<sub>L</sub>2-14/J<sub>H</sub>3 genes was demonstrated in 61 cases from all included countries (Germany, n = 24; Sweden, n = 24; Italy, n = 5; United States, n = 4; Australia, n = 2; and Finland, n = 2). The LCRD3 of the V<sub>L</sub>2-14* cases had a median length of 12 codons (range, 11-12 codons) and showed high sequence homology in the cases with functional rearrangements. The most common LCRD3 sequence, detected in 45 cases, was Glu-Val-Trp-Asp-Ser-(Ser/Gly)-Ser-Asp-Val-Val (QVWDS[S/G]SDHPWV), where the sixth amino acid could be either a serine (S) or a glycine (G). In 14 cases, 1 additional difference from either of these 2 sequences was detected, and an asterisk (*) indicates that the case used a V<sub>L</sub> gene other than V<sub>L</sub>2-14. Geographic origin of the patient is indicated in the case number: Germany (G), Sweden (S), Italy (I), United States (U), Finland (F), and Australia (A).
Germany (n/H11005) are shown in Table S2. The configuration was found in all analyzed samples. The KDE data showed highly homologous HCDR3 and LCDR3 sequences and mutated and 3 unmutated VH3-21 alleles. A nonfunctional VHJ3-21/D2-14 together with Vλ3-21/D−/Jλ6 was found in 41% of the patients (n = 46), of which 34 cases displayed highly homologous HCDR3 and LCDR3 sequences and 12 patients moderate HCDR3 homology and highly homologous LCDR3s (Figure 1; Table 1). The conserved Ig sequences were detected in patients from all 6 included countries: Sweden (n = 19), Germany (n = 15), Italy (n = 5), United States (n = 4), Finland (n = 2), and Australia (n = 1).

Analysis of kappa deleting element (KDE) rearrangements

Forty-two of the cases, with known λ light-chain expression, were examined for the presence of a rearranged KDE, as well as the germ-line configuration of KDE. Rearrangement of the KDE, either to the Jλ-Cλ intron RSS or to a Vλ RSS (Figure S1), was detected in 41 (98%) of 42 cases, on one (38%) or both (60%) alleles. A nonfunctional Vλ gene rearrangement together with 1 or 2 KDE rearrangements was demonstrated in 15 and 16 patients, respectively. PCR product showing germ-line KDE configuration was found in all analyzed samples. The KDE data are shown in Table S2.

Relation to other prognostic markers

CD38 expression data were available for 43 patients; in 20 cases (12 mutated and 8 unmutated) the CD38 expression level was more than 20%, whereas 23 cases (14 mutated and 9 unmutated) showed less than 20% CD38 expression (Figure 2A). ZAP-70 expression was assessed in 10 patients, of which 7 cases (5 mutated and 2 unmutated) demonstrated more than 20% ZAP-70+ cells, while the remaining 3 cases (2 mutated and 1 unmutated) had less than 20% ZAP-70+ cells (Figure 2B). FISH analysis was performed in 55 cases (34 mutated and 21 unmutated); 13q− was the most common aberration (n = 25, 18 mutated and 7 unmutated), followed by 11q− (n = 15, 8 mutated and 7 unmutated), 17p− (n = 5, 3 mutated and 2 unmutated), and +12 (3 unmutated). In 7 cases, none of the analyzed cytogenetic aberrations was detected.

Survival analysis

Survival data were available for 64 patients from Sweden (n = 32), Germany (n = 14), Italy (n = 9), United States (n = 4), Finland (n = 3), and Australia (n = 2), and the overall survival was 88 months (range, 37-198 months). No difference in survival was found between mutated (41 cases) and unmutated (23 cases) Vλ3-21+ patients, with a median survival of 79 months for the mutated, whereas the median survival was not reached for the unmutated cohort (log-rank test, P = .17; Figure 3A). Also, no significant difference in overall survival was evident when comparing survival in patients with highly homologous HCDR3 (30 cases) versus nonhomologous HCDR3 (34 cases) (104 vs 70 months, P = .32; Figure 3B), short (41 cases) versus long (23 cases) HCDR3 (92 vs 65 months, P = .60), and Vλ2-14 use (48 cases) versus use of other Vλ genes (16 cases) (85 vs 77 months, P = .81). Different borders for the HCDR3 homology were also applied (0,

