Somatic Diversification and Selection of Immunoglobulin Heavy and Light Chain Variable Region Genes in IgG+CD5+ Chronic Lymphocytic Leukemia B Cells

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Summary

Chronic lymphocytic leukemia (CLL) is characterized by the clonal expansion of CD5-expressing B lymphocytes. Most studies have found that these leukemic CD5+ B cells, like their normal counterparts, use immunoglobulin (Ig) variable (V) region genes that exhibit minimal, if any, somatic diversity. These and other observations have suggested that CD5+ B cells may be incapable of generating Ig V gene diversity, and therefore may not be able to develop higher affinity binding sites that could be selected by antigen. However, most of the studies of CLL and normal CD5+ B cells have focused on IgM-producing cells. Since somatic mutations are most often seen in B cells that have undergone an isotype class switch, we analyzed the Ig heavy (H) and light (L) chain variable region genes of seven IgG+CD5+ CLL B cells to determine if somatic diversification and antigen selection had occurred. The data derived provide evidence for skewed use, somatic diversification, and antigenic selection of the Ig V region genes. Nonrandom use of both H and L chain variable region genes was manifested by an overrepresentation of VH4 and VL family genes and the underrepresentation of the JH4 gene segment. Furthermore, VH4 gene use was restricted to only two family members (4.21 and 4.18). In four of the seven cases, the VH and VL genes displayed >5% difference from the most homologous known germline counterparts. Polymerase chain reaction and Southern blot analyses performed in two of these patients demonstrated that their unique VH CDR2 and adjacent sequences were not present in their germline DNA. In addition, a significant level of diversity was seen in the rearranged DJ8 segments and at the VL-JL junctions of every patient that occurred both at the time of recombination and subsequently. The localization of replacement changes to complementarity determining regions of some patients suggested that antigen selection had occurred. Furthermore, the mutations identified in the VH and VL genes of each individual patient were strikingly similar, both in number and location. Collectively, the data indicate that a subset of CD5+ CLL B cells can display Ig V region gene mutations. In addition, they are consistent with the notions that in some cases antigen selection of these mutations may have occurred, and that antigen stimulation may be a promoting factor in the evolution of certain CLL clones.

Abbreviations used in this paper: CDR, complementarity determining region; CLL, chronic lymphocytic leukemia; FR, framework region; RT, reverse transcriptase.

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tation (2), this principle was challenged by the study of Cai et al. (6), which documented V\textsubscript{\mu} gene mutations among a cohort of CLL patients whose malignant clone used the V\textsubscript{\mu}251 gene. Surprisingly, however, a follow-up study (7) of a larger, well-characterized group of patients whose leukemic cells used the same gene failed to confirm these findings, thus leaving this dilemma unresolved.

It is important to note, however, that most of these studies of CD5\textsuperscript{+} CLL B cells have dealt with IgM-producing cells. Since antigen stimulation resulting in somatic diversification may occur more often at the time of or among isotype-switched B cells (8), CD5\textsuperscript{+} B cells producing non-IgM antibodies may exhibit different features, both at the protein and nucleic acid levels. To test this hypothesis and to help resolve the issue of the capacity of these cells to somatically diversify their Ig V genes, we have analyzed the Ig V genes produced by CD5\textsuperscript{+} CLL cells that produce IgG antibodies. The data demonstrate an overrepresentation of Ig V\textsubscript{\mu} and V\textsubscript{\gamma} family genes and an underrepresentation of a frequently employed J\textsubscript{\delta} segment. In addition, there is strong evidence that family genes and an underrepresentation of a frequently employed J\textsubscript{\delta} segment. In addition, there is strong evidence that these mutations may have been antigen driven and selected.

Materials and Methods

**CLL Cells and Heterohybridomas.** Seven patients with CLL whose CD5\textsuperscript{+} leukemic B cell clones produced IgG were studied. As recently reported (9), each patient exhibited clinical features typical for CLL, with stages ranging from 0 to IV (Rai classification; 10). In addition, all exhibited an expanded population of circulating IgG\textsuperscript{+}CD5\textsuperscript{+} B cells as determined by immunofluorescence, antibodies were used by the heterohybridomas were derived from the CLL clones overexpanded in vivo. All seven hybridomas expressed V\textsubscript{\gamma} family genes and an underrepresentation of a frequently employed J\textsubscript{\delta} segment. In addition, there is strong evidence that these mutations may have been antigen driven and selected.

**Reverse Transcriptase (RT) PCR.** The primers for the V\textsubscript{\mu} and V\textsubscript{\gamma} families and C\textsubscript{\mu} and C\textsubscript{\nu} amplification were as reported in references 13 and 14, respectively. Total RNA (500 ng) was reverse transcribed by use of avian myeloblastosis virus RT (GIBCO BRL, Gaithersburg, MD) and the appropriate C\textsubscript{\mu} or C\textsubscript{\nu} primer, and then specific amplification of the cDNA was accomplished as described (15).

