RELATIONSHIP OF VARIABLE REGION GENES EXPRESSED BY A HUMAN B CELL LYMPHOMA SECRETING PATHOLOGIC ANTI-PR_2 ERYTHROCYTE AUTOANTIBODIES

By LESLIE E. SILBERSTEIN,* SAMUEL LITWIN,‡ and CONDIE E. CARMACK‡

*From the *Department of Pathology and Laboratory Medicine, School of Medicine, University of Pennsylvania 19104; and the ‡Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111*

Many autoimmune diseases are associated with autoantibody formation. Yet in only a few instances has it been clearly established that the autoantibody associated with the disease contributes to the pathogenic process. Cold agglutinins are autoantibodies that preferentially bind to RBC membrane antigens at low temperatures (1–3). Cold agglutinins may be pathologic, found in association with cold agglutinin disease (CAD)[1] or benign, found in the sera of healthy individuals. The antigenic specificities of benign and pathologic cold agglutinins are similar and include various glycolipids and glycoproteins (1, 4). CAD has been classified as either idiopathic (i.e., not associated with an underlying disease) or secondary to lymphoid neoplasms or infections (2). In cold agglutinin disease, whether idiopathic or secondary to lymphoid neoplasms, cold agglutinins are typically IgMκ monoclonal autoantibodies, as defined by a homogeneous peak on the serum electrophoretic pattern.

To further examine the clonality and cellular origin of pathologic cold agglutinins we have studied B cell clones from a patient (RR) with a splenic lymphoma associated with immune hemolysis due to an anti-Pr_2 cold autoantibody (5). Cytogenetic studies of splenic lymphocytes demonstrated an abnormal karyotype (51, XX, +3, +9, +12, +13, +18). After EBV transformation of splenic lymphocytes, seven clones were isolated; each clone had the same abnormal karyotype and secreted an IgMκ anti-Pr_2 cold agglutinin. Further studies of surface phenotype, Ig gene rearrangements, and antibody specificity suggested that the EBV-transformed clones secreted an RBC autoantibody that was identical to the pathogenic autoantibody causing immune hemolysis in the patient (6). However, while the IEF spectrotypes of the autoantibodies derived from five of the seven B cell clones were identical to the cold agglutinin isolated from the patient's serum, two of seven clones had distinctive spectrotypes. This finding indicated that the EBV-transformed B cell clones were structurally heterogeneous, even though they retained the same autoreactive specificity.

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[1] *Abbreviations used in this paper:* ALL, acute lymphoblastic leukemia, CAD, cold agglutinin disease; CDR, complementarity-determining region; CLL, chronic lymphocytic leukemia; m/bp/d, mutations/base pair/division; m/c/d, mutations/cell/division; MLE, maximum likelihood estimate; sIg, surface Ig.

Idiotypic heterogeneity of human follicular lymphomas has been ascribed to a high rate of somatic mutations in the V region genes (7–10). If a similar somatic mutational process were taking place in the autoreactive tumor described here, we would predict that sequence variants could account for the observed spectrotypic diversity. In this report, we have defined the molecular basis of autoantibody heterogeneity of B cell tumor origin through nucleotide sequence analysis of both heavy (V_H) and light (V_L) chain variable region genes. By comparing the junctional sequences formed by the joining of V_H, D_H, J_H, as well as V_κ and J_κ gene segments, we have evaluated the clonality of the seven clones at a molecular level. Additionally, a comparison of the V region sequences has allowed us to examine the frequency of nucleotide substitutions and to determine if the deduced amino acid sequences can explain the different IEF spectrotypes observed.

Materials and Methods

RNA Isolation. RNA was prepared from LS clones (1, 2, 4, 5–8) that had been established by EBV transformation as previously described (5). Cells (~3–5 × 10^8) were grown in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) with 10% FCS. Cells were washed twice in PBS and frozen as pellets in liquid nitrogen and stored at −70°C. Total cellular RNA was prepared by the guanidinium isothiocyanate method as described by Chirgwin et al. (11). Poly(A)$^+$ RNA was isolated on oligo(dT)-cellulose columns (12).

