Extensive and Selective Mutation of a Rearranged V_{H}5 Gene in Human B Cell Chronic Lymphocytic Leukemia

By Jilian Cai, Caroline Humphries, Andrea Richardson, and Philip W. Tucker

From the Department of Microbiology and Program in Immunology, The University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75235

Summary

B cell chronic lymphocytic leukemia (CLL) is the malignant, monoclonal equivalent of a human CD5^+ B cell. Previous studies have shown that the V_\mu and V_\lambda genes rearranged and/or expressed in CLL have few and apparently random mutations. However, in this study, we have found that the rearranged V_{H}251 gene, one of the three-membered V_{H}5 family, has extensive and selective mutations in B-CLL cells. Somatic mutation at the nucleotide level is 6.03% in B-CLLs whereas the somatic mutation levels are much lower in CD5^+ and CD5^- cord B cells, adult peripheral blood B cells, and Epstein-Barr virus-transformed CD5^+ B cell lines (0.45, 0.93, and 1.92%, respectively). Complementary determining region 1 (CDR1) mutation in CLLs is particularly prevalent, and interchanges in CDRs often lead to acquisition of charge. Analysis of somatic mutations and mutations to charged residues demonstrated that the mutations in CLLs are highly selected.

In the human, the estimated 100–200 V_\mu gene segments on chromosome 14 can be divided into six families based on nucleotide sequence homology (for review see reference 1). Family size ranges from greater than 25 (V_{H}3 and V_{H}1) to one (V_{H}6), with the “smaller” families (V_{H}4, V_{H}5, and V_{H}6) displaying unexpectedly low polymorphism (2). We discovered the V_{H}5 family in a case of familial B cell chronic lymphocytic leukemia (B-CLL)^1 (3) and demonstrated that one (V_{H}251) of two functional (V_{H}251 and V_{H}32) and one pseudo (V_{H}15) members is rearranged in about 20% of CLLs examined (4). Others have shown that the V_{H}3 family is the most commonly used in CLL, in proportion to its relative size, and have confirmed that usage of V_{H}5 and the other small families is biased (5–10). Although the V_{H}1 family is underrepresented in CLL with respect to its germ-line complexity, one member (51P1) is observed in 10% of CLLs and constitutes 60% of the total V_{H}1 contribution (5). Preferential usage of a V_{\kappa}3b gene and the associated cross-reactive idiotype has been observed (11, 12). In cases where the germline equivalents could be identified, V_\mu usage in CLL often reflects the fetal repertoire; i.e., restricted usage of “developmentally regulated” genes (for review see reference 13) displaying little or no somatic mutation. This is in contrast to the relatively high frequency of V_\mu and V_\lambda mutations seen in follicular lymphoma (14).

Most B-CLL express CD5^+, a marker present on <15% of normal PBLs or splenocytes, but present on most cord blood B cells (for review see references 15 and 16). Human CD5^+ B cells, and the corresponding Ly-1 B subset in mice, appear to constitute a distinct developmental lineage and share important functional similarities. Human and mouse CD5^+ B cells produce a disproportionate level of low affinity, polyclonal autoantibodies (15–17). It has been proposed (18) that CLL could be a consequence of the unique ontogeny of the Ly1/CD5 lineage in that repertoires become progressively restricted, inevitably leading to monoclonality and clones that eventually transform. As with B-CLL, murine Ly-1 B cells have generally demonstrated restricted (19) if not unique (20) V_\mu repertoires with limited to no somatic mutation (16).

The prevailing speculation from the above considerations is that Ig genes expressed in CLL lack mutations and encode polyspecific autoreactivity. However, data we present here raise questions as to the generality of that opinion. A prototypic developmentally regulated V_{H}5 gene (V_{H}251) is extensively mutated in most CD5^+ B-CLLs in a manner consistent with antigen drive, whereas little, if any, mutation of V_{H}251 is seen in preimmune and postimmune CD5^+ or CD5^- compartments.

Materials and Methods

Patient Materials and Cells. Frozen CLL lymphocytes were obtained from 40–60-yr-old patients from W. Blattner (Environmental Epidemiology Branch, National Cancer Institute, Frederick, MD),
R. G. Smith (Department of Internal Medicine, The University of Texas Southwestern), and C. Lutz (Department of Pathology, the University of Iowa Cancer Center, Iowa City, IA). All samples were established (21) by these investigators to be >95% leukemic and >90% CD5+. Two EBV-transformed cell lines (22) obtained from CD5+ adult PBLs, were provided by P. Casali (New York University School of Medicine, NY). Cord blood B cells, collected from four delivering mothers, and PBLs, donated from normal adults (30–40-yr-old), were provided by P. Lipsky (The University of Texas Southwestern). Cord Samples were further fractionated into CD5+ and CD5− fractions by doublestaining with CD20 (B1; Coulter Electronics Inc., Hialeah, FL) and Leu-1 (Becton Dickinson & Co., Mountain View, CA) as previously described (22). CD5 positivity of unfractonated cord samples ranged from 50 to 70%.