| Table 1. Ig gene characteristics for Vλ3-21+ patients in each country |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                           | Total          | Germany       | Sweden        | United States  | Finland        | Australia      |
| No. of Vλ3-21 cases        | 90             | 37            | 32            | 9              | 7             | 3             | 2             |
| Frequency, %*              | 6/33           | 2/13          | 22/10         | 5/4            | 4/3           | 2/1           | 1/1           |
| Mutated/unmutated          | 32/14          | 14/10         | 10            | 4/3            | 3/1           | 1             | 0             |
| Vλ gene homology           | At least 98%   | 17/8          | 3             | 1              | 1             | 1             | 1             |
| 96% to 98%                 | 41             | 17/14         | 4             | 4              | 1             | 1             | 1             |
| Less than 96%              | 17             | 8             | 1             | 0              | 1             | 1             | 1             |
| μ/λ expression             | 10/58          | 1/14          | 5/27          | 1/8            | 3/4           | 0/3           | 0/2           |
| Highly homologous HCDR3    | 36             | 10/16         | 5             | 3              | 1             | 1             | 1             |
| Moderately homologous HCDR3| 14             | 6/5           | 1             | 1              | 1             | 0             | 0             |
| Vλ2-14 functional          | 65             | 26/24         | 6             | 4              | 3             | 2             |               |
| Homologous HCDR3 and Vλ2-14| 46             | 15/19         | 5             | 4              | 2             | 1             |               |

*Percent Vλ3-21 using cases; that is, the number of Vλ3-21+ cases out of the total number of CLL cases investigated by VH gene sequencing in each cohort.

Figure 2. CD38 and ZAP-70 expression in Vλ3-21+ CLL. (A) CD38 expression in 26 mutated and 17 unmutated Vλ3-21+ CLL patients. (B) ZAP-70 expression in 7 mutated and 3 unmutated Vλ3-21+ CLL patients.

Figure 3. Kaplan-Meier survival curve analysis in Vλ3-21+ CLL. (A) Comparison of Vλ3-21+ patients with mutated and unmutated VH rearrangements. No significant difference could be detected between the 2 subsets (log-rank test, P = .17); the median survival was 74 months for patients with mutated VH3-21 genes, whereas the median survival was not reached for patients with unmutated VH3-21 genes. (B) Comparison of Vλ3-21+ patients with highly homologous HCDR3s versus nonhomologous HCDR3s. The highly homologous HCDR3s had a median overall survival of 104 months, while patients with nonhomologous HCDR3s had a median survival of 70 months (log-rank test, P = .32).
Discussion

We have previously identified a V_{H}3-21–using subset of CLL patients that displayed peculiar characteristics of the Ig genes and had a poor outcome.\textsuperscript{13,14} To further study the Ig gene features in V_{H}3-21\(^+\) CLL and the prognostic impact of V_{H}3-21 use, we extended the V_{H}3-21\(^+\) material and included CLL patients from 6 different countries around the world (ie, Germany, Sweden, Italy, United States, Finland, and Australia). In parallel with our previous studies,\textsuperscript{13,14} we could demonstrate the characteristic Ig gene rearrangements from all included countries. A large proportion (56%) of V_{H}3-21\(^+\) CLL patients showed gene rearrangements with very short and homologous HCDR3s, where many of these also had the amino acid motif, DANGMDV. Furthermore, a majority (72%) of V_{H}3-21\(^+\) patients displayed preferential V_{H} gene use of the V_{S}2-14-14 gene. The LCDR3s were very similar in the majority (91%) of the V_{H}3-21\(^+\)/V_{S}2-14\(^+\) patients and showed a conserved amino acid sequence, QVWDS(S/G)SDHPWV. The combination of homologous HCDR3/LCDR3 was detected in samples from all countries. Hence, according to our data, conserved V_{H}3-21\(^+\) BCRs are most probably a general phenomenon worldwide, which is further supported by the recent study of Mediterranean V_{H}3-21\(^+\) patients that showed cases with similarly restricted Ig gene characteristics.\textsuperscript{16}

The uniformity of the BCRs found in the V_{H}3-21 CLL cases strongly indicates that antigen selection is involved in CLL development via recognition of common antigenic epitopes. The fact that very similar V_{H}3-21\(^+/\)V_{S}2-14\(^+\) rearrangements can be found in different countries in a considerable number of patients strengthens the theory of antigen selection, since this is unlikely to have occurred by chance. One current theory proposes that an antigen most likely promotes proliferation of a V_{H}3-21\(^+\) B cell followed by clonal expansion and acquisition of genetic events before leukemia transformation (see the recent review by Chiorazzi et al\textsuperscript{13}). Although, to date the potential antigen(s) is unknown, previous\textsuperscript{10,31} and recent studies\textsuperscript{32} suggest that autoantigens may be involved in this process. Further research into finding such causative antigen(s) is necessary.

