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

By Vladimir Cherepakhin, Stephen M. Baird, Grant W. Meisenholder, and Thomas J. Kipps

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 IgM reactive with Lcl1, a murine monoclonal antibody specific for a supratypic cross-reactive idio-
type encoded by a subset of human Ig variable region genes of the V4 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.

© 1993 by The American Society of Hematology.

IN 1928, MAURICE N. RICHTER described an aggressive lymphoma as “Reticular cell sarcoma” that developed in a patient with chronic lymphocytic leukemia (CLL).\(^1\) Descriptions of such lymphomas vary from what the Working Formulation now describes as either small noncleaved cell lymphoma, large-cell lymphoma (cleaved or noncleaved), or large-cell lymphoma with tumor giant cells.\(^2\) However, all these types are considered high-grade lymphomas in both the Working Formulation and the Kiel classification, and are distinguished readily from the small well-differentiated lymphocytic lymphoma that typifies the tissue phase of CLL. Now known as Richter’s syndrome (RS), this transition from an indolent leukemia to a high-grade B-cell lymphoma (RS lymphoma) occurs infrequently in patients with long-standing CLL.\(^3\)

Controversy exists as to whether such lymphomas arise from the original CLL clone or from a de novo lymphoproliferation in the setting of CLL. In support of the former, several studies have noted that the leukemia and RS lymphoma cells from the same patient express the same Ig light chains and have identical Ig gene rearrangements by Southern analyses.\(^4-9\) However, as noted, the histology of RS lymphomas is distinct from that of the primary B-cell leukemia. Also, there may be differences between lymphoma and leukemia cells in their expression of surface antigens. In particular, in contrast to the leukemic B cells, the high-grade B-cell lymphomas of RS often do not express the CD5 surface antigen.\(^10-15\) Finally, several studies have noted that the leukemia and RS lymphoma cells from the same patient may express different Ig light chain isotypes and/or have distinct Ig gene rearrangements by Southern analyses.\(^6,11,13-18\) These studies have fueled speculation that RS lymphomas may originate from a B-cell clone distinct from that of the original CLL.

However, conclusions based only on surface antigen expression, Ig light chain expression, and/or Ig gene rearrangements patterns are not definitive. B cells may lose expression of surface antigens such as CD5 under a variety of conditions, such as after transformation with Epstein-Barr virus or exposure to cytokines such as interleukin-4.\(^19-22\) Also, B cells originating from the same clone may have disparate Ig gene rearrangements by Southern analyses and/or express different Ig light chains secondary to Ig somatic mutation and/or reiterative Ig gene rearrangements, as occurs in Ig “receptor editing.”\(^23-25\) On the other hand, different B-cell clones fortuitously may share expression of the same Ig light chain and have similar Ig gene rearrangements by Southern analyses, particularly when a limited number of restriction enzymes are used.

To determine whether RS lymphoma is derived from a preexisting CLL B-cell clone, we analyzed the Ig expressed by CLL and RS lymphoma cells from a CLL patient who developed RS. Our results show that both the high-grade B-cell lymphoma and the CLL B cells of this patient share a common clonal origin.

MATERIALS AND METHODS

Patient material. COT, a 56-year-old Caucasian man, was diagnosed as having Rai stage I B-cell CLL in 1984, when he presented with immune thrombocytopenia that required splenectomy that same year. He needed no further therapy until July 1991, when he...
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.

Immunohistochemistry and flow cytometry. Lc1, a murine IgG1 monoclonal antibody (MoAb), was provided by Dr J.G. Sissons (Cambridge University, Cambridge, UK) and conjugated with phycocerythrin (PE) in our laboratory. PE-conjugated mouse IgG1, antihuman CD5 and mouse IgG1, antihuman CD3, and fluorescein isothiocyanate (FITC)-conjugated mouse IgG2a, antihuman CD20 are as described. Conjugates of an irrelevant mouse IgG2a (MOPC21; Caltag, South San Francisco, CA) were used to control for specificity. Immunohistochemistry and flow cytometry analyses were performed as described.

Polymerase chain reaction (PCR). Genomic DNA preparations extracted from peripheral blood lymphocytes (PBL) or from cryostat tissue sections were used as templates. To amplify Ig VH4 regions of the rearranged Ig heavy-chain genes, we used the subgroup-specific leader sense oligonucleotide primer 5'-gtgaattcaagcttTTGACYCAWCGSYCTCAG-3' and 5'-ctcgaattcTTACCTAGGACGGTGAGCTTGGCT-Y, corresponding to an Ig Vδ first framework region (FR1) sense strand consensus sequence and a Jδ antisense consensus sequence, respectively. The amplified fragments were ligated, cloned, and sequenced as described. DNA hybridization. Amplified Ig VH4 genes were detected in 10-μL samples from the PCR reaction mixtures by agarose gel electrophoresis and subsequent Southern blot analyses, essentially as described, but performing final stringent washes at 65°C in 0.2X 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 VH4 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 VH4 genes were registered as distinct bands of expected size (410 bp) in agarose gel electrophoresis.