Oligonucleotide Primers. Human μ and κ oligonucleotide primers were synthesized using phosphotriester chemistry by an oligonucleotide synthesizer (Applied Biosystems, Inc., Foster City, CA). Oligonucleotides were purified by electrophoresis through 20% polyacrylamide/7 M urea preparative gels, followed by passive elution and separated on Sep-Pak C_{18} cartridges (Waters Associates, Milford, MA). Sequences used for Poly(A)$^+$ RNA priming were (μ) 5′-CAGGAGACGAGGGGGAA-3′ (9) and (κ) 5′-AGATGGCGGGAAGATGAAGAC-3′.

cDNA Synthesis and Cloning. Double-stranded cDNA was made by the method described by Gubler and Hoffman (13) as modified for Ig genes (14) using 5 μg of LS series RNA. Blunt-ended double-stranded cDNA was ligated into the phosphatased SmaI site of phagemid pBS M13- (Stratagene, San Diego, CA). *Escherichia coli* strain bSJ72 was transformed by recombinant phagemid DNA. Even though a highly enriched library containing human immunoglobulin genes was generated, it was necessary to screen the partial library with V_H (15) and V_L (16) gene probes. Northern blot analysis of isolated RNA (data not shown) indicated that the expressed V genes belonged to the V_{HI} and $V_{\kappa III}$ families. Agarose gel purification of probes proved essential since plasmid DNA would crosshybridize to the phagemid DNA on the filters.

Sequencing of cDNA Clones. After clones were identified as containing a human heavy or light chain cDNA insert, the phagemid clones, which contain the M13 origin of replication were grown with helper phage K07 (17) to rescue ssDNA. Since the pBS M13$^-$ vector yields negative stranded ssDNA, the M13 reverse primer was used for sequencing. cDNA sequences were determined with the method of Sanger (18) using Sequenase (United States Biochemical Corp., Cleveland, OH) with [^{35}S]dATP. 5% PAGE/8 M urea gels (Model STS 45; IBI, New Haven, CT) were run according to manufacturer's recommendation. The gels were fixed, dried, and exposed overnight to XAR-5 film (Eastman Kodak Co., Rochester, NY). Sequences were then determined and analyzed with the Wisconsin Sequence Package (19) running on a VAX computer (Digital, Burlington, MA).

Statistical Analysis of Observed Somatic Mutations. Seven cells produced a pattern of mutations having three cells either unmutated or with identical mutations and four cells each having its own unique set of mutations. This 3-1-1-1-1 pattern was used to determine a maximum likelihood estimate (MLE) as well as confidence bounds on the underlying mutation rate. This was done by simulating the clonal proliferation process for this situation by adapting a previously developed general Monte-Carlo model (20). The computer model of cell proliferation starts with one cell and produces a random succession of left or right progeny until

a total of 40 divisions is reached (Fig. 1 *A*). Then the same tree is restarted picking left or right random progeny as before. This time the tree is merely followed if the chosen left or right progeny already exists. If the tree fails to contain the requested random branch, then a new cell is added in that direction and more new cells are again added until the 40th division is reached. This is continued until seven strands of the tree have been extended to 40 divisions. Note that the tree would contain 2^{40} (1.1×10^{12}) cells at its last level if completed, but our simulation generates only seven. These seven are a random selection of the entire set of 2^{40}. Each new cell acquires a random number of mutations determined from the Poisson distribution. The parameter or the mean of the Poisson distribution is the mutation rate. The mutation rate is defined by the user and we used a range to investigate the ability of clonal development to create the observed pattern of mutations. Since each cell contains a large number of independently mutating bases, each with very low probability (see Results), the Poisson distribution is appropriate for the number of mutations per cell per division.

The chance of silent, neutral, or defunctionalizing mutations has been determined both theoretically and empirically (20, Shlomchik, M. J., S. Litwin, and M. Weigert, manuscript in preparation). The probability of each type is taken into account in generating the tree so that the likelihood of a lineage is determined. If a pathway incurs a lethal mutation, then the program inserts a dead cell in the tree. The creation of dead cells puts blockages in the tree structure, since dead cells don't proliferate. Subsequent passes to generate the needed seven cells may encounter a dead cell. If this happens the program restarts the pass. In the

