Extensive and Selective Mutation of a Rearranged V_H5 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_H and V_L genes rearranged and/or expressed in CLL have few and apparently random mutations. However, in this study, we have found that the rearranged V_H251 gene, one of the three-membered V_H5 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_H 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_H3 and V_H1) to one (V_H6), with the "smaller" families (V_H4, V_H5, and V_H6) displaying unexpectedly low polymorphism (2). We discovered the V_H5 family in a case of familial B cell chronic lymphocytic leukemia (B-CLL)[1] (3) and demonstrated that one (V_H251) of two functional (V_H251 and V_H32) and one pseudo (V_H15) members is rearranged in about 20% of CLLs examined (4). Others have shown that the V_H3 family is the most commonly used in CLL, in proportion to its relative size, and have confirmed that usage of V_H5 and the other small families is biased (5–10). Although the V_H1 family is underrepresented in CLL with respect to its germline complexity, one member (51P1) is observed in 10% of CLLs and constitutes 60% of the total V_H1 contribution (5). Preferential usage of a $V\kappa3b$ gene and the associated cross-reactive idiotype has been observed (11, 12). In cases where the germline equivalents could be identified, V_H 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_H and V_L 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, polyspecific 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_H 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_H5 gene (V_H251) is extensively mutated in most $CD5^+$ B-CLLs in a manner consistent with antigen drive, whereas little, if any, mutation of V_H251 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),

[1] *Abbreviations used in this paper:* B-CLL, B cell chronic lymphocytic leukemia; R/S mutation, replacement/silent mutation.

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 unfractionated cord samples ranged from 50 to 70%.

cDNA 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 λgt10 and libraries, propagated as previously described (24). Resulting libraries were doublescreened for V_H251 and $C\mu$ by plaque hybridization. Positive clones were subcloned into 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, dGCACTGAATTCCCTGATTCAAATTTTGTGTCTCC, corresponds to the V_H251 leader intron (4) preceded by an EcoRI cloning site. The 3′-antisense primer, dTACAGGATCCTGAGGAGACGGTGACCAGGGT, corresponds to identical J_H1-J_H6 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 Taq 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 $CaCl_2$-competent BSJ_{72} 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 analysis of their N-D-J segments. Sequencing data were analyzed with DNAstar programs (DNAstar Inc., Madison, WI).

Analysis of Mutations. All mutations in one set of B cells were evaluated as a pool since each set contains a number of clones (11 in CLLs, 19 in cord B cells, 8 in EBV-transformed B cells, and 13 in PBL B cells). A sensitive binomial probability model used previously 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: $p_{RCDR} = n!/[R_{CDR}!(n-R_{CDR}!)] \times p^{RCDR} \times (1-p)^{n-RCDR}$; where R_{CDR} = no. of replacement mutation in CDRs; p = 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 = $R_{CDR} + S + 2R_{FR}$. The same method was also used to evaluate the frequency of mutations leading to charged residues in CDRs.

Results

V_H251 Rearranged in CLL B Cells Are Extensively Mutated Relative to Cord Blood and Adult PBLs. We screened 40 CLLs and obtained 11 that rearranged V_H251, 1 that rearranged V_H32 and none that rearranged V_H15 (data not shown). This biased percentage is consistent with what we (4) have previously observed. Since the focus here is on V_H251 mutation, the PCR reaction used for cloning from the normal B cell population was specific for V_H251. We previously showed that there were essentially no differences within V_H251 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 germline sequences.

Complete nucleotide sequences of rearranged V_H251 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 V_H251 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 V_H251 rearranged in CLLs. In contrast, the somatic mutation of rearranged V_H251 in $CD5^+$ or $CD5^-$ cord blood B cells and adult unfractionated 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 V_H bases) of V_H251 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 unfractionated 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 Intraclonal Variability. The excessive and unprecedented levels of mutation observed in the V_H251 CLL sequences raised the possibility of an ongoing mutational process. We chose two samples (CLLs 2 and 10) whose rear-