DNA Synthesis and Genomic DNA Isolation. Total RNA was isolated from CLL cells (CLL1–9) and EBV-transformed CD5+ B cells (VERG1–14), primed for first-strand synthesis with oligo(dT), and converted to double-stranded cDNA (23). cDNA was cloned into AgeI and libraries, propagated as previously described (24). Resulting libraries were double-screened for V5251 and Cµ by plaque hybridization. Positive clones were subcloned in pUC vectors for subsequent analyses. Genomic DNA was isolated from CLL10–11, cord blood B cells, and adult PBLs by the method of Blin and Stafford (25).

PCR Amplification and Cloning. Primers used in the PCR amplifications were synthesized on a DNA synthesizer (Applied Biosystems Inc., Foster City, CA). The 5'-sense primer, dGCACTGAAATCCCTGATTCAAATTGTTGTCTCC, corresponds to the V5251 leader intron (4) preceded by an EcoRI cloning site. The 3'-antisense primer, dTACAGGATCCTGAGGAGACGGTGACCAGGOT, corresponds to identical Jµ– Jgµ sequences (26) followed by a BamHI cloning site. PCR was performed according to recommendations of the manufacturer (Perkin-Elmer Cetus, Norwalk, CT). DNA templates were mixed with 50 pmol of each primer, 100 µmol dNTPs, and 0.5 U Tag DNA polymerase. Samples were amplified for 30–35 cycles as follows: denaturation at 94°C for 1 min, annealing at 63°C for 2 min, and extension at 72°C for 3 min. The reaction was extended for 10 min at 72°C after the last cycle.

Cloning and Sequencing. PCR products were digested and fractionated on 1% low-melting agarose gels, ligated into EcoRI and BamHI sites of pUC19, and transformed into CaCl2–competent BSI2 bacteria. All cDNA and PCR-generated clones were sequenced on both strands by the dideoxy chain termination method (27) using the M13 universal and reverse-universal primers. For CLLs, multiple colonies were sequenced. For PBL B cells and cord B cells, clones were randomly chosen and sequenced. Clones with identical mutations or germline sequences were verified as distinct by Shlomchik et al. (28) was used to evaluate whether the observed replacement mutations in CDRs were selective. That is, the probability of the number of R mutations in CDRs is: 

\[
p_{R_{\text{CDR}}} = n! / R_{\text{CDR}}! (n - R_{\text{CDR}})! \times p_{\text{KCDR}}^{R_{\text{CDR}}} \times (1 - p)^{n - R_{\text{CDR}}},
\]

where \(R_{\text{CDR}}\) = no. of replacement mutation in CDRs; \(p_{\text{KCDR}}\) = expected probability of R mutations which is the product of the relative size of CDRs and the expected R mutations; and \(n\) = total mutation occurred = \(R_{\text{CDR}} + S + 2R_{\text{FR}}\). The same method was also used to evaluate the frequency of mutations leading to charged residues in CDRs.

Results

V5251 Rearranged in CLL B Cells Are Extensively Mutated Relative to Cord Blood and Adult PBLs. We screened 40 CLLs and obtained 11 that rearranged V5251, 1 that rearranged V32 and none that rearranged V15 (data not shown). This biased percentage is consistent with what we (4) have previously observed. Since the focus here is on V5251 mutation, the PCR reaction used for cloning from the normal B cell population was specific for V5251. We previously showed that there were essentially no differences within V5251 sequences obtained from the livers of 10 adult donors (2). This lack of polymorphism allows conclusions to be drawn regarding somatic mutation in the absence of individual germ-line sequences.

Complete nucleotide sequences of rearranged V5251 genes from CLLs, cord blood B cells, CD5+ B cell lines and adult PBL are shown in Figs. 1–4. The 11 CLL sequences derived from individual cDNA libraries (CLLs 1–9) or from PCR amplification of genomic DNA (CLLs 10–11). Genomic Southern analysis performed on CLL samples where adequate DNA was available revealed the V5251 rearrangement at >90% molarity (3, 4, and data not shown). This agreed with the morphological assignment (20, and see Materials and Methods) of leukemic mass, consistent with previous observations that CLL is macroscopically monoclonal. Regardless of the molecular cloning method, there are extensive somatic mutations in V5251 rearranged in CLLS. In contrast, the somatic mutation of rearranged V5251 in CD5+ or CD5− cord blood cells and adult unfractonated PBLs are much lower, whereas a relative high level of mutation occurs in EBV-transformed CD5+ B cell lines. Collectively, the average mutation level (base mutations/total Vµ bases) of V5251 utilized in CLLS is 6.03%, about 13 and 6.5-fold higher than those in cord blood B cells, and PBLs, respectively (Table 1). The average mutation level of eight sequences from EBV-transformed CD5+ B cell lines is 1.92%, about twofold greater than in PBL and fourfold greater than in cord blood B cells. These low mutation levels, which include several 100% germline sequences, strongly argue against the possibility that the extensive mutations in CLLS 1–9 resulted from PCR errors.