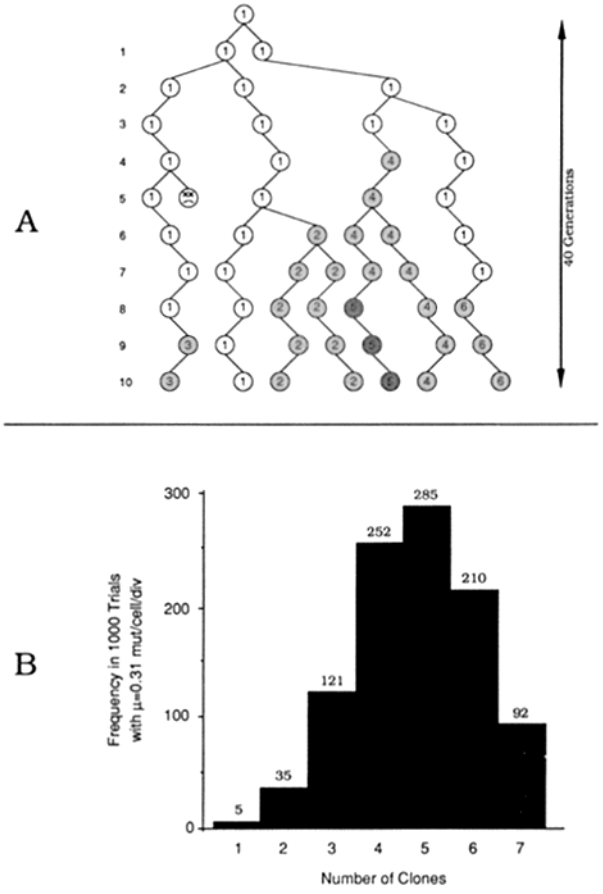

FIGURE 1. (*A*) Simulated clonal proliferation showing 10 cell divisions with random left/right daughters. Circles (representing individual cells) contain cell clone number. Open cells are unmutated, light gray cells contain one and darker gray cells contain two mutations. One cell at level 5 is dead. (*B*) Frequencies of all possible numbers of clones generated in 1,000 trials when the mutation rate is set at 0.031 mutations/cell/division.

event that no path to 40 divisions exists by virtue of dead cells, the entire tree is aborted. When seven cell lines have been extended to 40 divisions, the clonal identity of each cell is determined. Two cells in the final division are regarded as identical if and only if they have a common ancestor from which neither has mutated. The possibility that two final division cells contain identical, independently derived mutations is ignored since it is so improbable. However, with a little additional programming individual base mutations could be recorded and this possibility taken into account.

The program requests the user to specify a mutation rate, then it determines if the observed pattern is likely to occur. It does this by simulating the process of cell proliferation 1,000 times, all at the same mutation rate. In each repetition the program checks if the seven cells alive at division 40 match the observed pattern. At the conclusion of the 1,000 repetitions the number of times the pattern was observed is tabulated. If the seven simulated cells make up 3 identical ones, i.e., each with either no mutations, or the same set of mutations, and four others that are each unique, then this simulation matches the observed outcome. The number of occurrences of this pattern among the 1,000 repetitions divided by 1,000 is an estimate of the probability of the observed outcome for this value of the mutation rate. By running the program several times, entering different mutation rates each time, we can determine what rate gives the biggest tally, and hence biggest estimate of the probability of the observation. The 1,000 trials are regarded as Bernoulli trials, and we are estimating the chance of success, in this case, the chance of the original observation of 3-1-1-1-1. The probability for success in a trial is the likelihood function that is to be maximized. It depends on the mutation rate, and would be conditioned on the fact that trials that are aborted should not be considered, since they cannot account for the observed data. It would also account for the patterns of all possible cell lineages after 40 divisions. We note that trial abortion is very rare at the mutation rates we are using (see Results), namely p (aborted tree) <0.00178 (computation not shown). However, we can investigate the underlying process without explicitly knowing the likelihood function by using a computer simulation. When we run the program at a very low mutation rate (0.001 mutations per cell per division) it rarely produces five different clones, whereas at a high mutation rate (0.15 mutations per cell division) it produces six or seven different clones. Thus we adjust the mutation rate to an intermediate value so that the program reflects the observed data. By applying a series of different values for the mutation rate we identify one that maximizes the program's production of mutational patterns similar to that observed. This rate is our approximation to the maximum likelihood estimate of the true rate. Three similar statistics are collected. First, the outcome is tested for being identical to the observed 3-1-1-1-1 pattern. Next, the outcome is tested for containing at least five clones, finally it is tested for containing no more than five clones. The last two statistics are used to determine ~95% confidence bounds on the true mutation rate. These three statistics are tallied and the entire procedure is repeated 1,000 times. Finally, the program outputs the number of times the observed mutational pattern was obtained as well as the tallies of the other two statistics.

Results

Nucleotide Sequences of V_H and V_L Regions. The V_H and V_L region gene segments of the seven EBV lines were cloned as cDNAs by extending an oligonucleotide primer homologous to the 5′ region of the human heavy and light chain constant regions (Figs. 2 and 3). The use of the μ chain primer has been previously reported (8, 9). The choice of the κ primer was based on identifying a human C_κ region that was homologous to an evolutionary conserved C_κ sequence from the mouse (21). The κ primer consisted of a 21mer, 18 nucleotides from the 3′ end of the J segment of a rearranged gene. Two or more independent cDNA clones were isolated and sequenced because of concern for sequencing or cloning artifacts. Clones were determined to be of independent origins on the basis of different cDNA sizes and orientation of inserts. Sequences of cDNA clones isolated from a particular LS cell line

were all identical indicating high fidelity of the reverse transcriptase. Of the several thousand nucleotides sequenced, no mutation due to polymerization of the reverse transcriptase was seen. Furthermore, to assess for mutations occurring due to tissue culture, cDNA clones of cell lines LS2 and 5 were isolated and sequenced at 6-mo intervals; no changes in nucleotide sequences were seen among repeat isolates.