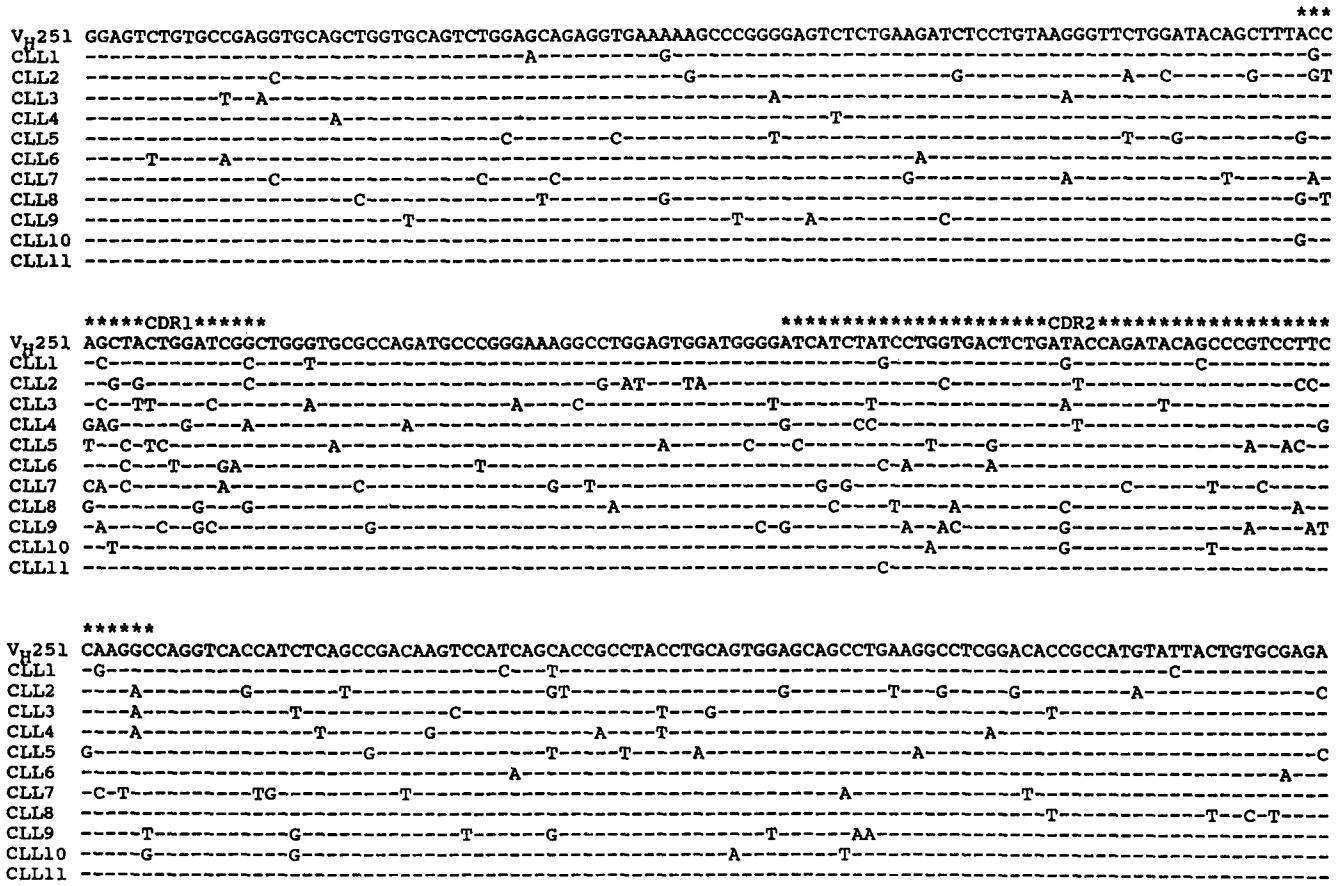

Figure 1. Nucleotide sequences of rearranged V_H251 genes in CLLs. (*) CDR1 and CDR2. (- - -) identities.

ranged V_H251 genes were highly mutated and were molecularly cloned by different methods. Five to six independent sequences obtained from independent bacterial plaques or colonies were identical within 1–2 bases (data not shown). We conclude that as in other $CD5^+$ CLL analyses, intraclonal variability is improbable, suggesting that the observed mutations occurred before the transformation process.