We previously noted the C to G polymorphism at position 54 in 29% of adults (2, and data not shown). We find the equivalent value in adult PBLs (23%), double that value in unfractonated cord (53%) but none of the 11 CLLS, 8 EBV-transformed CD5+ B cells, nor 4 CD5+ cord sequences carried this polymorphism. Although the database must be expanded, a CD5+, subset restricted bias is evident. There are no features that distinguish the populations from which these samples were selected.

Lack of Intrachromosomal Variability. The excessive and unprecedented levels of mutation observed in the V5251 CLLS sequences raised the possibility of an ongoing mutational process. We chose two samples (CLLs 2 and 10) whose rear-
**Mutation within V\textsubscript{\gamma}251 Genes Utilized in CLLs Are Highly Selected.** Table 1 summarizes the replacement and silent mutations and their ratios with respect to location. The average ratio of replacement to silent mutation in CDRs relative to framework regions (FWRs) in CLLs is significantly higher than those in PBLs, cord B cells, and EBV-transformed CD5\textsuperscript{+} B cells, and suggests a higher selection level. In both CD5\textsuperscript{+} and CD5\textsuperscript{-} B cells, EBV-transformed CD5\textsuperscript{+} B cells and PBL B cells, several rearranged V\textsubscript{\gamma}251 genes are identical to germline sequences. Using a binomial probability model to analyze differences among their selection levels, we calculated the p values for finding the number of observed R\textsubscript{CDR} at random (Table 2). Since we have several sequences within each set of B cells (11 CLLs, 19 cord B cells, 8 EBV-transformed B cells, and 13 PBL B cells), we evaluated all mutations within a set of B cells as one pool. The p values of cord B cells and PBL B cells (0.133 and 0.136) are very close to the expected p value (0.173), which suggests there is little, if no, selection operating on those B cells. The small p value (0.034) of EBV-transformed CD5\textsuperscript{+} B cell lines is indicative of selection. However, the very very small p value of CLL samples strongly indicates that selection occurred.

Usage of J\textsubscript{n} and D Segments. Consistent with the data of others (5, 10), most CLLs used J\textsubscript{4} with rearranged V\textsubscript{\gamma}251 genes (Table 3). In PBLs, about the same fraction (75%) used J\textsubscript{4} and 17% used J\textsubscript{5}, which was similar to the percentages seen for other V\textsubscript{n} genes (29). However, CD5\textsuperscript{+} PBL lines and cord blood B cells use J\textsubscript{4} (50%) less frequently but J\textsubscript{5} (40%) considerably more frequently. The similar J\textsubscript{4} usage in cord (mostly CD5\textsuperscript{+}) and the adult CD5\textsuperscript{+} lines is expected if the adult CD5\textsuperscript{+} population derives from the cord CD5\textsuperscript{+} population which has undergone self-renewal over an extended lifetime. From the same logic, discordance between cord and CLL usage raises the possibility of different precursors for the normal and neoplastic adult pool.

As described by others (29), we found inverted D joining, D-D joining, inverted D-D joining, and double inverted D joining. It is unusual that the rearranged V\textsubscript{\gamma}251 gene in PBL5 (Fig. 4) is recessed 10 nucleotides into the 3' end and abutted directly (without N bases) to DN4. Given the low numbers analyzed, we did not see any biased usage of D seg-
Figure 2. Nucleotide sequences of rearranged V_{251} genes in cord blood B cells unfractionated (A) and fractionated (B) for CD5.
Figure 3. Nucleotide sequences of rearranged V_{251} genes in EBV-transformed CD5^+ B cells from adult PBL.

Figure 4. Nucleotide sequences of rearranged V_{251} genes in adult PBL. In PBL5, 10 nucleotides at the 3' end were recessed.
Table 1. Nucleotide Changes in Rearranged \( V_{251} \) Genes From Different Types of B-Cells