**Cloning and Sequencing of PCR Products.** PCR products were force cloned into the pUC19 vector after digestion with EcoRI and HindIII. Clones were isolated and sequenced in both directions by the Sanger dideoxy chain termination method (16), either manually or by an automated sequenator (model 373A; Applied Biosystems, Inc., Foster City, CA) using the DyeDeoxy™ terminator kit (Applied Biosystems Inc.).

**Southern Blot Analysis.** Genomic DNA was amplified by PCR with selected primers, electrophoresed through 1% agarose gel, transferred onto nylon membranes (Separation, Inc., Westboro, MA), and UV cross-linked as described previously (15). Blots were hybridized with 32P-labeled probes in hybridization solution (QuickHyb, Stratagene Inc., La Jolla, CA) according to the manufacturer's instructions. When appropriate, stripping was accomplished by incubation at 72°C for 1 h in 2.5 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, and 0.5 mM sodium pyrophosphate. After washing, blots were exposed overnight on photographic film (XAR5; Eastman Kodak Co., Rochester, NY).

Results

**V\textsubscript{\mu} Gene Segment Analyses.** Fig. 1 lists the Ig V\textsubscript{\mu} gene sequences determined. Computer comparisons (17) of these with their presumed germline counterparts indicated the following features.

**Biased V\textsubscript{\mu} Gene Usage.** Only two gene families were used, with five patients' cells using V\textsubscript{\mu}4 family genes and two V\textsubscript{\mu}3 family genes. Of the 12 or more members of this moderately sized family (18), only 2 were represented in this group: V\textsubscript{\mu}4.21 (n = 3; patients 001, 033, and 055) and V\textsubscript{\mu}4.18 (n = 2; 039 and 057). Because of the small number of V\textsubscript{\mu}3 genes found, no conclusions about gene frequency use can be drawn.

**Different Degrees of Similarity with Germline Genes.** Two of the V\textsubscript{\mu}4 CLL samples (patients 039 and 057) exhibit a high degree of homology (>98.5%) with the presumed germline ancestor (Fig. 1), whereas the other three (001, 033 and 055) display considerably less identity (95, 92, and 91%, respectively). Similarly, one of the V\textsubscript{\mu}3 cases (040) is highly homologous with a germline gene (>98.5%), whereas much less similarity (94%) is observed in the other (030). The three V\textsubscript{\mu}4 CLL B cells that show significant differences from their ancestral gene (<95% homology; 001, 033, and 055) all use the V\textsubscript{\mu} 4.21 gene. Those patients using the V\textsubscript{\mu} 4.18 gene exhibit >98.5% homology to the germline.

**Differences in Distribution of Nucleotide Changes.** For certain patients (001 and 033), the differences from the ancestral genes are located throughout the V\textsubscript{\mu} segment (Fig. 1), whereas for others (030 and 055), differences are nonrandomly distributed. In these latter two patients, the differences cluster predominantly within complementarity determining region (CDR)2 for patient 030 and within CDR2 and FR3 for patient 055.

**Differences in Deduced Amino Acid Sequences.** When the nucleotide differences of the patients are analyzed for their effects on the amino acid sequence of the deduced Ig H chain proteins (Fig. 2A), there are striking differences in replacement/silent (R/S) substitution ratios for certain patients. For example, for patient 030, the R/S of CDR 1,2 is 7.0 and that of framework regions (FR) 1,2,3 is 0.6. In contrast, for patient 033, the R/S in CDR 1,2 is 0.8 and in FR 1,2,3 is 1.3. Patient 055 illustrates an overall R/S for CDR 1,2 of 1.7 and for FR 1,2,3 of 1.3, although most of these latter substitutions occur in a defined region of FR3.
Absence of the Patient-specific Sequences in Germline DNA

Next we investigated whether the differences in the V4 gene segments observed in patients 030 and 055 were somatically generated. These patients were chosen since their V4 sequences were significantly different from the presumed germline counterparts, and these differences clustered within areas of the gene that would likely directly influence the shape of the antigen-binding groove.

**PCR Analyses.** The first approach involved the construction of two sets of PCR primer pairs, one that would allow amplification of a region encompassing the entire CDR2 and portions of FR2 and FR3 of all genes of the two families involved (V43 and V44), and another set for amplification of only the unique sequences from the two individual patients (CDR2 for patient 030 and CDR2 plus part of FR3 for patient 055). The former are designated G for general primers, and the latter S for specific primers. These primer pairs were used to amplify either unrearranged neutrophil DNA or rearranged lymphocyte DNA. Fig. 3 indicates the results obtained for patient 055.