Mutation within V_H251 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 $CD5^+$ and suggests a higher selection level. In both $CD5^+$ and $CD5^-$ B cells, EBV-transformed $CD5^+$ B cells and PBL B cells, several rearranged V_H251 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_{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 $CD5^+$ 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_H and D Segments. Consistent with the data of others (5, 10), most CLLs used J_H4 with rearranged V_H251 genes (Table 3). In PBLs, about the same fraction (75%) used J_H4 and 17% used J_H5, which was similar to the percentages seen for other V_H genes (29). However, $CD5^+$ PBL lines and cord blood B cells use J_H4 (50%) less frequently but J_H5 (40%) considerably more frequently. The similar J_H usage in cord (mostly $CD5^+$) and the adult $CD5^+$ lines is expected if the adult $CD5^+$ population derives from the cord $CD5^+$ 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_H251 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-

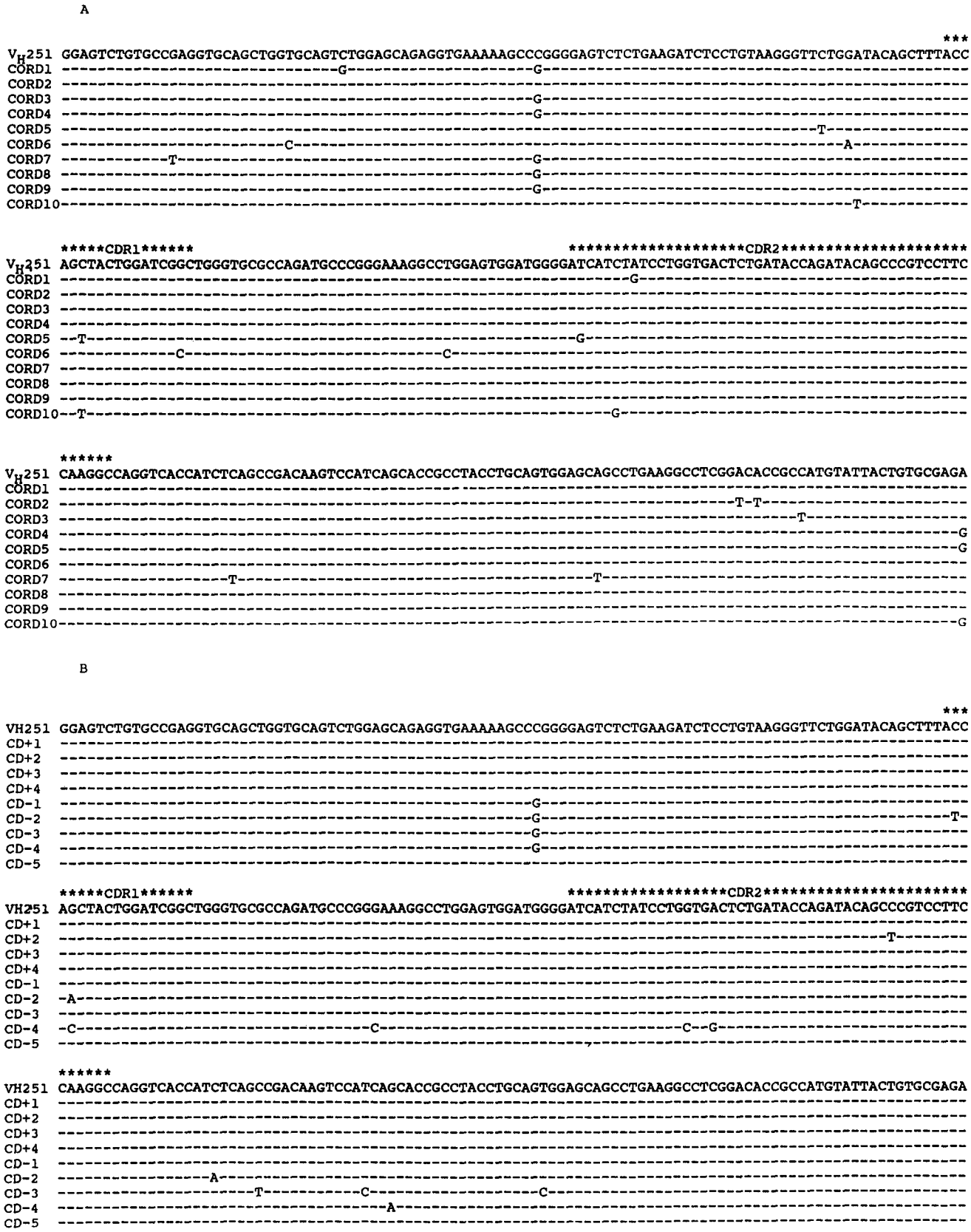

Figure 2. Nucleotide sequences of rearranged V_H251 genes in cord blood B cells unfractionated (*A*) and fractionated (*B*) for CD5.

