Common Clonal Origin of Chronic Lymphocytic Leukemia and High-Grade Lymphoma of Richter’s Syndrome

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Patients with B-cell chronic lymphocytic leukemia (CLL) infrequently may develop high-grade B-cell lymphoma, or Richter’s syndrome lymphoma (RS lymphoma). Such lymphomas differ from the original leukemia in both histology and clinical behavior. Studies seeking to define the clonal relationship between the cells of the two malignancies in any one patient have yielded conflicting reports. We examined the clonal relationship between the early and late neoplastic cells of a patient who underwent Richter’s transformation. In contrast to the original leukemia cells, the secondary high-grade lymphoma was CD5-. However, both the leukemia cells and the evolved RS lymphoma expressed surface lgM reactive with Lc1, a murine monoclonal antibody specific for a supratypic cross-reactive idio-
type encoded by a subset of human Ig variable region genes of the V_{\mu}A subgroup. Nucleic acid sequence analyses of the heavy and light chain variable region genes expressed by both leukemia and lymphoma cells show that the CD5- B-cell lymphoma constitutes a clonal expansion of mutant cells derived from the original CD5+ B-cell leukemia. Moreover, certain sets of somatic mutations distinguish the Ig variable region genes used by RS lymphoma from those expressed by the CLL B cells. This is the first study to establish the clonal relationship between CLL and RS lymphoma through primary structural analyses of the expressed Ig genes.

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began monthly chemotherapy with CVP (cyclophosphamide, vincristine, and prednisone) because of bulky adenopathy. In November 1991, a peripheral blood sample was taken for the analyses we report here. Because of progressive diffuse adenopathy, he underwent diagnostic cervical and inguinal lymph node biopsies in January 1992. Despite aggressive therapy, the patient experienced a clinical course typical for that of patients with RS and died 5 months later.2,3

**Immunohistochemistry and flow cytometry.** Lc1, a murine IgG, monoclonal antibody (MoAb), was provided by Dr J.G. Sissons (Cambridge University, Cambridge, UK)38 and conjugated with phycoerythrin (PE) in our laboratory. PE-conjugated mouse IgGα, anti-human CD5 and mouse IgGκ, anti-human CD3, and fluorescein isothiocyanate (FITC)-conjugated mouse IgG1 anti-human CD20 are as described.27 Conjugates of an irrelevant mouse IgG1 (MOPC21; Caltag, South San Francisco, CA) were used to control for specificity. Immunohistochemistry and flow cytometry analyses were performed as described.27,28

**Polymerase chain reaction (PCR).** Genomic DNA preparations extracted from peripheral blood lymphocytes (PBL) or from cryopreserved tissue sections were used as templates. To amplify Ig V_{H}4 regions of the rearranged Ig heavy-chain genes, we used the sub-group-specific leader sense oligonucleotide primer 5'-gtcgaattcTTGACYCAWCGSYCTCAG-3' and 5'-ctcgaattcTTACCTAGGACGGTGAGCTTGGCT-Y, corresponding to an Ig V_{H} first framework region (FR1) sense strand consensus sequence and a J_{H} antisense consensus sequence, respectively. The amplified fragments were ligated, cloned, and sequenced as described.27 DNA hybridization. Amplified Ig V_{H}4 genes were detected in 10-μL samples from the PCR reaction mixtures by agarose gel electrophoresis and subsequent Southern blot analyses, essentially as described,32 but performing final stringent washes at 65°C in 0.2× SSC (20× SSC is 3 mol/L NaCl, 0.3 mol/L sodium citrate, pH 7.4). A 660-bp fragment containing the entire human Ig V_{H}4 gene was used to probe PCR fragments, as described. This probe was labeled with 32P using the Oligolabelling Kit (Pharmacia, Uppsala, Sweden). The amplified Ig V_{H}4 genes were registered as distinct bands of DNA of expected size (410 bp) in agarose gel electrophoresis.