Fig. 3, Lanes 1 and 2 indicate the positions in the gel of

1509 Hashimoto et al.
the V\textsubscript{H} 4.21 segment amplified from RNA by RT/PCR with the G and S primer pairs, respectively. Amplification of both germline (lane 3) and rearranged (lane 5) DNA with the G primer set resulted in appropriately sized products. Amplification of the same DNA with the S primer pair (lanes 4 and 6) failed to reveal readily detectable bands. Since the inability to consistently amplify a product may be due to gene copy number, aliquots (1 \mu l) of the products of the G primer amplifications from both unrearranged and rearranged DNA, which should have increased specifically the frequency of all V\textsubscript{H} 4. CDR2 regions, were reamplified with the S primers. After reamplification, only the rearranged DNA yielded an appropriate product (lane 8). Products corresponding in size to the specific mutated sequence never were detected with germline DNA (lane 7). Faint bands corresponding to the G products can be seen for each sample (lanes 7 and 8) because of the transfer of G primers from the original reactions of lanes 3 and 5. Most importantly, DNA sequence analyses of the isolated G and S bands from lanes 7 and 8, respectively, revealed identity with those presented in Fig. 1 (data not shown).

As a further specificity control, these primers were used to amplify the G and S sequences from another patient whose CLL B cells used the same Ig V\textsubscript{H} 4.21; patient 001. An appropriately sized product was obtained from rearranged DNA with the G primers (lane 11), but not with the S primers (lane 12). In addition, products could not be found when the G product was reamplified with the S primers (lane 13), indicating that the S sequence was uniquely expressed only in CLL 055 B cells and not in a patient using the same ancestral V\textsubscript{H} 4. gene. Identical results were obtained by performing similar studies with patient 030 (data not shown).

**Southern Blotting Analyses.** A second approach involved the probing of unrearranged DNA with radiolabeled oligonucleotides specific for the mutation-specific or conserved se-
quences of each patient. In these studies, germline and rearranged DNA were amplified with the same sets of general G primers described above and then exposed to probes complementary to either the unique CDR2 sequence or the conserved FR sequence as a positive control. Fig. 4 indicates the results of these experiments for patient 030. Hybridization with the mutation-specific probes occurred only with the rearranged DNA, whereas hybridization of the conserved probe was seen with both the unarranged and rearranged DNA. These differences in binding of the mutation-specific probes occurred despite the use of longer incubation times (16 vs. 2 h) and less stringent hybridization buffers and washing regimens (2 vs. 0.1 × SSC; room temperature vs. 40°C) than those used for the conserved probes.

D and Jḥ Gene Segment Analyses

The sequences of the D and Jḥ segments expressed by the CD5+ B cells are listed in Fig. 5, A and B.

Nonrandom Jḥ Gene Usage. Jḥ5 and Jḥ6 gene segments were used by six of the seven patients. Surprisingly, there were no examples of Jḥ4 gene use in this cohort, even though this is the most commonly used Jḥ segment in healthy adults (19). D gene use appeared random, although homologous germline D segments could not be accurately defined in two patients (030 and 055).

Somatic Diversification of D and Jḥ Segments. Among those CD5+ B cells with clearly definable D segments, varying degrees of identity with the germline gene segments existed (Fig. 5 A), with patients 001 and 057 being the most homologous (92.8 and 90.5%) and patients 033, 039, and 040 being the least (67.7, 58.8, and 47.7%, respectively). Ancestral germline D segments could not be assigned for patients 030 and 055. Indeed, in these cases, it appeared that the majority (030) or the entire (055) D segment was deleted at the time of recombination and replaced by N addition. It is noteworthy that the V h genes of these patients were two of the most divergent from their germline counterparts (Fig. 1).

The Jḥ segments also show significant alterations from the germline, with examples of both trimming at the D-J joints and internal deletion (Fig. 5 B). Furthermore, significant numbers of N additions were identifiable for all patients except 040.

Finally, presumptive nucleotide substitutions were found in the Jḥ and D segments of all patients in whom likely germline progenitor D segments could be identified. Patients 039 and 040 are noteworthy since in both cases the level of V h gene diversification was negligible, whereas the levels of DJ, mutation were considerable.

Deduced Amino Acid Differences in CDR3. The nucleotide changes in the D and Jḥ segments resulted in CDR3 lengths that were quite disparate, ranging from 11 to 23 amino acids (Fig. 2 A). A dramatic similarity in amino acid sequence was found in the CDR3 of patients 039 and 057. This sequence similarity includes an identical stretch of 6 amino acids that is separated by four mismatches from another identical stretch of 5 amino acids.

Reidentification of CLL-unique Sequences in Freshly Isolated CD5+ CLL B Cells

Since this study spanned 3 yr, attempts were made to determine if new mutations had occurred in vivo over this time frame. Patients 001, 030, 033, 039, and 057 were reanalyzed. Patients 055 and 040 could not be included in this evaluation since the former was treated with fludarabine that essentially eliminated circulating CLL B cells, and the latter had died. The unique V gene arrays that had been created by mutation, N addition, and DJ, segment substitution and deletion served as unequivocal signatures of the CLL clones.

RNA was obtained from freshly isolated B cells, reverse transcribed, amplified with appropriate patient-specific FR2 upstream primer and the Cy downstream primer, and sequenced. In three patients (001, 033, and 039), the newly defined sequences were identical to those listed in Fig. 1 (data not shown). A single-point alteration that was not productive of an amino acid change was found in patient 030. The most significant differences were found in patient 057, in whom three point mutations in CDR 2 and 3 were detected among two clones; one of these (G → A) resulted in an amino acid change (Ser → Asn) at codon 52 of CDR2.