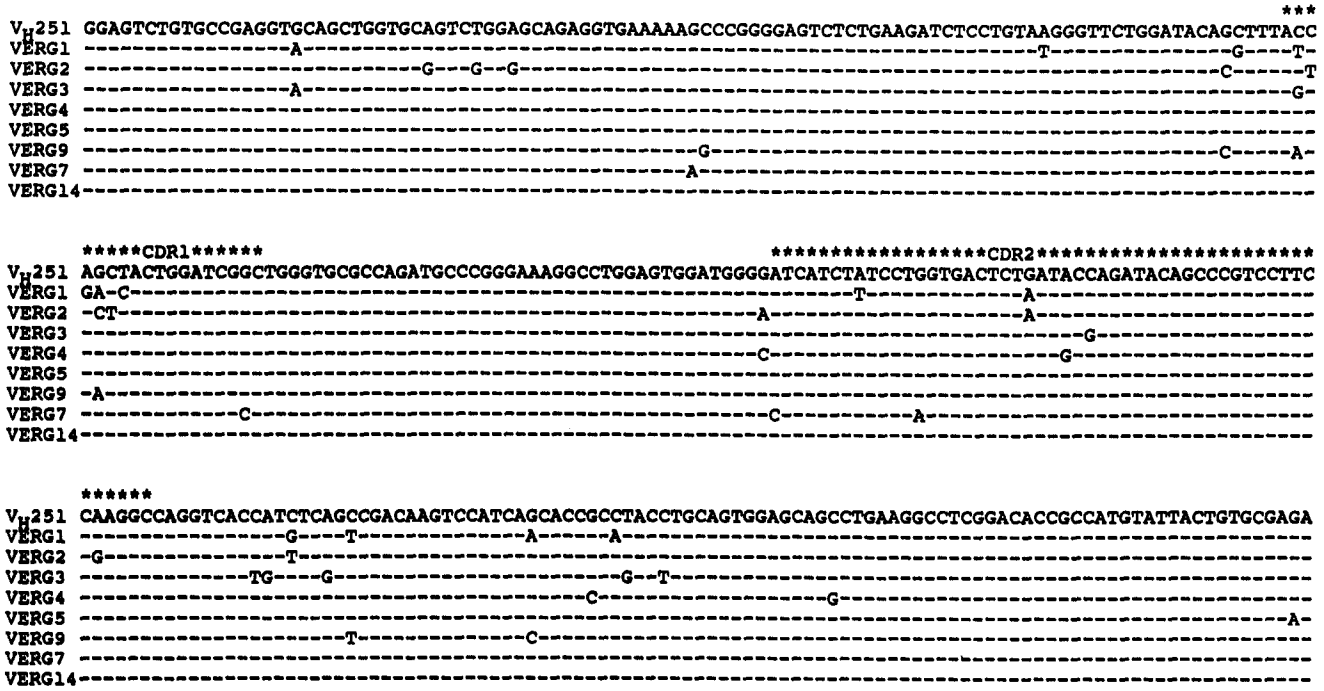

Figure 3. Nucleotide sequences of rearranged V_H251 genes in EBV-transformed CD5$^+$ B cells from adult PBL.