The nucleotide sequences of the seven V_H and V_K genes are shown in Figs. 2 and 3, respectively. It is evident that except for a few nucleotide substitutions, the sequences are almost identical. Identical V_H-D_H, D_H-J_H, V_K-J_K, and N gene segments are all consistent with a clonal origin. Clonality is further supported by the unusual karyotype associated with the tumor and EBV-transformed cells, as well as the Southern blot analyses of heavy and light chain loci showing identical Ig gene rearrangements (6).

Although the sequences are nearly identical, several nucleotide substitutions are seen that could only be attributed to somatic mutations if all cells were in fact derived from a single progenitor cell. Single T substitutions at amino acid positions 72 (LS4) and 78 (LS1) in the heavy chain gene and position 92 (LS6) in the light

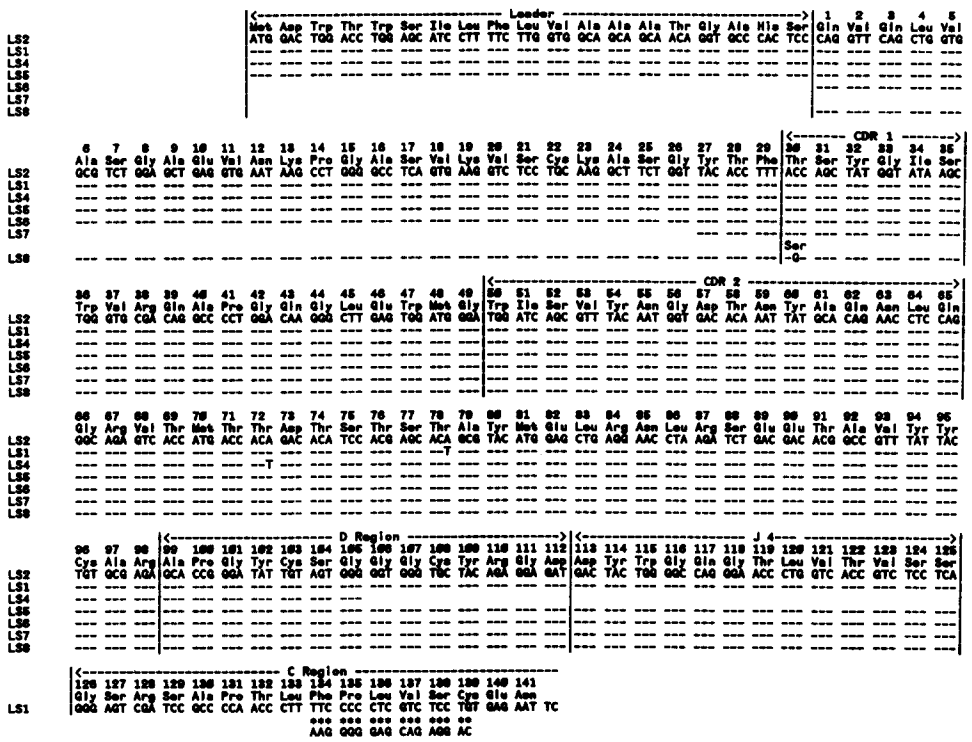

FIGURE 2. Nucleotide sequences of V_H region from anti-Pr_2 EBV clones. Amino acid translation is given above nucleotide sequence and numbered according to reference 22. Complementarity-determining regions (CDR1, CDR 2, and CDR 3), D and J4 gene segments are as indicated. Differences from the consensus sequence (LS2) are indicated in both nucleotide and amino acid sequences. Homology of the nucleotide with the consensus sequence on the top line is shown as a dash. The μ oligonucleotide sequence used to prime cDNA clones is indicated with an asterisk. These sequence data have been submitted to the EMBL/GenBank Data Libraries under accession number Y00652.