V, Gene Segment Analyses

Fig. 6 lists the Ig V, gene sequences determined and the computer comparisons with the presumed germline counterparts.

Nonrandom V, Gene Family Use. Of the six CLL clones expressing κ chains, five used a V,1 family gene (patients 030, 040, 055, and 057). However, unlike the Ig V,4 gene use, which was restricted to only two individual family members, four different V,1 family genes were represented (Fig. 6 and Table 1).

Different Degrees of Similarity with Germline V, Genes That Parallel Those of the Corresponding V h Gene. The V,1 genes
used by patients 040, 039, and 057 displayed complete identity with the germline genes L9 and O2, respectively, and therefore are not listed on Fig. 6. In contrast, the V_{L} genes used by patients 001, 030, 033, and 055 exhibited >5% divergence from the most homologous germline counterparts. These levels of difference parallel those seen among the Ig V_{H} genes of each patient.

Figure 5. Nucleotide sequences of the CDR3 and FR4 regions of the H chains. (A) Sequences of the CLL D gene segments compared with their most likely germline counterpart. Those listed as "unassignable" were not sufficiently similar to 24 reported sequences to make a comparative ancestral assignment. (B) Comparison of germline and CLL J_{\mu} gene segments.

Figure 6. Nucleotide sequences of V_{L} genes. Since the V_{L} gene sequence for patient 040 is identical to the L9 germline gene and those of patients 039 and 057 are identical to the O2 germline gene, they are not listed. These sequence data are available from EMBL/GenBank/DDBJ under accession numbers X84340-X84346.
Table 1. Serologic and Genetic Characteristics of IgG\(^+\)CD5\(^+\) CLL B Cells*

| Patient | C\(_n\) | CR1\(^*\) | V\(_n\) family | Most homologous germline | V\(_n\) gene | V\(_n\) gene difference | Likely germline D segment | J\(_n\) | N addition/trimming\(^5\) | Somatic\(^1\) mutation V\(_n\)/D\(_n\) | Possible antigenic selection |
|---------|--------|----------|---------------|--------------------------|-------------|-------------------------|---------------------------|-------|-----------------|-----------------------------|-----------------------------|
| CLL 001 | γ1     | –        | 4             | 4.21                     | 5           | D21/9                   | 6                         | +/+   | +/+/+           | -                           | -                           |
| CLL 033 | γ1     | F4       | 4             | 4.21                     | 8           | DLR2                    | 6                         | +/+   | +/+/+           | -                           | -                           |
| CLL 055 | γ1     | F4       | 4             | 4.21                     | 9           | ?                       | 5                         | +/+   | +/+/+           | +/−                         | +/−                         |
| CLL 039 | γ3     | –        | 4             | 4.18                     | 1           | DM1                     | 2                         | +/+   | −/+++           | +                           | +                           |
| CLL 057 | γ3     | –        | 4             | 4.18                     | 1           | DN1                     | 5                         | +/+   | −/+             | +                           | +                           |
| CLL 030 | γ1     | F4       | 3             | H11                      | 6           | ?                       | 5                         | +/+   | +/+/+           | +                           | +                           |
| CLL 040 | γ1     | –        | 3             | 1.9III                   | 2           | DLR2                    | 6                         | −/+   | −/+/+           | +                           | +                           |

**H chain**

| Patient | C\(_i\) | CR1\(^*\) | V\(_i\) family | Most homologous germline | V\(_i\) gene | V\(_i\) gene difference | J\(_i\) | N addition/trimming\(^5\) | Somatic\(^1\) mutation V\(_i\)/J\(_i\) | Possible antigenic selection |
|---------|--------|----------|---------------|--------------------------|-------------|-------------------------|-------|-----------------|-----------------------------|-----------------------------|
| CLL 001 | λ      | –        | λ1-c          | DPL8                     | 6           | JA2/3                   | −/+   | +/+             | −                           | -                           |
| CLL 033 | κ     | 3I       | κIIb          | A27                      | 5           | JK1                     | −/+   | +/+             | −                           | -                           |
| CLL 055 | κ     | 3I       | κI            | L19                      | 7           | JK4                     | +/+   | +/+/+           | +/−                         | +/−                         |
| CLL 039 | κ     | –        | κI            | O2                       | 0           | JK2                     | +/+   | −/+             | +                           | +                           |
| CLL 057 | κ     | –        | κI            | O2                       | 0           | JK1                     | +/+   | −/+             | +                           | +                           |
| CLL 030 | κ     | 3I       | κI            | L12a                     | 7           | JK4                     | −/+   | +/+/+           | +                           | +                           |
| CLL 040 | κ     | 3I       | κI            | L9                       | 0           | JK1                     | −/+   | −/+             | −                           | -                           |

* Adapted, in part, from references 9 and 22.

\(^\dagger\) Cross-reactive idiotypic (CR1) determinants detected by solid-phase RIA as described (33, 34).