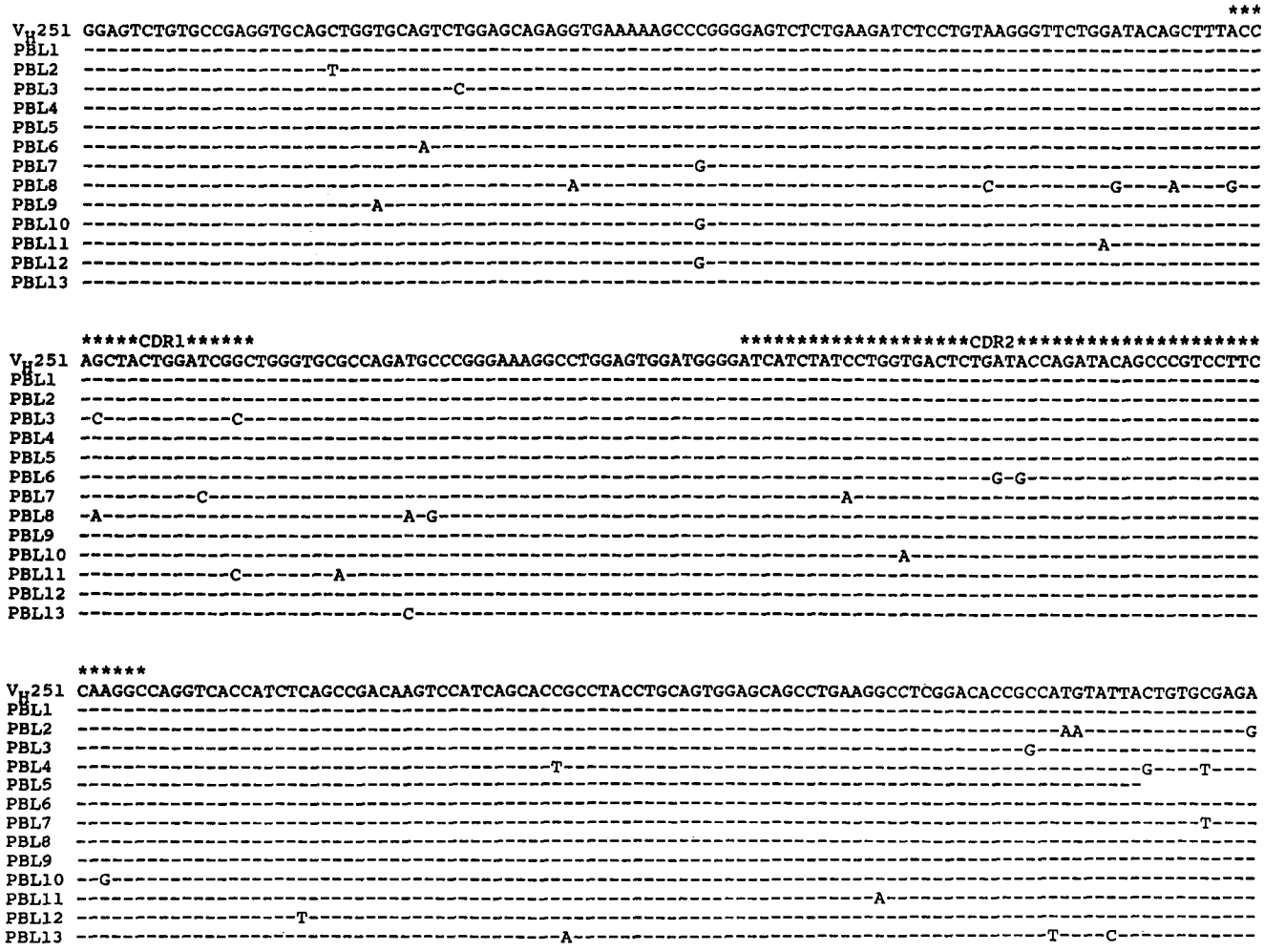

Figure 4. Nucleotide sequences of rearranged V_H251 genes in adult PBL. In PBL5, 10 nucleotides at the 3′ end were recessed.

Table 1. *Nucleotide Changes in Rearranged V_H251 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	CDR1 (18)	34	17.2	8	4.04	42	21.2	4.26	
cells	FWR2 (42)	10	2.17	11	2.38	21	4.35	0.91	6.03
(11)	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	CDR1 (18)	9	6.25	2	1.39	11	7.64	4.50	
cells	FWR2 (42)	0	0.00	2	0.60	2	0.60	0.00	1.92
(8)	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	CDR1 (18)	6	2.56	0	0.00	6	2.56	ND‡	
cells	FWR2 (42)	3	0.55	1	0.18	4	0.73	3.00	0.93
(13)	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	CDR1 (18)	4	1.17	2	0.58	6	1.75	2.00	
cells	FWR2 (42)	1	0.13	1	0.13	2	0.26	1.00	0.45
(19)	CDR2 (51)	5	0.52	0	0.00	5	0.52	ND‡	
	FWR3 (96)	8	0.44	5	0.27	13	0.71	1.00	

* 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.

ments in the CLLs. In PBLs and cord B cells, the DXP and DN families are most commonly used, both at 23%.