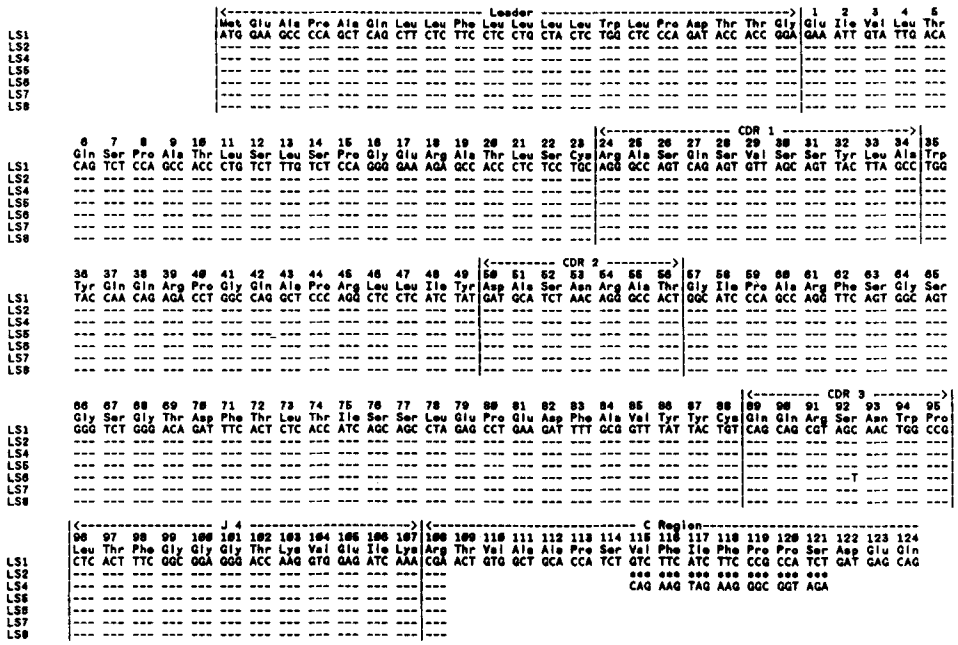

FIGURE 3. Nucleotide sequences of V_L regions from anti-Pr_2 EBV clones. Amino acid translations is given above nucleotide sequence and numbered according to reference 22. Complementary-determining regions (CDR 1 and CDR2) and J4 gene segment are as indicated. Differences from the consensus sequence (LS1) are indicated. Homology of the nucleotide with the consensus sequence on the top line is shown as a dash. The κ oligonucleotide sequence used to prime cDNA clones is indicated with an asterisk. These sequence data have been submitted to the EMBL/GenBank Data Libraries.

chain gene all are silent changes and produce no predicted amino acid changes. The only nucleotide substitution that changes the predicted amino acid sequence is at amino acid position 30 (LS8) in the heavy chain gene. The change (Thr→Ser) is considered a conservative substitution since both have aliphatic hydroxyl side chains and no change in the net charge of the deduced protein is expected.

The gene sequences were compared for homology to previously published V_H and V_κ genes and in particular to other human RBC autoantibody sequences. The V_H sequences belong to the V_{HI} gene family as demonstrated by Northern blot analysis (data not shown) and were found to be 88% homologous to a germline V_{HI} gene (Fig. 4 *A*) (22). The LS light chain sequences were 97% homologous to a germline $V_{\kappa III}$ gene (Fig. 4 *B*) (23). We have surveyed the D gene segments known to date and have not found identical sequences (24, 25). The V region gene sequences of the LS autoantibodies differ from another human anti-Pr_2 RBC autoantibody, which by NH_2-terminal sequencing uses V_{HIII} and $V_{\kappa IV}$ family genes (26). The use of different V genes by anti-Pr_2 autoantibodies suggests that the human response to this antigen is not highly restricted.

Confidence Interval for the Observed Mutation Rate. To estimate the mutation frequency, we assumed that a tumor mass of at least 10^{12} cells (based on weight and immunohistochemistry of the spleen) existed at the time of splenectomy and EBV transformation. From this cell mass number, we calculated that ~40 ($2^{40} = 1.1 \times 10^{12}$) cell