| Samples (No.) | Region (Bases) | Percent base change/bp* | Replacement | Silent | Total | Replacement/Silent | Average mutation (percent Base mutation) |
|---------------|----------------|-------------------------|-------------|--------|-------|-------------------|------------------------------------------|
|               |                |                         | No. | %   | No. | %   | No. | %   |                     |                                          |
|               |                |                         | FWR1 (99) | 17 | 1.56 | 18 | 1.65 | 35 | 3.54 | 0.94               |
| CLL B cells   |                |                         | CDR1 (18) | 34 | 17.2 | 8 | 4.04 | 42 | 21.2 | 4.26               |
| (11)          |                |                         | FWR2 (42) | 10 | 2.17 | 11 | 2.38 | 21 | 4.35 | 0.91               |
|               |                |                         | CDR2 (51) | 44 | 7.84 | 12 | 2.14 | 56 | 9.98 | 3.67               |
|               |                |                         | FWR3 (96) | 28 | 2.27 | 21 | 2.37 | 49 | 4.64 | 1.33               |
|               |                |                         | FWR1 (99) | 6  | 0.76 | 5  | 0.63 | 11 | 1.11 | 1.20               |
| CD5+ B cells  |                |                         | CDR1 (18) | 9  | 6.25 | 2  | 1.39 | 11 | 7.64 | 4.50               |
| (8)           |                |                         | FWR2 (42) | 0  | 0.00 | 2  | 0.60 | 2  | 0.60 | 0.00               |
|               |                |                         | CDR2 (51) | 7  | 1.72 | 1  | 0.25 | 8  | 1.96 | 7.00               |
|               |                |                         | FWR3 (96) | 11 | 1.43 | 4  | 0.52 | 15 | 1.95 | 2.75               |
|               |                |                         | FWR1 (99) | 5  | 0.39 | 4  | 0.31 | 9  | 0.70 | 1.25               |
| PBL B cells   |                |                         | CDR1 (18) | 6  | 2.56 | 0  | 0.00 | 6  | 2.56 | ND                   |
| (13)          |                |                         | FWR2 (42) | 3  | 0.55 | 1  | 0.18 | 4  | 0.73 | 3.00               |
|               |                |                         | CDR2 (51) | 4  | 0.60 | 1  | 0.15 | 5  | 0.75 | 4.00               |
|               |                |                         | FWR3 (96) | 10 | 0.80 | 3  | 0.24 | 13 | 1.04 | 3.33               |
|               |                |                         | FWR1 (99) | 4  | 0.21 | 2  | 0.11 | 6  | 0.32 | 2.00               |
| Cord B cells  |                |                         | CDR1 (18) | 4  | 1.17 | 2  | 0.58 | 6  | 1.75 | 2.00               |
| (19)          |                |                         | FWR3 (96) | 5  | 0.52 | 0  | 0.00 | 5  | 0.52 | ND                   |
|               |                |                         | FWR2 (42) | 1  | 0.13 | 1  | 0.13 | 2  | 0.26 | 1.00               |
|               |                |                         | FWR2 (42) | 8  | 1.17 | 2  | 0.58 | 6  | 1.75 | 2.00               |
|               |                |                         | FWR3 (96) | 5  | 0.52 | 0  | 0.00 | 5  | 0.52 | ND                   |

* Percent base change/bp was derived by adding the replacement or silent mutations in all sequences for each region of one sample and dividing by the total number of base pairs in each region.

† ND means no calculation of replacement/silent mutation because of no silent mutation.

Table 2. Analysis of Mutation of Rearranged \( V_{251} \) Genes

| Total mutations | Replacement in CDRs (R<sub>CDR</sub>) | Probability |
|-----------------|--------------------------------------|-------------|
| Expected        |                                      | 0.173       |
| Cord B cells    |                                      | 0.133       |
| B-CLLs          |                                      | 1.31 x 10^-7|
| CD5+ B cells    |                                      | 0.034       |
| PBL B cells     |                                      | 0.136       |

No Preference in L Chain Rearrangements in CLLs that Use \( V_{251} \). The demonstrated preference for \( V_\kappa 3b \) (11) and our observation of selective mutation and \( J_\kappa 4 \) bias prompted an examination of \( V_\kappa /V_\lambda \) status in our CLLs. Using PCR primers previously shown (30) to be specific for \( V_\kappa 1-V_\kappa 4 \) families, we amplified family-specific bands for \( V_\kappa 1-4 \) with no particular preferences (data not shown). Failure to obtain amplification signals in several samples was consistent with

Table 3. Usage of \( J_n \) Segments in Rearranged \( V_{251} \) Genes

| \( J_n \) segments | CLL B cells | PBL B cells | Cord B cells | CD5+ B cells | Total usage |
|---------------------|-------------|-------------|--------------|--------------|-------------|
| \( J_{11} \)        | 0           | 1           | 0            | 1            | 2           |
| \( J_{12} \)        | 0           | 0           | 1            | 0            | 1           |
| \( J_{13} \)        | 0           | 0           | 0            | 0            | 0           |
| \( J_{14} \)        | 8 (73%)     | 9 (75%)     | 5 (50%)      | 4 (50%)      | 26          |
| \( J_{15} \)        | 2 (18%)     | 2 (17%)     | 4 (40%)      | 3 (38%)      | 11          |
| \( J_{16} \)        | 1 (9%)      | 0           | 0            | 0            | 1           |

1078 Mutation of \( V_\kappa 5 \) Gene in Human B Cell Chronic Lymphocytic Leukemia
B cell populations studied here (Tables 1 and 2) or in the V.251 gene rearranged in different cell populations without for the high ratio of CDR replacement with charged residues. These mutations are also highly selected and occur at much higher than expected frequency. In CDR1, half of the germline neutral residues are replaced, and a third of these replacements are with charged residues. A similar CDR1 focus was observed in the two V.251-utilizing antibodies previously reported (31, 32). Both had four CDR1 interchanges. All are found among our CLL pool although none are charged.