\(^5\) Notations preceding the slash (/) relate to the relative numbers of N additions; notations after the slash relate to degrees of trimming at joints.

\(^1\) Notations preceding the slash relate to the relative number of mutations in V\(_n\) or V\(_i\); those after the slash relate to DJ\(_n\) and J\(_i\) segments.

R/S substitution ratios >3 for the CDR and <1 for the FR (Fig. 2 B). These patterns closely resemble those seen for the corresponding H chain gene in almost every case.

**Random Use of J\(_i\) Gene Segments.** Unlike the nonrandom use of J\(_n\), J\(_i\) families are used in an apparently stochastic fashion (Fig. 7). In every case, there is evidence for trimming of the J\(_i\) segments as well as examples of a limited number of N additions in patients 039, 040, and 057 (Fig. 7). The combination of trimming and N addition has resulted in V\(_i\)-J\(_i\) junctional diversity in each case.

**Discussion**

The preceding data demonstrate that certain CD5\(^+\) CLL B cells display skewed use, somatic diversification, and evidence consistent with antigenic selection of their Ig H and L chain V region genes. Table 1 collates the genetic data from this study and the serologic data from a companion study (9) to provide a comprehensive view of the characteristics of these cells.

**Gene Segment Use.** Evidence for nonrandom use of both H and L chain V region genes was manifested as an over-representation of V\(_n\)\(_4\) and J\(_i\)I family genes and under-representation of the J\(_i\)\(_4\) gene segment. V\(_n\)\(_4\) gene use was restricted further to only two (4.21 and 4.18) of the 12-16 members of the family (18). A restriction to specific gene members was not seen for J\(_i\)I, although the O2 gene was expressed in two patients.

The frequency of V\(_n\)\(_4\) gene use among our patients is
not from a composite of the available genes 4.21 and 4.18 is striking considering that two prior studies failed to document such a restricted use of individual genes within a V\textsubscript{H} family in CLL B cells (23, 24). Furthermore, the absence of J\textsubscript{4} use by our patients’ B cells is significantly different not only from healthy adult blood B cells (p < 0.003; 19) but also from most CLL patients (p < 0.002; 21). In addition, the overrepresentation of V\textsubscript{H} family genes in our patients differs from that of the V\textsubscript{H}IIIb gene humkv325/A27 reported in other cohorts with inflammatory diseases like rheumatoid arthritis (32).

Multiple somatic changes not related to the recombination process also occurred in regions of these genes. Four patients showed significant variations of their H and L chain V genes from the reported germline counterparts. The level and location of these differences in the H and L chains were quite similar within an individual patient’s leukemic clone. The possibility that these differences reflected the presence of heretofore unreported gene segments was ruled out for the two patients studied in detail (030 and 055). Interestingly, a cross-reactive idiotype found almost exclusively on mutated IgG autoantibodies and not within the germline (P4; 33) was expressed by these two IgG molecules as well as that produced by patient 033 (9 and Table 1), providing serologic corroboration of the molecular data obtained.

However, mutations were not seen in the V\textsubscript{H} genes of all patients studied. Specifically the two patients using the V\textsubscript{H}4.18 gene demonstrated very minor differences from the germline counterpart. In contrast, the three patients that used the V\textsubscript{H}4.21 gene demonstrated significant somatic diversification. This observation confirms those recently reported (21, 35) on the discordant occurrence of mutations in these two gene segments in CLL.

The diversification detected in the IgG-producing B cells of these patients is not restricted to the V\textsubscript{H} or V\textsubscript{L} segments. Essentially all of the patients demonstrated differences from the germline D and J\textsubscript{H} segments, and every patient showed diversity at the V\textsubscript{H}-J\textsubscript{H} junctions. These latter changes are striking since they result in the acquisition of a positively charged arginine residue at the joints in two patients (039 and 057) and the creation of a threonine residue one position downstream from the joints in five patients (030, 033, 040, 055, and 057).

Most previous reports suggest that CD5\textsuperscript{+} CLL B cells undergo little somatic diversification (2), although certain studies have contained occasional patients whose V genes diverge from the presumed germline counterparts (for review...
junctions. Although the H chain disparities could have arisen by V. gene replacement, the lack of mutation seen in the corresponding VL segments of these same patients suggests that this mechanism is a less likely explanation.

In support of the latter possibility are reports of V. (36) and V. (37) gene mutations in CLL patients whose B cells lacked CD5 expression. Although our patients display typical features of CLL and their expanded leukemic clones express CD5, they do comprise a subset defined by the production of IgG antibodies (9). Thus, our data are compatible with both the studies of Cai et al. and Rassenti and Kipps, providing clear evidence for somatic diversification of V region genes in a subset of leukemic CD5^+ B cells that have undergone an isotype class switch. Furthermore, our studies indicate that mutations can occur in the V genes of both the H and L chains and that the numbers and locations of these changes are closely paralleled in individual patients. However, it should be noted that the extent of mutation documented in these leukemic CD5^+ B cells is not as extensive as that observed in B cells secreted by these CD5^+ CLL cells did not react significantly with a panel of six classical autoantigens (9). This observation appears to relate at least in part to antibody valency (9). However, in addition, the somatic mutations documented here may have sufficiently altered their binding sites away from reactivity with an original (auto)antigen in favor of another or of an exoantigen (46, 47).