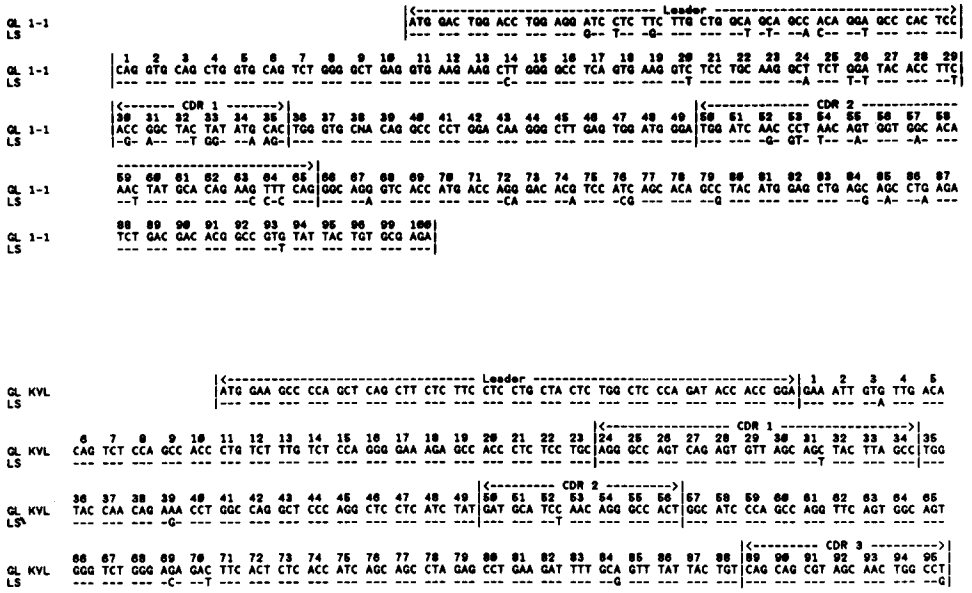

FIGURE 4. Comparison of the V_H and V_K nucleotide sequences of the anti-Pr_2 autoantibodies reported here (LS lines) and (*A*) GL 1-1, germline V_{HI} gene (23); (*B*) GL-KVL, germline V_{KIII} gene (24). Dashes indicate sequence homology with the LS sequence. Listed above the nucleotide sequence are the position numbers of the corresponding amino acids listed according to reference 22. Leader and CDR regions are as indicated.

divisions had taken place and on the average, 4/7 (0.571) somatic mutations per cell had accumulated from the single tumor cell to the time of splenectomy and transformation. Since there are 696 bp in both heavy and light chain genes, these figures allow us to estimate a mutation frequency of 0.01427 mutations/division or 2.05×10^{-5} mutations/base pair/division (m/bp/d) (0.571/(696 bps × 40 divisions)). With the mutation rate of 0.031 mutations per cell (4.45×10^{-5} m/bp/div) per division, the chance of the observed pattern was maximized and was about 27%, i.e., the 1,000 repetitions produced 266 outcomes each of which contained a set of three identical cells plus four completely unique cells (Fig. 1 *B*). This is the MLE for the mutation rate. Setting the mutation rate to 0.0085 mutations per cell per division (1.22×10^{-6} m/bp/div) the chance of observing at least five clones was reduced to 0.028 and by setting it to 0.12 mutations per cell per division (1.72×10^{-4} m/bp/div) the chance of observing five or fewer clones was reduced to 0.023. Thus an approximate 95% confidence interval for the mutation rate is (0.0085, 0.12) mutations per cell per division and (1.22×10^{-6}, 1.72×10^{-4}) mutations per base pair per division.

Our estimate of tumor cell mutation rate rests on the assumption that tumor cells do not die. Thus, it must be taken as an upper band until better approximations to tumor cell birth and death rates are available.

Discussion

The humoral immune repertoire is produced by B lymphocytes that have the ability to respond to a wide range of antigenic specificities. The differentiation of B lymphocytes can be divided into two stages. The first stage is antigen independent in-

volving the development of stem cells into B cells. After acquiring antigen binding receptors, B cells migrate to the peripheral organs such as lymph nodes and spleen where they encounter various antigens. The second stage involves the proliferation and subsequent differentiation of B cells into Ig-secreting plasma cells. Since autoantigens are ubiquitous, it follows that autoimmune responses must be controlled by regulatory mechanisms such as clonal deletion and/or T cell suppression.

Although autoantibodies are associated with many autoimmune disorders, their role in the pathogenesis of disease in most cases is unclear. In contrast, the pathologic role of RBC autoantibodies in immune hemolysis is well established (1). To study the biology of these pathogenic RBC autoantibodies, we have established seven EBV-transformed B cell clones from a patient (RR) with splenic lymphoma and immune hemolysis due to an anti-Pr_2 RBC autoantibody. Previously, we had demonstrated that these EBV-transformed clones secreted the same pathogenic autoantibody as present in the serum of the patient. The sequence data of this report confirm the clonal relatedness of these lines. By IEF analysis, however, two of seven clones secreted autoantibodies with different spectrotypes. The different IEF banding patterns could be explained by a post-translational event, such as glycosylation, or by somatic mutation of the primary nucleotide sequence. Altered glycosylation could result from a change in primary sequence leading to the appearance or disappearance of glycosylation sites, or by variable glycosylation at a particular amino acid without a change in the primary amino acid sequence (27). The observed somatic mutations in the LS cell lines are either silent or conservative and thus do not account for any charge differences. Although the conservative, serine to threonine substitution (LS8; V_H; position 30), occurred in one of the autoantibodies with a different spectrotype, this somatic mutation did not involve an Asn-X-Ser/Thr recognition sequence required for *N*-linked glycosylation (27). *O*-linked glycosylation of a serine residue in the V region has been reported only once in an abnormal human myloma λ light chain (28). It is therefore unlikely that the two distinctive spectrotypes result from the nucleotide substitutions found in the V_H and V_L region genes of the seven clones. Variable glycosylation and/or mutations in the Ig constant regions are alternative causes for spectrotypic differences and cannot be ruled out.

The small number of somatic mutations, i.e., only 4 in >5,000 bases sequenced, shows that at the time of sampling, a low somatic mutation frequency existed in this B cell tumor population. One can speculate that the mutation rate may have been high at one point but then slowed or even stopped. Alternatively, a high somatic mutation rate may never have existed.