Colonies of transformed bacteria were screened for human Ig VH4 inserts by Southern blot hybridization of denatured DNA from colonies grown on Hybond-N filters.
leader oligonucleotide sense primer and a JH consensus antisense primer to amplify the rearranged Ig VH 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 32P-labeled human Ig VH4 gene probe. These fragments were cloned for sequence analyses. Two clones from each PCR reaction were sequenced. Each contained a functionally rearranged Ig VH4 gene. All shared the same Ig VH4DJH, indicating that they were derived from a common clonal ancestor. The rearranged Ig VH segment belongs to the Ig VH4 family, and has the closest homology (96%) with a previously published germline sequence V71-4.31 The region deduced as being encoded by a JH segment is most homologous to JH3, with only one base difference from that of the reported germline JH3 sequence (Fig 4). The G → C substitution noted at position 102 also has been noted in other reported JH6 gene segments and may represent a genetic polymorphism.27,32,33 Of all the known germline D segments, the nucleic sequence between that encoded by Ig VH4 and JH6 had the highest homology with the D2 minigene, sharing greater than 91% sequence homology over a stretch of 23 bp.

The sequences of Ig VH4 genes of the CLL and RS lymphoma share several nucleotide codons that are distinct from those of the putative germline Ig VH4 gene, V71-4. However, the Ig VH4 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 VH4 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 Ig VH4 genes from other CLL and RS lymphoma-derived 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 VH4 genes were cloned in a similar way via PCR using a consensus sense primer for the FR1 of human Ig VH and a consensus antisense primer for JH. Each clone contained a functionally rearranged Ig VH4 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 VH4 genes rearranged with the same JACX1 cluster. Moreover, the Ig VXJ4 junctions are identical. Deduced amino acid sequences are shown in Fig 7.

A computer search for known germline VH genes in GenBank identified the cloned Ig VH4 genes to have the highest homology (86%) to V1S2, an Ig VH4 gene of the V1X1 subgroup.35 However, the differences between the cloned Ig VH4 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 VH4 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 large well-differentiated lymphocytes obtained from the cervical lymph node, of which 91% were high-grade lymphoma cells expressing CD20, but not CD3 or CD5. Immunohistochemical analyses showed that the original leukemia B-cell population. Nucleotide changes in codons 82A and 82C that are not present in the Ig VH4 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 Ig VH4 genes from other CLL and RS lymphoma-derived 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 VH4 genes were cloned in a similar way via PCR using a consensus sense primer for the FR1 of human Ig VH and a consensus antisense primer for JH. Each clone contained a functionally rearranged Ig VH4 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 VH4 genes rearranged with the same JACX1 cluster. Moreover, the Ig VXJ4 junctions are identical. Deduced amino acid sequences are shown in Fig 7.

A computer search for known germline VH genes in GenBank identified the cloned Ig VH4 genes to have the highest homology (86%) to V1S2, an Ig VH4 gene of the V1X1 subgroup.35 However, the differences between the cloned Ig VH4 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 VH4 genes and a functionally rearranged Ig

**Sequence analysis of the Ig V genes expressed by CLL and RS lymphoma cells.** We used an Ig VH4 subgroup-specific
Fig 4. Nucleotide sequences of the rearranged Ig Vδ4 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 Vδ4 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.41 Base differences resulting in amino acid substitutions are shown in upper case; silent base changes are in lower case.

Vδ gene, designated 1B9/F2.36 Consequently, the Ig Vδ genes expressed by the CLL population and the RS lymphoma most probably are derived from a germline Ig Vδ gene other than V1S2. The RS lymphoma-derived Ig Vδ 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 Vδ clones in codon 48 (clone ne4x) and in codon 59 (clone pe22d).

DISCUSSION

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

Fig 5. Deduced amino acid sequences of the cloned Ig Vδ4 genes. The clones are indicated as in Fig 4.
Cells express Ig bearing the Lcl sCRI (Fig 2). This sCRI agrees with the known molecular basis for the RS lymphoma. We found that both CLL and RS lymphoma share a common clonal origin. We found that Ig heavy chains expressed by these two Lcl-reactive B-cell clones, the Ig heavy chains noted in this study apparently use genetic elements that form the heavy-chain CDR3.

Similarly, the cells of the two malignancies express Ig genes with closest sequence homology are shown below (see also legend for 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 Lcl sCRI (Fig 2). This sCRI was previously identified on human IgGs encoded by a subset of Ig V \(_{H}\) genes belonging to the Ig \(\text{S}_{\text{S}}\)-region reactive B-cell clones, the Ig \(\text{V}_{\text{H}}\) genes identified in this study actually use the Ig \(\text{V}_{\text{H}}\) genes of WRD and COT share highest homology. The isolated Ig \(\text{V}_{\text{H}}\) genes have the highest homology with WRD (95%), a functionally rearranged Ig \(\text{V}_{\text{H}}\) gene isolated from either the CLL cells or RS lymphoma lesions in a cervical lymph node (clone \(\text{pe22d}\)).