Several groups have studied V. and Vn genes utilized in human CLLs and found little or no mutations (5–11). As with V.251, some of the Vn genes analyzed are considered to be developmentally regulated, in that they are over-represented in the fetal repertoire (13). Therefore, V.251 is a member of the small human V.5 gene family (4) that displays remarkably little polymorphism (2). That allowed us to compare the nucleotide sequences of the V.251 gene rearranged in different cell populations without the corresponding germline counterparts. 9 of 11 Vn251 genes utilized in CLLs have clusters of extensive mutation. Using a binomial probability model to assess this observation, we obtained a p value of 3.88 × 10^{-15}, whereas the expected p value is 10^{-2}. This indicates that a strong selective force is operative in generating charged residues within CDRs. The combined effect of the extensive mutations in CDRs and FWRs leads to a significant increase in overall positive charge.

Discussion

V.251 is a member of the small human V.5 gene family (4) that displays remarkably little polymorphism (2). That allowed us to compare the nucleotide sequences of the V.251 gene rearranged in different cell populations without the corresponding germline counterparts. 9 of 11 Vn251 genes utilized in CLLs have clusters of extensive mutation. Using a binomial probability model, we found that mutations within CDRs of CLLs are highly selective. The selection level is much higher than those observed in the other B cell populations studied here (Tables 1 and 2) or in the two previous reports of antigen-specific EBV-transformants (31) or hybridomas (32) that express a V.251 H chain. The deduced amino acid sequences of the CLL were remarkable for the high ratio of CDR replacement with charged residues. The binomial probabilities confirmed that these mutations are also highly selected and occur at much higher than expected frequency. In CDR1, half of the germline neutral residues are replaced, and a third of these replacements are with charged residues. A similar CDR1 focus was observed in the two V.251-utilizing antibodies previously reported (31, 32). Both had four CDR1 interchanges. All are found among our CLL pool although none are charged.

Several groups have studied V. and Vn genes utilized in human CLLs and found little or no mutations (5–11). As with V.251, some of the Vn genes analyzed are considered to be developmentally regulated, in that they are over-represented in the fetal repertoire (13). Therefore, V.251 is a member of the small human V.5 gene family (4) that displays remarkably little polymorphism (2). That allowed us to compare the nucleotide sequences of the V.251 gene rearranged in different cell populations without the corresponding germline counterparts. 9 of 11 Vn251 genes utilized in CLLs have clusters of extensive mutation. Using a binomial probability model, we found that mutations within CDRs of CLLs are highly selective. The selection level is much higher than those observed in the other B cell populations studied here (Tables 1 and 2) or in the two previous reports of antigen-specific EBV-transformants (31) or hybridomas (32) that express a V.251 H chain. The deduced amino acid sequences of the CLL were remarkable for the high ratio of CDR replacement with charged residues. The binomial probabilities confirmed that these mutations are also highly selected and occur at much higher than expected frequency. In CDR1, half of the germline neutral residues are replaced, and a third of these replacements are with charged residues. A similar CDR1 focus was observed in the two V.251-utilizing antibodies previously reported (31, 32). Both had four CDR1 interchanges. All are found among our CLL pool although none are charged.

Several groups have studied V. and Vn genes utilized in human CLLs and found little or no mutations (5–11). As with V.251, some of the Vn genes analyzed are considered to be developmentally regulated, in that they are over-represented in the fetal repertoire (13). Therefore, V.251 is a member of the small human V.5 gene family (4) that displays remarkably little polymorphism (2). That allowed us to compare the nucleotide sequences of the V.251 gene rearranged in different cell populations without the corresponding germline counterparts. 9 of 11 Vn251 genes utilized in CLLs have clusters of extensive mutation. Using a binomial probability model, we found that mutations within CDRs of CLLs are highly selective. The selection level is much higher than those observed in the other B cell populations studied here (Tables 1 and 2) or in the two previous reports of antigen-specific EBV-transformants (31) or hybridomas (32) that express a V.251 H chain. The deduced amino acid sequences of the CLL were remarkable for the high ratio of CDR replacement with charged residues. The binomial probabilities confirmed that these mutations are also highly selected and occur at much higher than expected frequency. In CDR1, half of the germline neutral residues are replaced, and a third of these replacements are with charged residues. A similar CDR1 focus was observed in the two V.251-utilizing antibodies previously reported (31, 32). Both had four CDR1 interchanges. All are found among our CLL pool although none are charged.

Several groups have studied V. and Vn genes utilized in human CLLs and found little or no mutations (5–11). As with V.251, some of the Vn genes analyzed are considered to be developmentally regulated, in that they are over-represented in the fetal repertoire (13). Therefore, V.251 is a member of the small human V.5 gene family (4) that displays remarkably little polymorphism (2). That allowed us to compare the nucleotide sequences of the V.251 gene rearranged in different cell populations without the corresponding germline counterparts. 9 of 11 Vn251 genes utilized in CLLs have clusters of extensive mutation. Using a binomial probability model, we found that mutations within CDRs of CLLs are highly selective. The selection level is much higher than those observed in the other B cell populations studied here (Tables 1 and 2) or in the two previous reports of antigen-specific EBV-transformants (31) or hybridomas (32) that express a V.251 H chain. The deduced amino acid sequences of the CLL were remarkable for the high ratio of CDR replacement with charged residues. The binomial probabilities confirmed that these mutations are also highly selected and occur at much higher than expected frequency. In CDR1, half of the germline neutral residues are replaced, and a third of these replacements are with charged residues. A similar CDR1 focus was observed in the two V.251-utilizing antibodies previously reported (31, 32). Both had four CDR1 interchanges. All are found among our CLL pool although none are charged.