Finally, these studies suggest that our understanding of the triggering capabilities and requirements for CLL cells may be too simplistic. Somatic diversification of normal CD5^+ B cells appears to be initiated by antigen, require T cell help, and occur in germinal centers, whereas CD5^+ B cells are considered to be triggered by T cell-independent antigens, presumably in the mantle zones of lymph nodes (for review...
see reference 48). However, although studies in humans support this general view (49), others also indicate that human CD5+ B cells, both normal and leukemic, can respond to T cell–derived stimuli (50, 51). Indeed, the preferential use by our patients’ CD5+ B cells of γ1 and γ3 subclass genes and the absence of γ2 (Table 1; 9) might favor the latter mechanism, since studies suggest that, in general, T cell–dependent protein antigens induce switching to IgG1 and IgG3, while switching to IgG2 frequently can be a function of T cell–independent polysaccharide antigens (for a review see reference 52). Finally, since at least in the murine system it is thought that CD5+ B cells may represent a different cellular lineage (53) that follows a distinct differentiation pathway, such cells might use different mechanisms or anatomic sites to achieve somatic diversification. Similarly, diversification might occur in a distinct subset of these cells, due either to inherent subset differences or differences in accessory cell or T cell helper function. The latter might be especially relevant since we have shown previously that most IgM-producing CLL patients have diminished T cell helper function (54). The surface phenotypes of these IgG+CD5+ CLL B cells and their T cell functions are being evaluated to determine whether unique features exist for these particular cases.

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References

1. Dighiero, G., P. Travade, S. Chevret, P. Fenaux, C. Chastang, and J.L. Binet. 1991. B-cell chronic lymphocytic leukemia: present status and future directions. Blood. 78:1901–1914.

2. Kipps, T. 1993. Immunoglobulin genes in chronic lymphocytic leukemia. Blood Cells. 19:615–625.

3. Broker, B.M., A. Klajman, P. Youinou, J. Jouquan, C.P. Worman, J. Murphy, L. Mackenzie, R. Quatray-Papafso, M. Blaschek, P. Collins, et al. 1988. Chronic lymphocytic leukemia cells secrete multispecific antibodies. J. Autoimmun. 1:469–481.

4. Sthoeger, Z.M., M. Wàkai, D.B. Tse, V.P. Vinciguerra, S.L. Allen, D.R. Budman, S.M. Lichtman, P. Schalman, L.R. Weiselberg, and N. Chiorazzi. 1989. Production of autoantibodies by CD5-expressing B lymphocytes from patients with chronic lymphocytic leukemia. J. Exp. Med. 169:255–268.

5. Borche, L., A. Lim, J.L. Binet, and G. Dighiero. 1990. Evidence that chronic lymphocytic leukemia B lymphocytes are frequently committed to production of natural autoantibodies. Blood. 76:562–569.

6. Cai, J., C. Humphries, A. Richardson, and P.W. Tucker. 1992. Extensive and selective mutation of a rearranged V H gene in human B cell chronic lymphocytic leukemia. J. Exp. Med. 176:1073–1081.

7. Rassenti, L.Z., and T.J. Kipps. 1993. Lack of extensive mutation in the V H genes used in common B cell chronic lymphocytic leukemia. J. Exp. Med. 177:1039–1046.

8. Gearhardt, P.J., N.D. Johnson, R. Douglas, and L. Hood. 1981. IgG antibodies to phosphorylcholine exhibit more diversity than their IgM counterparts. Nature (Lond.). 291:29–34.

9. Wàkai, M., S. Hashimoto, M. Omata, Z.M. Sthoeger, S.L. Allen, S.M. Lichtman, P. Schalman, V.P. Vinciguerra, B. Diamond, M. Dono, et al. 1994. IgG*, CD5+ human chronic lymphocytic leukemia B cells. Production of IgG antibodies that exhibit diminished autoreactivity and IgG subclass skewing. Autoimmunity. 19:39–48.

10. Rai, K.R., A. Sawitsky, E.P. Cronkite, A.D. Chanana, R.N. Levy, and B.S. Pasternak. 1975. Clinical staging of chronic lymphocytic leukemia. Blood. 46:219–234.

11. Deane, M., and J.D. Norton. 1991. Immunoglobulin gene “fingerprinting”: an approach to analysis of B lymphocyte clonality in lymphoproliferative disorders. Br. J. Haematol. 77:274–281.

12. Hingorani, R., I.H. Choi, P. Akolkar, B. Gulwani-Akolkar, R. Pergolizzi, J. Silver, and P.K. Gregersen. 1993. Clonal predomination of T cell receptors within the CD8+ CD45RO+ subset in normal human subjects. J. Immunol. 151:5762–5769.