More recently, the cells of the two malignancies express Ig genes with closest sequence homology are shown below (see also legend for 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 Lcl sCRI (Fig 2). This sCRI was previously identified on human IgGs encoded by a subset of Ig \(\text{V}_{\text{H}}\) genes belonging to the Ig \(\text{S}_{\text{S}}\)-region reactive B-cell clones, the Ig \(\text{V}_{\text{H}}\) genes identified in this study actually use the Ig \(\text{V}_{\text{H}}\) genes of WRD and COT share highest homology. The isolated Ig \(\text{V}_{\text{H}}\) genes have the highest homology with WRD (95%), a functionally rearranged Ig \(\text{V}_{\text{H}}\) gene isolated from either the CLL cells or RS lymphoma lesions in a cervical lymph node (clone \(\text{pe22d}\)).

More recently, the cells of the two malignancies express Ig genes with closest sequence homology are shown below (see also legend for 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 Lcl sCRI (Fig 2). This sCRI was previously identified on human IgGs encoded by a subset of Ig \(\text{V}_{\text{H}}\) genes belonging to the Ig \(\text{S}_{\text{S}}\)-region reactive B-cell clones, the Ig \(\text{V}_{\text{H}}\) genes identified in this study actually use the Ig \(\text{V}_{\text{H}}\) genes of WRD and COT share highest homology. The isolated Ig \(\text{V}_{\text{H}}\) genes have the highest homology with WRD (95%), a functionally rearranged Ig \(\text{V}_{\text{H}}\) gene isolated from either the CLL cells or RS lymphoma lesions in a cervical lymph node (clone \(\text{pe22d}\)).

More recently, the cells of the two malignancies express Ig genes with closest sequence homology are shown below (see also legend for 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 Lcl sCRI (Fig 2). This sCRI was previously identified on human IgGs encoded by a subset of Ig \(\text{V}_{\text{H}}\) genes belonging to the Ig \(\text{S}_{\text{S}}\)-region reactive B-cell clones, the Ig \(\text{V}_{\text{H}}\) genes identified in this study actually use the Ig \(\text{V}_{\text{H}}\) genes of WRD and COT share highest homology. The isolated Ig \(\text{V}_{\text{H}}\) genes have the highest homology with WRD (95%), a functionally rearranged Ig \(\text{V}_{\text{H}}\) gene isolated from either the CLL cells or RS lymphoma lesions in a cervical lymph node (clone \(\text{pe22d}\)).
the same Ig V\lambda gene and have identical Ig V\lambda J\lambda gene rearrangements. Formed during the process of B-cell differentiation, such Ig rearrangements generate novel CDR3 junctional sequences between the Ig V\mu, D, and J\mu gene segments for the Ig heavy chain and the V and J genes for Ig light chain. Furthermore, D and J\mu gene segments of the Ig heavy chain generally undergo N-terminal nucleic acid base insertion immediately before V\mu gene rearrangement.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+ B cells in the peripheral blood expressed CD5, whereas greater than 99% of the B cells in the RS lymphoma population were CD5+ (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 V genes. Four unique nucleotide substitutions (in codons 21, 71, 82A, and 82C) are identified in each of the Ig V\mu genes cloned from the RS lymphoma, but not in the CLL-derived Ig V\mu genes (Fig 4). Also, each of the Ig V\lambda genes isolated from the lymphoma shared the same base substitution in codon 83, distinguishing these genes from CLL-derived Ig V\lambda genes (Fig 6). Furthermore, the Ig V gene clones of the CLL were isolated from two independent PCR amplifications, and the rearranged Ig V 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 V genes should share the same base substitutions from the CLL-derived Ig V 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 V 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.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.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 reiterated Ig V gene rearrangements subsequent to successful expression of Ig heavy and light chain genes.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.8,18

In this regard it is noteworthy that the sequence diversity is much greater between the CLL Ig V 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 V 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 V\mu and Ig V\lambda RS 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.40 However, the RS lymphoma-derived Ig V\mu,\lambda genes have an average of 5.25 base differences per clone from the consensus sequence of the CLL-derived Ig V\mu genes (clone pb1, Fig 4). On the other hand, the CLL-derived Ig V\mu genes have an average of only 1.25 base changes per clone from this same consensus sequence. Similarly, Ig V\lambda 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 V\lambda genes (pb8x, Fig 6), whereas the CLL-derived Ig V\lambda genes have an average of only 0.5 base differences per clone from this same consensus sequence. Thus, the sequence diversity noted between Ig V genes of the CLL versus that of the RS lymphoma exceeds the diversity noted in the Ig V genes used by either population alone. As such, it appears that the nucleic acid base substitutions noted in the Ig V 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 V 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.

REFERENCES

6. Bertoli LF, Kuhagawa H, Borzillo GV, Mayumi M, Prchal JT, Kearney JF, Durant JR, Cooper MD: Analysis with antiidiotype...
antibody of a patient with chronic lymphocytic leukemia and a large cell lymphoma (Richter's syndrome). Blood 70:45, 1987
Common clonal origin of chronic lymphocytic leukemia and high-grade lymphoma of Richter's syndrome

V Cherepakhin, SM Baird, GW Meisenholder and TJ Kipps