The idea that the mutation rate can vary in the expansion and maturation of a B cell clone is supported by data from murine cell lines with various specificities (including autoantigens) and representing different stages of B cell differentiation (20, 29, 30). Mutations appear to be infrequent in the preimmune repertoire and primary immune response (estimated at $<10^{-5}$ m/bp/d) (31, 32). However, during subsequent steps of B cell maturation, characteristic of secondary immune responses, somataic point mutations are introduced in a stepwise fashion at a rate approximating 10^{-3} m/bp/d (33, 34). At later stages of B cell differentiation, as demonstrated in studies of transformed plasma cells, somatic mutations are considered to occur again at a lower rate (estimated mutation frequency between 10^{-6} and 10^{-7} m/bp/d) (35).

The type of lymphoma analyzed in this report differs in several aspects from other

B cell malignancies of which V regions have been analyzed (36, 37). First, the B cell tumor of the patient in this report consists of early plasmacytoid cells, which represent a more mature stage of B cell differentiation than the types involved in acute lymphoblastic leukemia (pre-B cell) (38), chronic lymphocytic leukemia (immature-mature B cell) (39), and follicular lymphoma (follicular center cell, activated B cell) (8, 9). Second, the B cell tumor described here, is unique in that its specificity is well defined.

Based on the available V region sequences from these four different types of B cell lymphomas, a correlation is proposed between the stage of B cell ontogeny and the estimated mutation frequencies (see Fig. 5). In a recent study of V_H sequence analysis from patients of acute lymphoblastic leukemia (ALL), no somatic mutations were found in >15,000 nucleotide sequences (38). Based on this finding it was calculated that the prevalence of mutations in these ALL tumors was $<6.7 \times 10^{-6}$ m/bp/d. In the two cases of CLL, no evidence for sequence heterogeneity of expressed V genes was observed; the V_κ sequences isolated from two unrelated individuals are highly homologous to each other and to a previously published germline

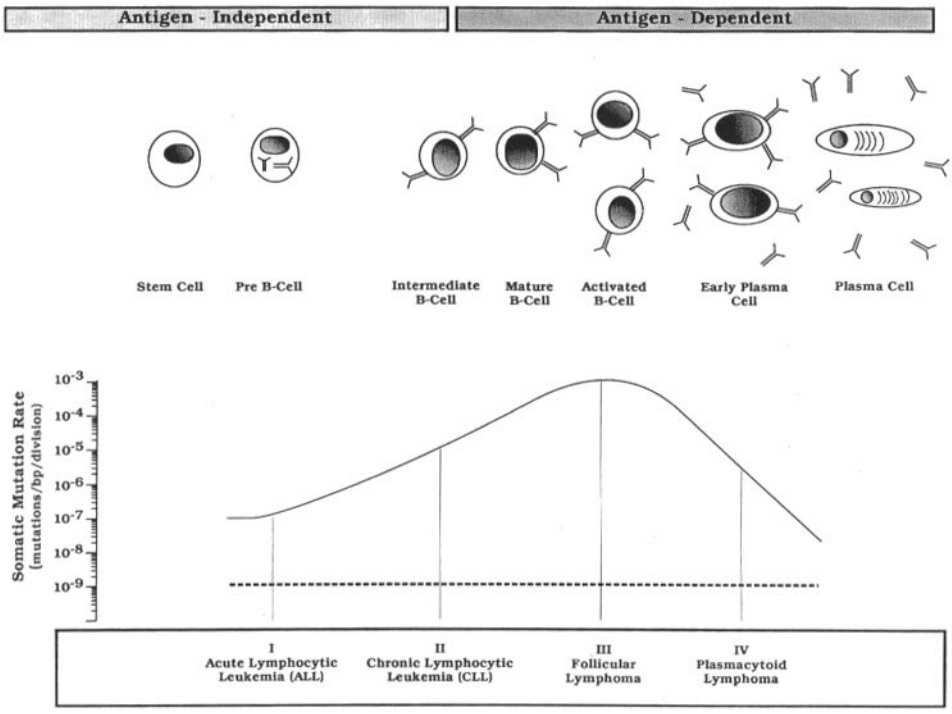

FIGURE 5. Hypothetical correlation of differentiation stage of different B cell tumors and somatic mutation rate. I. ALL; pre-B cell; low somatic mutation rate estimated (39). II. CLL; immature-mature B cell; low somatic mutation rate estimated (40, 41). III. Follicular lymphomas; activated B cell, likely of follicular center cell origin; somatic mutation rate is high (8, 9). IV. Well-differentiated lymphoma; anti-Pr_2 secreting B cell lymphoma, early plasmacytoid cell type; low somatic mutation rate estimated (this paper). Solid line indicates somatic mutation rates estimated for four types of B cell lymphomas representing different stages of B cell differentiation. The dotted line reflects the rare occurrence of somatic mutations in non-Ig variable region loci (32).