Several groups have studied V. and Vn genes utilized in human CLLs and found little or no mutations (5–11). As with V.251, some of the Vn genes analyzed are considered to be developmentally regulated, in that they are over-represented in the fetal repertoire (13). Therefore, V.251 is a member of the small human V.5 gene family (4) that displays remarkably little polymorphism (2). That allowed us to compare the nucleotide sequences of the V.251 gene rearranged in different cell populations without the corresponding germline counterparts. 9 of 11 Vn251 genes utilized in CLLs have clusters of extensive mutation. Using a binomial probability model, we found that mutations within CDRs of CLLs are highly selective. The selection level is much higher than those observed in the other B cell populations studied here (Tables 1 and 2) or in the two previous reports of antigen-specific EBV-transformants (31) or hybridomas (32) that express a V.251 H chain. The deduced amino acid sequences of the CLL were remarkable for the high ratio of CDR replacement with charged residues. The binomial probabilities confirmed that these mutations are also highly selected and occur at much higher than expected frequency. In CDR1, half of the germline neutral residues are replaced, and a third of these replacements are with charged residues. A similar CDR1 focus was observed in the two V.251-utilizing antibodies previously reported (31, 32). Both had four CDR1 interchanges. All are found among our CLL pool although none are charged.

Figure 5. Deduced amino acid sequences of V.251 genes utilized in CLLs.
do. The latter are the precursor lineage for most of the V\textsubscript{251}-utilizing CLL (with CLL being an exception) and potentially may carry additional biases, such as J\textsubscript{\textalpha} preference. The antigen drive apparently operative on V\textsubscript{251} may derive from self-reactivity. Most human autoantibodies are produced by CD5\textsuperscript{+} B cells that preferentially use small V\textsubscript{\textalpha} families (15–17). Although not the rule with IgMs, extensively mutated sequences have been served in some autoantibodies (38–40). The unusual acquisition of charged residues we observed is reminiscent of high affinity antinucleic acid responses (41). Some CD5\textsuperscript{+} B cells expressing V\textsubscript{251} may be stimulated and selected by self-antigens to proliferate constantly, subjecting these cells to abnormal expansion and eventual transformation into tumors. Alternatively, our data is equally compatible with a foreign antigen providing the drive.

The potential for V\textsubscript{251} diversification in the normal preimmune environment is extremely limited, irrespective of the CD5\textsuperscript{+}/CD5\textsuperscript{−} compartment. That V\textsubscript{251} in adult EBV-transformed CD5\textsuperscript{+} cells show relative high mutation extends findings that human CD5\textsuperscript{+} cells are susceptible to an antigen-driven diversification mechanism (31, 38–40). As with limited germline polymorphism, absence of mutation could be an advantage for a developmentally regulated V\textsubscript{\textalpha} gene. In the case of V\textsubscript{251} expressers, CLL could be a natural consequence of repertoire restriction via expansion of given clones by a “super” antigen. Thus the normal inability to develop this high affinity response would divert the inevitable disaster of monoclonality.

We thank Drs. W. Blattner, R. G. Smith, and C. Lutz for providing well-characterized CLLs; Dr. P. Lipsky for providing CD5 fractionated cord blood; and Dr. P. Casali for providing EBV-transformed CD5\textsuperscript{+} PBL lines. We appreciate the comments and suggestions of Drs. D. Capra and V. Pascual. We thank Utpal Das for help with the manuscript.

This work was supported by National Institutes of Health grant CA-44016.

Address correspondence to Dr. Phillip W. Tucker, Department of Microbiology and Program in Immunology, The University of Texas, Southwestern Medical Center at Dallas, Dallas, TX 75235.

Received for publication 23 January 1992 and in revised form 10 July 1992.