** Colony screening.** Colonies of transformed bacteria were screened for human Ig V_{H}4 inserts by Southern blot hybridization of denatured DNA from colonies grown on Hybond-N filters.
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leader oligonucleotide sense primer and a J\textsubscript{H} consensus antisense primer to amplify the rearranged Ig \( V_{\text{H}} \) genes from genomic DNA isolated from the patient's CLL cells or RS lymphoma. Two separate PCR were performed on the genomic DNA of the CLL cells, and on the genomic DNA of the RS lymphoma obtained from each of two disparate anatomic sites, eg, cervical (ne) and inguinal (pe) lymph nodes. In all cases, such PCR generated fragments of approximately 660 bp that hybridized specifically with a \( ^{32} \text{P} \)-labeled human Ig \( V_{\text{H}}4 \) gene probe. These fragments were cloned for sequence analyses. Two clones from each PCR reaction were sequenced. Each contained a functionally rearranged Ig \( V_{\text{H}}4 \) gene. All shared the same Ig \( V_{\text{H}}4DJ_{\text{H}} \), indicating that they were derived from a common clonal ancestor. The rearranged Ig \( V_{\text{H}} \) segment belongs to the Ig \( V_{\text{H}}4 \) family, and has the closest homology (96%) with a previously published germline sequence \( V71-4 \).\textsuperscript{31} The region deduced as being encoded by a \( J_{\text{H}} \) segment is most homologous to \( J_{\text{H}}6 \), with only one base difference from that of the reported germline \( J_{\text{H}}6 \) sequence (Fig 4). The G \( \rightarrow \) C substitution noted at position 102 also has been noted in other reported \( J_{\text{H}}6 \) gene segments and may represent a genetic polymorphism.\textsuperscript{27,32,33} Of all the known germline D segments, the nucleic sequence between that encoded by Ig \( V_{\text{H}} \) and \( J_{\text{H}} \) had the highest homology with the D2 minigene,\textsuperscript{34} sharing greater than 91% sequence homology over a stretch of 23 bp.

The sequences of Ig \( V_{\text{H}}4 \) genes of the CLL and RS lymphoma share several nucleotide codons that are distinct from those of the putative germline Ig \( V_{\text{H}}4 \) gene, \( V71-4 \). However, the Ig \( V_{\text{H}}4 \) genes of the RS lymphoma share a set of four additional substitutions in codons 21, 71, 82A, and 82C that are not present in the Ig \( V_{\text{H}}4 \) genes expressed by the leukemia B-cell population. Nucleotide changes in codons 82A and 82C result in amino acid substitutions (Fig 5). The sequences of \( V_{\text{H}}4 \) genes from other CLL and RS lymphoma-derived clones and from the putative germline gene. The other Ig \( V_{\text{H}} \) gene clones share more than 99.8% homology with the consensus sequence, with only two base differences: one in codon 3 of the clone ne5, and another in codon 44 of clone pb4. The rearranged Ig \( V_{\lambda} \) genes were cloned in a similar way via PCR using a consensus sense primer for the FR1 of human Ig \( V_{\lambda} \) and a consensus antisense primer for \( J_{\lambda} \). Each clone contained a functionally rearranged Ig \( V_{\lambda} \) gene (Fig 6). Sequence analyses of the clones derived from CLL or RS lymphoma genomic DNA showed that both malignant B-cell populations expressed the same Ig \( V_{\lambda} \) genes rearranged with the same \( JAC1 \) cluster. Moreover, the Ig \( V_{\lambda}XJ \) junctions are identical. Deduced amino acid sequences are shown in Fig 7.

A computer search for known germline \( V_{\lambda} \) genes in GenBank identified the cloned Ig \( V_{\lambda} \) genes to have the highest homology (86%) to V1S2, an Ig \( V_{\lambda} \) gene of the \( V_{\lambda}1 \) subgroup.\textsuperscript{35} However, the differences between the cloned Ig \( V_{\lambda} \) genes and V1S2 include an in-frame one codon deletion in the first complementarity determining region (CDR). Such deletions generally do not arise through the process of Ig somatic mutation. Also, higher homology (90%) is observed between these Ig \( V_{\lambda} \) genes and a functionally rearranged Ig

RESULTS

Histology and immunophenotype. In contrast to lymph nodes typically observed from patients with CLL, the enlarged lymph nodes of COT were effaced by a diffuse proliferation of large lymphoid cells admixed with a few small lymphocytes (Fig 1). This histology is typical for that of RS.\textsuperscript{24} In contrast, the leukemia B cells had the morphology of small well-differentiated lymphocytes (not shown). Immunohistochemical analyses showed that the original leukemia B cells and the RS lymphoma B cells expressed surface IgM\( \lambda \) (not shown). Flow cytometric analyses showed that both neoplastic B-cell populations react specifically with Lc1, a murine MoAb specific for a supratypic cross-reactive idiotype (sCRI) encoded by Ig \( V_{\text{H}}4 \) genes of the Ig \( V_{\text{H}}44 \) subgroup\textsuperscript{28} (Fig 2). CLL cells were noted to coexpress CD5 and pan-B-cell surface antigens, such as CD20; however, the RS lymphoma cells were CD5\textsuperscript{−} (Fig 3).