13. Larrick, J.W., L. Danielsson, C.A. Brenner, E.F. Wallace, M. Abrahamson, K.E. Fry, and C.A.K. Borrebaeck. 1989. Polymerase chain reaction using mixed primers: cloning of human monoclonal antibody variable region genes from single hybridoma cells. Biotechnology. 7:934–938.

14. Harindranath, N., H. Ikematsu, A.L. Notkins, and P. Casali. 1993. Structure of the V H and V L segments of polyreactive and monoreactive human natural antibodies to HIV-1 and E. coli B-galactosidase. Int. Immunol. 5:1523–1533.

15. Hashimoto, S., P.K. Gregersen, and N. Chiorazzi. 1993. The human Ig-β cDNA sequence, a homologue of murine B29, is identical in B cell and plasma cell lines producing all the human Ig isotypes. J. Immunol. 150:491–498.

16. Sanger, F., S. Nicklen, and A.R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA. 74:5463–5467.

17. Elgavish, R., and H.W. Schroeder, Jr. 1993. SAW: a graphical user interface for the analysis of immunoglobulin variable domain sequences. Biotechniques. 15:1066–1071.

18. Willems van Dijk, K., E.H. Sasso, and E.C.B. Milner. 1991. Polymorphism of the human Ig V H4 gene family. J. Immunol. 146:3646–3651.

19. Yamada, M., R. Wasserman, B.A. Reichard, S. Shane, A.H.
Caton, and G. Rovera. 1991. Preferential utilization of specific immunoglobulin heavy chain diversity and joining segments in adult peripheral blood B lymphocytes. J. Exp. Med. 173: 395–407.

20. 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. 75:1518–1524.

21. Schroeder, H.W., and G. Dighiero. 1994. The pathogenesis of chronic lymphocytic leukemia: analysis of the antibody repertoire. ImmunoL Today. 15:288–294.

22. Hashimoto, S., M. Wakai, J. Silver, and N. Chiorazzi. 1992. Biased usage of variable and constant region genes by IgG*, CD5 human leukemia B cells. Ann. NY Acad. Sci. 651: 477–479.

23. Stevenson, F.K., M.B. Spellerberg, J. Treasure, C.J. Chapman, L.E. Silberstein, T.J. Hamblin, and D.B. Jones. 1993. Differential usage of an Ig heavy chain variable region gene by human B-cell tumors. Blood. 82:224–230.

24. Ebeling, S.B., M.E.M. Schutte, K.E. Akkermans-Koolhaas, A.C. Bloem, F.H.J. Gmelig-Meyling, and T. Logtenberg. 1992. Expression of members of the Ig V~3 gene families is not restricted at the level of individual genes in human chronic lymphocytic leukemia. Int. Immunol. 4:313–320.

25. Kipps, T.J., S. Fong, E. Tomhave, P.P. Chen, R.D. Goldfein, and D.A. Carson. 1987. High frequency expression of a conserved x light chain variable region gene in chronic lymphocytic leukemia. Proc. Natl. Acad. Sci. USA. 84:2916–2920.

26. Mayumi, M., T. Kuritani, H. Kubagawa, and M.D. Cooper. 1983. IgG subclass expression by human B lymphocytes and plasma cells: B lymphocytes precommitted to IgG subclass can be preferentially induced by polyclonal mitogens with T cell help. J. Immunol. 130:671–677.

27. Prolland, S.S., and J.B. Nutvig. 1972. Class, subclass and allelic exclusion of membrane-bound Ig on human B lymphocytes. J. Exp. Med. 136:409–414.

28. Norris, H.H. 1992. Surface markers, heavy chain sequences and B cell lineages. Int. Rev. Immunol. 8:225–246.

29. Haughton, G., L.W. Arnold, A.C. Whitmore, and S.H. Clarke. 1992. The immunoglobulin kappa light chain repertoire in chronic lymphocytic leukemia and displaying autoantibody activity. Eur. J. Immunol. 15:288–294.

30. Victor, K.D., and J.D. Capra. 1994. An apparently common idiotype expressed in the synovium of a patient with rheumatoid arthritis. Arthritis Rheum. 35:905–913.

31. Lee, S.K., S.L. Bridges, Jr., W.J. Koopman, and H.W. Schroeder. 1992. The immunoglobulin kappa light chain repertoire expressed in the synovium of a patient with rheumatoid arthritis. Mol. Immunol. 31:39–46.

32. Lee, S.K., S.L. Bridges, Jr., W.J. Koopman, and H.W. Schroeder. 1992. The immunoglobulin kappa light chain repertoire expressed in the synovium of a patient with rheumatoid arthritis. Mol. Immunol. 31:39–46.

33. Davidson, A., A. Smith, J. Katz, J.L. Preud’homme, A. Solomon, and B. Diamond. 1989. A cross-reactive idiotype on DNA antibodies defines a heavy chain determinant present almost exclusively on IgG antibodies. J. Immunol. 143:174–180.