References

1. Capra, J.D., and P.W. Tucker. 1989. Human immunoglobulin heavy chain genes. J. Biol. Chem. 264:12745.
2. Sanz, I., P. Kelly, C. Williams, S. Scholl, P.W. Tucker, and J.D. Capra. 1989. The smaller human V\textsubscript{\textalpha} gene families display remarkably little polymorphism. EMBO (Eur. Mol. Biol. Organ.) J. 8:3741.
3. Shen, A., C. Humphries, P.W. Tucker, and F.R. Blattner. 1986. Human heavy chain variable region gene family nonrandomly rearranged in familial chronic lymphocytic leukemia. Proc Natl. Acad. Sci. USA. 84:8563.
4. Humphries, C.G., A. Shen, W.A. Kuziel, J.D. Capra, F.R. Blattner, and P.W. Tucker. 1987. A new human immunoglobulin V\textsubscript{\textalpha} family preferentially rearranged in immature B-cell tumour. Nature (Lond.). 331:446.
5. Kipps, T.J., E. Tomhave, L.F. Pratt, S. Duffy, P.P. Chen, and D.A. Carson. 1989. Developmentally restricted immunoglobulin heavy chain variable gene expressed at high frequency in chronic lymphocytic leukemia. Proc Natl. Acad. Sci. USA. 86:5913.
6. Logtenberg, T., M.E.M. Schutte, G. Inghirami, J.E. Berman, F.H.J. Mgelin-Meyling, R.A. Insel, D.M. Knowles, and F.W. Alt. 1989. Immunoglobulin V\textsubscript{\textalpha} gene expression in human B cell lines and tumours: biased V\textsubscript{\textalpha} gene expressed in chronic lymphocytic leukemia. Int. Immunol. 1:362.
7. Mayer, R., T. Logtenberg, J. Strauchen, A. Dimitriu-Bona, L. Mayer, S. Mechanic, N. Chiorazzi, L. Borche, G. Dighiero, A. Mannheimer-Lory, et al. 1990. CD5 and immunoglobulin V gene expression in B-cell lymphomas and chronic lymphocytic leukemia. Blood. 75:1518.
8. Deane, M., and J.D. Norton. 1990. Immunoglobulin heavy chain variable region family usage is independent of tumor cell phenotype in human B lineage leukemias. Eur. J. Immunol. 20:2209.
9. Logtenberg, T., F.M. Young, J.H. Van Es, F.H.J. Mgelin-Meyling, and F.W. Alt. 1989. Autoantibodies encoded by the most J\textsuperscript{\textalpha}-proximal human immunoglobulin heavy chain variable region gene. J. Exp. Med. 170:1347.
10. Meeker, T.H., J.C. Grimaldi, R. O'Rourke, J. Loeb, G. Juliusson, and S. Einhorn. 1988. Lack of detectable somatic hypermutation in the V region of the IgH chain gene of a human chronic B lymphocytic leukemia. J. Immunol. 141:3994.
11. Kipps, T.J., E. Tomhave, P.P. Chen, and D. Carson. 1988. Autoantibody-associated k light chain variable region gene expressed in chronic lymphocytic leukemia with little or no mutation: implication for etiology and immunotherapy. J. Exp. Med. 167:840.
12. Kipps, T.J., S. Fong, E. Tomhave, P.P. Chen, R.D. Goldfien, and D.A. Carson. 1987. High-frequency expression of a conserved k light chain variable region gene in chronic lymphocytic leukemia. Proc. Natl. Acad. Sci. USA. 84:2916.
13. Schroeder, H.W., and Y.Y. Wang. 1990. Preferential utilization of conserved immunoglobulin heavy chain variable gene segments during human fetal life. Proc. Natl. Acad. Sci. USA. 87:6146.
14. Levy, R., S. Levy, M.L. Cleary, W. Carroll, S. Kon, J. Bird, and J. Sklar. 1987. Somatic mutation in human B-cell tumors. *ImmunoL Res.* 96:43.
15. Casali, P., and A.L. Notkins. 1989. CD5+ B lymphocytes, polyclonal antibodies and the human B-cell repertoire. *ImmunoL Today.* 10:364.
16. Hardy, R.R., and K. Hayakawa. 1988. Normal, autoimmune and malignant CD5+ B cells: the Ly-1 B lineage? *Annu. Rev. ImmunoL.* 6:197.
17. Hardy, R.R., K. Hayakawa, M. Shimizu, K. Yamasaki, and T. Kishimoto. 1987. Rheumatoid factor secretion from human Leu1+ B cells. *Science (Wash. DC).* 236:81.
18. Stall, A.M., M.C. Farinas, D.M. Tarlinton, P.A. Lalor, L.A. Herzenberg, S. Strober, and L.A. Herzenberg. 1988. Ly-1 B-cell clones similar to human chronic lymphocytic leukemias routinely develop in older normal mice and young autoimmune (New Zealand Black-related) animals. *Proc. NatL Acad. Sci. USA.* 85:7312.
19. Pennell, C.A., L.W. Arnold, G. Haughton, and S.H. Clark. 1988. Restricted immunoglobulin variable region gene expression among Ly-1+ B cell lymphomas. *J. Immunol.* 141:2788.
20. Hardy, R.R., C.E. Carmack, S.A. Shinton, K.J. Riblet, and K. Hayakawa. 1989. A single V\(_\gamma\) gene is utilized predominantly in anti-BrMRBC hybridomas derived from purified Ly-1 B cells: definition of the V\(_\gamma\)11 family. *J. Immunol.* 142:3643.