Sequence analysis of the Ig \( V \) genes expressed by CLL and RS lymphoma cells. We used an Ig \( V_{\text{H}}4 \) subgroup-specific
Fig 4. Nucleotide sequences of the rearranged Ig VH4 genes cloned from CLL cells (clones pbl, pb2, pb3, and pb4), and from RS lymphoma lesions in a cervical lymph node (clones ne2 and ne5) and in an inguinal lymph node (clones pel and pe3). Also shown are previously reported germline (V71-4) and rearranged (WRD2) Ig VH4 genes with closest sequence homology, and the germline D2 and JH6 segments. Dots indicate homology with the clone pbl. Sequences corresponding to the primers used for PCR are double underlined. Amino acid residues are numbered according to Kabat et al. Base differences resulting in amino acid substitutions are shown in upper case; silent base changes are in lower case.

V\\\alpha gene, designated 1B9/F2.\textsuperscript{36} Consequently, the Ig V\\\alpha genes expressed by the CLL population and the RS lymphoma most probably are derived from a germline Ig V\\\alpha gene other than V\\\alpha S2. The RS lymphoma-derived Ig \textit{VX} clones share a common single-base silent mutation (codon 83), distinguishing these clones from the CLL-derived clones. Two other single-base substitutions were observed among the Ig V\\\alpha clones in codon 48 (clone ne4x) and in codon 59 (clone pe22d).

DISCUSSION

To determine the clonal relationship between a CD5\textsuperscript{+} B-cell CLL and the secondary CD5\textsuperscript{−} high-grade RS lymphoma, we examined the Ig expressed by the CLL cells and

\textbf{CDR1}  31-35  50->-----------65
\textbf{CDR2}  71-76  76->-----------81
\textbf{CDR3}  96-102

\textbf{Fig 5.} Deduced amino acid sequences of the cloned Ig VH4 genes. The clones are indicated as in Fig 4.
RS lymphoma of a patient who underwent Richter's transformation. We found that both CLL and RS lymphoma cells express Ig bearing the Lc sCRI (Fig 2). This sCRI previously was identified on human Igs encoded by a subset of rearranged Ig VX genes over, both Ig genes isolated from either the CLL cells or RS lymphoma lesions in a cervical lymph node (clone ne1 d, ne2x, ne4x) and from RS lymphocytic lymphoma that also was CD5- (Fig 2). The isolated Ig genes belong to the Ig VX gene subgroup.** This study indicates that CLL B cells and the morphologically distinct RS lymphoma share a common clonal origin. We found that both Ig heavy chains expressed by by both CLL and RS lymphoma that also was CD5- (Fig 4). The isolated Ig genes have the highest homology with WRD (95%), a functionally rearranged Ig Vh4 gene isolated from an Lc-reactive small lymphocytic lymphoma that also was CD5+. Moreover, both Ig Vh4 genes of WRD and COT share highest homology with the same germline Ig VX gene, V7-1-4. As such, the Ig Vh4 genes isolated from either the CLL cells or the RS lymphoma agree with the known molecular basis for the Lc sCRI. This substantiates the claim that the functionally rearranged Ig Vh4 genes identified in this study actually are the Ig VH genes expressed by these two Lc1-reactive neoplastic B-cell populations. However, unlike previously reported Ig heavy chains of Lc1-reactive B-cell clones, the Ig heavy chains noted in this study apparently use Ig JH. As such, the current data provide additional evidence that the molecular basis for the Lc sCRI is independent of the genetic elements that form the heavy-chain CDR3.

This study indicates that CLL B cells and the morphologically and phenotypically distinct RS lymphoma share a common clonal origin. We found that Ig heavy chains expressed by both CLL and RS lymphoma use the same Ig Vh4 gene and have identical Ig VDH4 gene rearrangements. Similarly, the cells of the two malignancies express

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the same Ig VX gene and have identical Ig VXJX gene rearrangements. Formed during the process of B-cell differentiation, such Ig rearrangements generate novel CDR3 junctional sequences between the Ig VX, D, and Jx genes for Ig light chain. Furthermore, D and Jx gene segments of the Ig heavy chain generally undergo N-terminal nucleic acid base insertion immediately before Vx gene rearrangement.\textsuperscript{37,38}

Because of the tremendous potential for diversity in these regions, the sequences that comprise the Ig heavy- and light-chain CDR3s generally are unique for each B-cell clone. Finding that the RS lymphoma has Ig light- and heavy-chain CDR3s that are identical to that of the original B-cell CLL population provides compelling evidence that these two neoplastic processes arose from a common clonal ancestor.