34. Davidson, A., J.L. Preud’homme, A. Solomon, M.-D. Chang, S. Beede, and B. Diamond. 1987. Idiotype analysis of myeloma proteins: anti-DNA activity of monoclonal immunoglobulins bearing an SLE idiotype is more common in IgG than IgM antibodies. J. Immunol. 138:1515–1518.

35. Pritsch, O., C. Magnac, G. Dumas, C. Egle, and G. Dighiero. 1993. V gene usage by seven hybrids derived from CD5^+ B-cell chronic lymphocytic leukemia and displaying autoantibody activity. Blood. 82:3103–3112.

36. Roudier, J., G.J. Silverman, P.P. Chen, D.A. Carson, and T.J. Kipps. 1990. Intraclonal diversity in the V gene expressed by CD5-negative chronic lymphocytic leukemia producing pathogenic IgM rheumatoid factor. J. Immunol. 144:1526–1529.

37. Wagner, S.D., and L. Luzzatto. 1993. V gene segments rearranged in chronic lymphocytic leukemia are distributed over a large portion of the V region and do not show somatic mutation. Eur. J. Immunol. 23:391–397.

38. Mantovani, L., R.L. Wilder, and P. Casali. 1993. Human rheumatoid B-1a (CD5^+ B) cells make somatically hypermutated high affinity IgM rheumatoid factors. J. Immunol. 151:473–488.

39. Jukes, T.H., and J.L. King. 1979. Evolutionary nucleotide replacements in DNA. Nature (Lond.). 281:605–606.

40. Radic, M.Z., M.A. Miscelli, J. Erikson, H. Shan, M. Shlomchik, and M. Weigert. 1989. Structural patterns in anti-DNA antibodies from MRL/lpr mice. Cold Spring Harbor Symp. Quant. Biol. 54:933–1001.

41. Radic, M.Z., J. Mackle, J. Erikson, C. Mol, W.F. Anderson, and M. Weigert. 1993. Residues that mediate DNA binding to autoimmune antibodies. J. Immunol. 150:4966–4977.

42. Bhat, T.N., G.A. Bentley, T.O. Fischmann, G. Boulot, and R.J. Poljak. 1990. Small rearrangements in structures of Fv and Fab fragments of antibody D1.3 on antigen binding. Nature (Lond.). 347:483–485.

43. Friedman, D.F., E.A. Cho, J. Goldman, C.E. Carmack, E.C. Besa, R.R. Hardy, and L.E. Silberstein. 1991. The role of clonal selection in the pathogenesis of an autoreactive human B cell lymphoma. J. Exp. Med. 174:525–537.

44. Bahler, D.W., and R. Levy. 1992. Clonal evolution of a follicular lymphoma: evidence for antigen selection. Proc. Natl. Acad. Sci. USA. 89:6770–6774.

45. Jain, R., S. Roncella, S. Hashimoto, A. Carbone, P. Francia di Celle, R. Foá, M. Ferrarini, and N. Chiorazzi. 1994. A potential role for antigen selection in the clonal evolution of Burt-kitt’s lymphoma. J. Immunol. 153:45–52.

46. Diamond, B., and M.D. Scharff. 1984. Somatic mutations of the T15 heavy chain gives rise to an antibody with autoantibody specificity. Proc. Natl. Acad. Sci. USA. 81:5841–5844.

47. Chen, C., V.A. Roberts, and M.B. Rittenberg. 1992. Generation and analysis of random point mutations in an antibody CDR2 sequence: many mutated antibodies lose their ability to bind antigen. J. Exp. Med. 176:855–866.

48. MacLennan, I.C.M. 1994. Germinal centers. Annu. Rev. Immunol. 12:117–139.

49. Zupo, S., M. Dono, L. Azzoni, N. Chiorazzi, and M. Ferrarini. 1991. Evidence for differential responsiveness of human CD5^+ and CD5^− B cell subsets to T cell-independent mitogens. Eur. J. Immunol. 21:351–359.

50. Fu, S.M., N. Chiorazzi, H.G. Kunkel, J.P. Halper, and S.R. Harris. 1978. Induction of in vitro differentiation and immunoglobulin synthesis of human leukemic B lymphocytes. J. Exp. Med. 148:1570–1578.

51. Zupo, S., M. Dono, R. Massara, G. Taborelli, N. Chiorazzi, and M. Ferrarini. 1994. Expression of CD5 and CD38 by human CD5^+ B cells: requirement for special stimuli. Eur. J. Immunol. 24:1426–1433.

52. Esser, C., and A. Radbruch. 1990. Immunoglobulin class switching: molecular and cellular analysis. Annu. Rev. Immunol. 8:717–735.

53. Hayakawa, K., R.R. Hardy, and L.A. Herzenberg. 1985. Progenitors for Ly-1 B cells are distinct from the progenitors of other B cells. J. Exp. Med. 161:1554–1568.

54. Chiorazzi, N., S.M. Fu, G. Montazieri, H.G. Kunkel, J.P. Halper, and S. Harris. 1979. T cell helper defect in patients with chronic lymphocytic leukemia. J. Immunol. 122:1087–1090.