No Preference in L Chain Rearrangements in CLLs that Use V_H251. The demonstrated preference for Vκ3b (11) and our observation of selective mutation and J_H4 bias prompted an examination of Vκ/Vλ status in our CLLs. Using PCR primers previously shown (30) to be specific for Vκ1-Vκ4 families, we amplified family-specific bands for Vκ1–4 with no particular preferences (data not shown). Failure to obtain amplification signals in several samples was consistent with

Table 2. *Analysis of Mutation of Rearranged V_H251 Genes*

	Total mutations	Replacement in CDRs (R_{CDR})	Probability
Expected			0.173
Cord B cells	45	9	0.133
B-CLLs	258	78	1.31×10^{-7}
$CD5^+$ B cells	64	16	0.034
PBL B cells	55	10	0.136

Table 3. *Usage of J_H Segments in Rearranged V_H251 Genes*

J_H segments	CLL B cells	PBL B cells	Cord B cells	$CD5^+$ B lines	Total usage
J_H1	0	1 (8%)	0	1 (12%)	2
J_H2	0	0	1 (10%)	0	1
J_H3	0	0	0	0	0
J_H4	8 (73%)	9 (75%)	5 (50%)	4 (50%)	26
J_H5	2 (18%)	2 (17%)	4 (40%)	3 (38%)	11
J_H6	1 (9%)	0	0	0	1

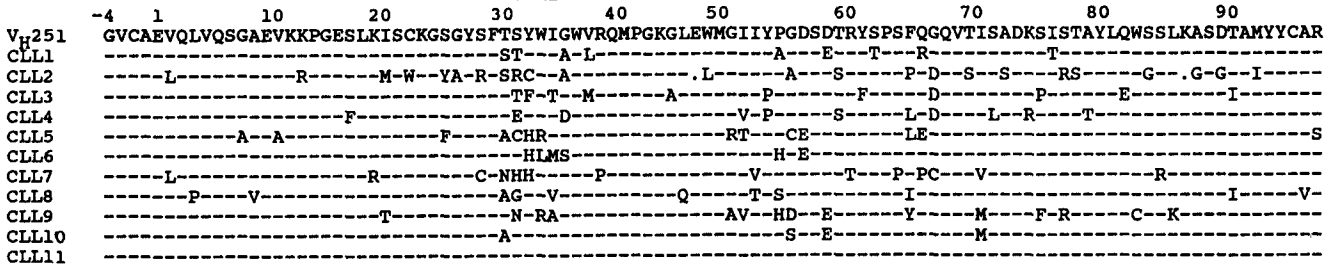

Figure 5. Deduced amino acid sequences of V_H251 genes utilized in CLLs.

the cell surface phenotypic assignment of λ. Although the sample size is relatively small, no biases are apparent.