It is not likely that cross-contamination of one malignant B-cell population with that of the other accounts for our identifying identical Ig gene rearrangements for both leukemia and lymphoma B-cell populations. Flow cytometric analyses showed that greater than 95% of the CD20\textsuperscript{+} B cells in the peripheral blood expressed CD5, whereas greater than 99% of the B cells in the RS lymphoma population were CD5\textsuperscript{-} (Fig 3). These data indicate that the studied neoplastic populations were homogeneous and exclusive of one another. Additional evidence is provided by the sequence analyses of the isolated Ig Vx genes. Four unique nucleotide substitutions (in codons 21, 71, 82A, and 82C) are identified in each of the Ig VX\textsubscript{HL} genes cloned from the RS lymphoma, but not in the CLL-derived Ig VX\textsubscript{HL} genes (Fig 4). Also, each of the Ig VX\textsubscript{HL} genes isolated from the lymphoma shared the same base substitution in codon 83, distinguishing these genes from CLL-derived Ig VX\textsubscript{HL} genes (Fig 6). Furthermore, the Ig VX gene clones of the CLL were isolated from two independent PCR amplifications, and the rearranged Ig VX genes of the RS lymphoma were isolated from independent PCR performed on DNA from RS lymphoma tissues obtained from two distinct anatomical sites. That all independently isolated lymphoma Ig VX genes should share the same base substitutions from the CLL-derived Ig VX gene consensus sequence argues strongly that these base differences cannot be secondary to mutations introduced by PCR. Rather, these base differences argue that the Ig VX genes isolated from the RS lymphoma are derived from a B-cell population that is distinct from that of the clonally related B-cell CLL.

Finding that an RS lymphoma is derived from the same clone as that of the original B-cell CLL supports earlier studies indicating that the RS lymphoma and CLL cells from any one patient may have similar Ig gene rearrangements by Southern blot analyses.\textsuperscript{69} However, in other studies, different patterns of Ig rearrangements were identified in DNA from CLL compared with that of the RS lymphoma of the same individual.\textsuperscript{11,13-15,17} The latter may be secondary to somatic mutations in or around the expressed Ig genes that fortuitously permute the recognition sequence(s) of the restriction enzyme(s) used in these analyses. In addition, recent studies have shown that fully differentiated B cells may undergo reiterative Ig VX gene rearrangements subsequent to successful expression of Ig heavy and light chain genes.\textsuperscript{23-25,39} Similarly, differences in the isotype of the Ig light chains expressed by CLL versus RS lymphoma may reflect a transformation-related genetic instability in the expression of Ig genes.\textsuperscript{4,18}

In this regard it is noteworthy that the sequence diversity is much greater between the CLL Ig VX genes and those of the RS lymphoma than between those of individual isolates of either population alone. Ignoring the regions that correspond to the PCR primers, the Ig VX genes cloned from the RS lymphoma are virtually identical. We observed a total of three nucleotide substitutions in more than 3,000 nucleotide bases in the 12 Ig VX\textsubscript{HL} and Ig VX\textsubscript{LS} lymphoma-derived clones studied. These substitutions result in only a 0.06% overall difference in nucleic acid sequence, and may be secondary to the known inaccuracy of PCR.\textsuperscript{40} However, the RS lymphoma-derived Ig VX\textsubscript{HL} gene sequences have an average of 5.25 base differences per clone from the consensus sequence of the CLL-derived Ig VX\textsubscript{HL} genes (clone pb1, Fig 4). On the other hand, the CLL-derived Ig VX\textsubscript{HL} genes have an average of only 1.25 base changes per clone from this same consensus sequence. Similarly, Ig VX genes isolated from the RS lymphoma, although virtually homologous to one another, have an average of 1.5 base differences per clone from the consensus sequence of the CLL-derived Ig VX genes (pb8x, Fig 6), whereas the CLL-derived Ig VX\textsubscript{LS} genes have an average of only 0.5 base differences per clone from this same consensus sequence. Thus, the sequence diversity noted between Ig VX genes of the CLL versus that of the RS lymphoma exceeds the diversity noted in the Ig VX genes used by either population alone. As such, it appears that the nucleic acid base substitutions noted in the Ig VX regions expressed by RS lymphoma may have been acquired abruptly during a period of genomic instability that may accompany Richter's transformation. Conceivably, such instability may be secondary to an abortive attempt to activate the process of Ig somatic hypermutation, resulting in Ig VX gene mutations and possibly other cytogenetic changes that contribute to the development of Richter's syndrome. Further investigation of the molecular events that occur during the early stages of Richter's transformation may show mechanisms of genetic instability important in the evolution of this disease.

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