V_κ sequence (39, 40). In contrast, a relatively high frequency of somatic mutation occurs in follicular B cell lymphomas similar to the mutational process found in normal differentiating B cells (8, 9). The mutation rate has not been determined for these human B cell tumors, because the number of cell divisions is not known. However, with some assumptions we have estimated the mutation rate of the lymphoma described in this report, to be 4.45×10^{-5} (see Results). Similarly, a mutation rate has also been estimated for cases of ALL. These estimated rates imply that somatic mutation rates that are significantly lower than those found in activated B cells would occur in lymphomas representing both earlier and more mature stages of differentiation (Fig. 5). We next determined if the prevalence of somatic mutations was significantly different among the various types of lymphomas by comparing the frequency of silent mutations found in the previously published V_H and V_L sequences (8, 9, 38–40). Only silent mutations were considered in order to exclude any bias due to selection. Using Fisher's exact test for the hypergeometric distribution, the frequency of silent mutations in the plasmacytoid B cell lymphoma (3/4,992 bp sequenced) was significantly lower than in follicular lymphoma (16/2,124 bp sequenced; $p < 0.00001$), but was not significantly different ($p > 0.5$) from the observed frequency in ALL (0/15,000) and CLL (0/1,428) (39). This difference can be explained as either a higher mutation rate in follicular lymphomas or by the possibility that these lymphomas represented a larger number of cell divisions allowing for a greater number of observed mutations. The result of this statistical analysis also fits with the proposed model illustrated in Fig. 5. Thus, this model proposes that B cell tumors with low somatic mutation frequencies will include cases of ALL and CLL, consisting of pre-B and intermediate B-cells, as well as lymphomas representing plasmacytic, more mature stages of B cell differentiation.

The role of exogenous and autoantigens in clonal selection and expansion of antigen-specific B cell during an immune response has been studied in several animal model systems (28–30). Although binding of sIg with autoantigen (i.e., Pr_2) may be important in driving a specific B cell clone to expand, other secondary factors such as increased oncogene expression are likely to contribute to the malignant transformation of these autoreactive B cell clones (41, 42). This view of lymphoma development, where clonal expansion and malignant transformation are separate and independent events, is supported by the observed clinical spectrum of cold hemagglutinin disease (1). At one end of the spectrum are patients with an expanded B cell clone producing a monoclonal cold agglutinin, identified as a homogeneous band on serum protein electrophoresis; these patients have no evidence for lymphoma and are diagnosed as having idiopathic cold hemagglutinin disease. At the other end of the spectrum are patients with the secondary form of cold hemagglutinin disease, whose expanded B cell clone has undergone malignant transformation; these patients present with or eventually develop clinical lymphoma.

In summary, the low mutation frequency observed in this Pr_2-specific B cell tumor, may not only be related to the tumor differentiation stage but also may reflect selection by autoantigen to retain Ig structure and specificity. The conserved nature of sIg receptors expressed by B cell tumors of this type would predict a good response to antiidiotype therapy (43). Additional studies of V genes from different types of B cell lymphomas, representing various stages of differentiation, will contribute to understanding the biology of B cell neoplasia and also define the potential for passive immunotherapy.

Summary

To study the biology of cold agglutinin disease we previously established EBV-transformed B cell clones isolated from a patient with splenic lymphoma of an early plasmacytic cell type and immune hemolysis due to an anti-Pr_2 cold agglutinin. These clones had an aberrant chromosomal marker identical to the patient's B cell lymphoma and each secreted IgM_k anti-Pr_2 similar to the pathologic autoantibody in the serum of the patient. In this study, we have further investigated the Pr_2-specific autoimmune response through nucleotide sequencing of V_H and V_L region genes. We have shown that the seven clones share the same VDJ/VJ gene segments and junctional elements confirming their clonal origin. The V_H sequences were 88% homologous to a V_{HI} germline gene while the V_L sequences were 97% homologous to a V_{kIII} germline gene. Only 4 somatic mutations (3 silent and 1 conservative) were found in >5,000 bp sequenced, suggesting that a low mutation rate existed. Based on a tumor mass of 10^{12} cells and a minimum of 40 divisions, we estimated the somatic mutation rate to be 4.45×10^{-5} m/bp/d.

This somatic mutation rate is similar to those estimated for acute lymphocytic leukemia (pre-B cell) and chronic lymphocytic leukemia (intermediate B cell), but significantly lower than the mutation frequency in follicular lymphomas (activated B cell). We propose that the difference in somatic mutation frequency of a B cell tumor may be related to the stage of B cell differentiation. In addition, the low mutation frequency observed in the Pr_2-specific B cell tumor may also reflect, in part, selection by autoantigen to conserve sIg structure and specificity.

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