Analysis of Deduced Amino Acid Sequences and Mutations to Charged Residues from V_H251 Utilized in CLLs. Comparison of the mutated V_H251 amino acid sequences derived from CLL cells with those derived from germline V_H251 revealed clustering of extensive changes in CDRs (Fig. 5). In CDR1, 54% of the amino acids are replaced, many with charged residues. All six CDR1 residues of germline V_H251 are uncharged, whereas 31% of the substituted residues are charged. Serine 31 is a hotspot, with 8/11 sequences substituted at this position. Particularly within CDRs, common mutations were shared among 2–3 sequences. Both conservative and nonconservative changes were observed, the most striking being the neutral to charge conversions at positions 33 (W→R), 67 (G→D), and 78 (S→R). The expected frequency of mutations leading to charged residues in FWRs is 4.6%, but there is a much higher percentage of observed mutations to charged residues in CDRs (23.7%), which is indicative of positive selection. Using the 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_H251 is a member of the small human V_H5 gene family (4) that displays remarkably little polymorphism (2). That allowed us to compare the nucleotide sequences of the V_H251 gene rearranged in different cell populations without the corresponding germline counterparts. 9 of 11 V_H251 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_H251 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_H251-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_L and V_H genes utilized in human CLLs and found little or no mutations (5–11). As with V_H251, some of the V_H genes analyzed are considered to be developmentally regulated, in that they are overrepresented in the fetal repertoire (13). There does not appear to be any phenotypic differences among the CLLs analyzed in other studies (5–11) and ours. Within our 11 samples, there were no features that distinguished the two CLLs that expressed V_H251 with only a few mutations. In all the studies, sequences were derived exclusively from IgMs. One feature that distinguishes the V_H5 family from all other human V_H families is that two members (V_H215 and V_H15) express abundant germline transcripts in CLLs (4, 33). The correlation between germline transcription and subsequent V to DJ recombination (34) conceivably could be extended to somatic mutation. Mutation of a productively regulated Vκ transgene requires its transcription (35). However, germline V_H251 transcripts in CLLs are not mutated (33), nor would one expect the selective mutation pattern we have observed to be generated from an antigen-independent mechanism.

Regardless of why V_H251 is susceptible to mutation, the result is at variance with the prevailing view of low affinity, polyspecific antibody expression in CLL. Instead, in the majority of our CLLs, the V_H251 sequences appear to be derived from prototypic high affinity antibodies. Selection could occur after the malignant transformation event. A model for B cell receptor (surface Ig idiotype)–mediated leukemogenesis, first elaborated by McGrath et al. (36) and potentially relevant to our situation, was provided by Mann et al. (37). In their analysis of B-CLL in HTLV-I endemic Caribbeans, a heterohybrid of one B-CLL demonstrated strict specificity for the gag 41 surface protein of HTLV-I. Inconsistent with this model is our lack of intraclonal sequence variability. Also we found no correlation of V_H usage with that of Vκ3b (or any other L chain), nor with that of D_H segment. This puts unprecedented requirements on V_H sequence/variation. We favor the alternative model that mutation precedes transformation. We envisage two general categories of $CD5^+$ B cells: those that do not somatically mutate and those that

do. The latter are the precursor lineage for most of the V_H251-utilizing CLL (with CLL being an exception) and potentially may carry additional biases, such as J_H preference.

The antigen drive apparently operative on V_H251 may derive from self-reactivity. Most human autoantibodies are produced by $CD5^+$ B cells that preferentially use small V_H 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 $CD5^+$ B cells expressing V_H251 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_H251 diversification in the normal preimmune environment is extremely limited, irrespective of the $CD5^+/CD5^-$ compartment. That V_H251 in adult EBV-transformed $CD5^+$ cells show relative high mutation extends findings that human $CD5^+$ 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_H gene. In the case of V_H251 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 $CD5^+$ 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_H 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_H 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 region 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. Gmelig-Meylig, R.A. Insel, D.M. Knowles, and F.W. Alt. 1989. Immunoglobulin V_H gene expression in human B cell lines and tumours: biased V_H 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. Gmelig-Meyling, and F.W. Alt. 1989. Autoantibodies encoded by the most J_H-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 Ig H 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 κ 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 κ 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. Rev.* 96:43.
15. Casali, P., and A.L. Notkins. 1989. CD5$^+$ B lymphocytes, polyreactive 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. Cleark. 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, R.J. Riblet, and K. Hayakawa. 1989. A single V_H gene is utilized predominantly in anti-BrMRBC hybridomas derived from purified Ly-1 B cells: definition of the V_H11 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).* 234:476.
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 Ble 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 idiotypic 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_H regions reveals apparent use of V_H 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_H transcripts. *J. Exp. Med.* 173:1529.
34. Yancopoulos, G.D., and F.W. Alt. 1985. Developmentally controlled and tissue-specific expression of unrearranged V_H 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 CLL: indirect role for retrovirus in leukemogenesis. *Science (Wash. DC).* 236:1103.
38. Burastero, S.E., and P. Casali. 1989. Characterization of human CD5 (Leu-1, OKT-1)$^+$ B lymphocytes and the antibodies they produce. *Contrib. Microbiol. 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.