21. Foon, K.A., and R.F. Todd. 1986. Immunologic classification of leukemia and lymphoma. *Blood.* 68:1.
22. Casali, P., G. Inghirami, M. Nakamura, T.F. Davies, and A.L. Notkins. 1986. Human monoclones from antigen-specific selection of B lymphocytes and transformation by EBV. *Science (Wash. DC).* 236:476.
23. Sambrook, J., E.F. Fritsch, and S. Maniatis. 1989. Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Press, Cold Spring Harbor, NY. 8.11–8.20.
24. Tucker, P.W. 1986. Construction and screening of recombinant DNA libraries in bacteriophage lambda. In *Handbook of Experimental Immunology*. Genetics and Molecular Immunology. Vol. 3. D.M. Weir, editor. Blackwell Scientific Publications, Ltd., Edinburgh. 1–16.
25. Blin, N., and D.W. Stafford. 1976. A general method for isolation of high molecular weight DNA from eukaryotes. *Nucleic Acids Res.* 3:2303.
26. Ravetch, J.V., U. Siebenlist, S. Korsmeyer, T. Waldmann, and P. Leder. 1981. Structure of the human immunoglobulin 1 locus: characterization of embryonic and rearranged J and D genes. *Cell.* 27:583.
27. Sanger, F.S., S. Nickler, and A.R. Coulson. 1977. DNA sequencing with chain termination inhibitors. *Proc. NatL Acad. Sci. USA.* 74:5463.
28. Shlomchik, M.J., A.H. Aucoin, D.S. Pisetsky, and M.G. Weigert. 1987. Structure and function of anti-DNA antibodies, derived from a single autoimmune mouse. *Proc. NatL Acad. Sci. USA.* 74:5463.
29. Yamada, M., R. Wasserman, B.A. Reichard, S. Shane, A.J. Caton, and G. Rovera. 1991. Preferential utilization of specific immunoglobulin heavy chain diversity and joining segments in adult human peripheral blood B lymphocytes. *J. Exp. Med.* 173:395.
30. Victor, K.D., I. Randen, K. Thompson, O. Forre, J.B. Natvig, S.M. Fu, and J.D. Capra. 1991. Rheumatoid factors isolated from patients with autoimmune disorders are derived from germline genes distinct from those encoding the Wa, Po and Blc cross-reactive idiotypes. *J. Clin. Invest.* 87:1603.
31. Van der Heijden, R.W., H. Bunschoten, V. Pascual, F.G. Uytdehaag, A.D. Osterhaus, and J.D. Capra. 1990. Nucleotide sequence of a human monoclonal anti-idiotypic antibody specific for a rabies virus-neutralizing monoclonal idiotype antibody reveals extensive somatic variability suggestive of an antigen-driven immune response. *J. Immunol.* 144:2835.
32. Sanz, I., P. Casali, J.W. Thomas, A.L. Notkins, and J.D. Capra. 1989. Nucleotide sequence of eight human natural autoantibody V\(_\gamma\) regions reveals apparent use of V\(_\gamma\)\(_{11}\) families. *J. Immunol.* 142:4054.
33. Berman, J.E., C.G. Humphries, J. Barth, F.W. Alt, and P.W. Tucker. 1991. Structure and expression of human germline V\(_\gamma\)\(_{11}\) transcripts. *J. Exp. Med.* 173:1529.
34. Yancopoulos, G.D., and F.W. Alt. 1985. Developmentally controlled and tissue-specific expression of unarranged V\(_\gamma\) gene segments. *Cell.* 40:271.
35. Umar, A., P.A. Schweitzer, N.S. Levy, J.D. Gearhart, and P.J. Gearhart. 1991. Mutation in a reporter gene depends on proximity to and transcription of immunoglobulin variable transgenes. *Proc. NatL Acad. Sci. USA.* 88:4902.
36. McGrath, M.S., G. Tamura, and I.L. Weissman. 1985. The receptor-mediated leukemogenesis hypothesis: a model of retrovirus oncogenesis by viral stimulation of cell-surface receptor. *In Leukemia.* I.L. Weissman, editor. Academic Press, NY. 235–249.
37. Mann, D.L., P. Desantis, G. Mark, A. Pfeifer, M. Newman, N. Gibbs, M. Popovic, M.G. Sarngadharan, R.C. Gallo, J. Clark, et al. 1987. HTLV-I-Associated B-cell Leukemia. I.L. Weissman, editor. Academic Press, NY. 235–249.
38. Burastero, S.E., and P. Casali. 1989. Characterization of human CD5 (Leu-1, OKT-1)+ B lymphocytes and the antibodies they produce. *ConcL Microb. Immunol.* 11:231.
39. Burastero, S.E., P. Casali, R.L. Wilder, and A.L. Notkins. 1988. Monoreactive high affinity and polyreactive low affinity rheumatoid factors are produced by CD5+ B cells from patients with rheumatoid arthritis. *J. Exp. Med.* 168:1979.
40. Harindranath, N., I.S. Goldfarb, H. Ikematsu, S.E. Burastero, R.L. Wilder, A.L. Notkins, and P. Casali. 1991. *Int. Immunol.* 3:865.
41. Shlomchik, M., M. Mascelli, H. Shan, M.Z. Radic, D. Pisetsky, A. Marshak-Rothstein, and M. Weigert. 1990. Anti-DNA antibodies from autoimmune mice arise by clonal expansion and somatic mutation. *J. Exp. Med.* 171:265.