An important finding, correlating to our previous studies,\textsuperscript{13,14} was the short overall survival (median survival, 88 months) of the V_{H}3-21\(^+\) patients. In CLL, the V_{H} gene mutation status is known to be of prognostic value, with an improved survival for patients with somatically hypermutated V_{H} genes.\textsuperscript{4,19} In this V_{H}3-21 cohort, 63% of patients had somatically hypermutated V_{H} genes but still had a poor prognosis similar to that of the unmutated V_{H}3-21\(^+\) CLls and unmutated CLls using other V_{H} genes,\textsuperscript{13,14,17} verifying that the mutation status is not applicable for this subset of patients. We also compared different subsets such as homologous HCDR3 versus nonhomologous HCDR3 and V_{S}2-14 use versus use of other V_{L} genes, but could not find any significant differences in overall survival. This is in contrast to the study of Mediterranean V_{H}3-21\(^+\) CLL where the aggressive disease was attributed mainly to cases with homologous HCDR3s.\textsuperscript{16} The low number of V_{H}3-21 cases analyzed (16 cases) in the Mediterranean cohort may explain this discrepancy. Furthermore, analysis of other prognostic markers showed that a considerable fraction of V_{H}3-21\(^+\) patients displayed CD38 and ZAP-70 expression, both mutated and unmutated cases.\textsuperscript{21} A slightly higher number of 11q\textsuperscript{-} aberrations was also found both in mutated and unmutated V_{H}3-21\(^+\) CLls (24% and 33%, respectively).

Studies from Sweden, Northern Ireland, and the United Kingdom have indicated a higher frequency (~9%-10%) of V_{H}3-21\(^+\) patients\textsuperscript{13,16,33,34} compared with CLL studies from other European countries and the United States (between 0% and 3%).\textsuperscript{2,4,16,33} What could be the reason for the difference in V_{H}3-21 frequencies in different materials? We believe that the selection of patient material may be one major cause influencing the results of the V_{H} gene analyses. The German and Swedish CLL cohorts used have been collected from different referral centers, leading to a patient cohort that in general has a worse prognosis compared with patient materials collected at local hospitals. Indeed, in our previous studies, the median overall survival of the CLL patient cohorts was significantly shorter than other studies with more stage A disease patients.\textsuperscript{4,13,14,17,19} Furthermore, most published studies on V_{H} mutation status/V_{H} gene use do not represent population-based material and have selected materials, to some extent at least. On the other hand, the fact that the frequency of V_{H}1-69 gene rearrangements appears more or less similar in different parts of the world supports the concept that regional differences in V_{H}3-21 frequency indeed exist. The frequency of V_{H}3-21\(^+\) CLls varied in this present material with the highest frequency in the Swedish cohort with decreasing frequencies as follows: Finland > Germany/Italy > United States/Australia. Hypothetically, a variation in exposure for a potential antigen(s) in different parts of the world could be an explanation for this finding. In the future, population-based investigations of V_{H} gene use in large series of CLL patients may reveal the true frequencies of different V_{H} genes.

Since the V_{H}3-21\(^+\) CLL subset is characterized by a strikingly biased expression of \(\lambda\) light chains, this led us to ask if these V_{H}3-21\(^+\) cases have undergone the traditional light-chain rearrangement pathway (IgK, Igk, Ig\(\lambda\)). In most of the 42 V_{H}3-21\(^+\) \(\lambda\)-expressing patients analyzed, the amplified V_{\lambda} gene rearrangements were nonfunctional due to KDE and/or out-of-frame V_{\lambda} rearrangements. This indicates that the light-chain rearrangements in the V_{H}3-21\(^+\) cases have followed the ordered recombination and have gone through several rearranging attempts before producing a functional light-chain. The reason for the multiple rearrangement events occurring in V_{H}3-21\(^+\) CLls is unknown, but it may be speculated that negative selection has acted on BCRs with a V_{H}3-21/V_{\lambda} rearrangement.

In conclusion, homologous V_{H}3-21/V_{S}2-14 rearrangements are found in CLls from various centers around the world, and the very similar HCDR3/LCDR3 strongly indicate that antigen selection is involved in development of V_{H}3-21\(^+\) CLL. V_{H}3-21\(^+\) gene use was also associated with increased ZAP-70 and CD38 expression and a more severe disease course, regardless of mutation status or HCDR3 homology.

References


Strikingly homologous immunoglobulin gene rearrangements and poor outcome in V_{\mu}3-21-using chronic lymphocytic leukemia patients independent of geographic origin and mutational status

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