Autoantibody-Associated Cross-Reactive Idiotypes Expressed at High Frequency in Chronic Lymphocytic Leukemia Relative to B-Cell Lymphomas of Follicular Center Cell Origin

By Thomas J. Kipps, Bruce A. Robbins, Patricia Kuster, and Dennis A. Carson

Using murine monoclonal antibodies (MoAbs) specific for immunoglobulin (Ig) cross-reactive idiotypes (CRIs), we performed immunohistochemical analyses on frozen tissue sections and cytocentrifuge preparations of Ig-expressing malignant cells from patients with chronic lymphocytic leukemia (CLL) and B-cell non-Hodgkin’s lymphomas (NHL) of follicular center cell origin. Twenty percent (4/20) of the Ig\(\times\) light chain–expressing CLL cells reacted with 17.109, a MoAb against a major CRI on human IgM autoantibodies that is encoded by a conserved Ig variable-region gene (V gene) of the V\(\times\)I11b sub-subgroup. Another MoAb specific for V\(\times\)11b framework determinant(s) reacted exclusively with all the 17.109-reactive CLL cells. Only one of 20 \(\times\) light-chain–expressing CLL cells reacted with 6B6.6, a monoclonal antibody specific for a CRI commonly found on rheumatoid factor (RF) paraproteins with light-chain variable regions of the V\(\times\)IIla sub-subgroup. Finally, greater than 20% (8/34) of all CLL reacted with G6, a MoAb specific for an Ig heavy chain–associated CRI present on several RF paraproteins. In contrast, these CRIs were expressed at significantly lower frequencies in NHL of follicular center cell origin. Only one of 30 NHL expressing \(\times\) light chains reacted with the 17.109 MoAb. Also, in contrast to the concordance between the 17.109-CRI and V\(\times\)11b framework determinant(s) in CLL, two lymphomas in addition to the 17.109-reactive lymphoma were recognized by the anti-V\(\times\)11b framework MoAb. None of the NHL reacted with either the 6B6.6 or the G6 MoAbs. These results are the first to demonstrate that CLL and NHL differ with respect to the expression of autoantibody-associated CRIs. The data support the notion that NHL of follicular center cell origin differs from CLL in its utilization and/or somatic mutation of Ig variable-region genes. The physiological and immunotherapeutic implications of these findings are discussed.

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of IgM RF paraproteins. G6-reactive RF paraproteins for which amino acid sequence data are available are noted to share considerable homology in the first, second, and fourth frameworks of the heavy-chain variable region. Therefore, it has been deduced that the heavy-chain variable regions of these paraproteins are encoded by a V<sub>H</sub> gene(s) of the V<sub>H</sub>1 subgroup, a relatively short D segment, and the J<sub>4</sub> gene segment. Similarly, Greenstein et al immunized mice with a human monoclonal RF and generated a MoAb that reacted specifically with all human IgG having V<sub>κ</sub>IIb κ light-chain variable regions regardless of the antibody's specificity. Finally, Schrohenloher and Koopman recently described another MoAb, designated 6B6.6, that defines a second CR1 on RF light chains derived from the V<sub>κ</sub>IIa sub-subgroup. As experiments with the 17.109 MoAb have shown, such antiidiotype reagents may provide highly useful serological probes for the expression of conserved human Ig V genes.

Because of the unexpectedly high prevalence of the 17.109-CRI in CLL, we sought to determine whether other autoantibody CRIs also were expressed in this malignancy. Furthermore, because of recent evidence suggesting that the expressed antibody repertoire of CD5 B cells may be distinct from that of other B lymphocytes, we also examined CD5-negative, slg-positive NHL for expression of the same CRIs. These experiments demonstrate that autoantibody-associated CRIs are common in CLL but are rare in slg-expressing NHL of follicular center cell origin. These data are consistent with the notion that the expression and/or processing of antibody V genes in CLL is distinct from that in CD5-negative NHL. The implications of these findings are discussed.

**MATERIALS AND METHODS**

**Tissue samples.** Fresh-frozen lymphoid tissue from patient biopsy specimens were stored at −70°C prior to preparation of cryostat sections. NHL specimens expressing slg with κ light chains were selected for analyses. Included in our survey were 11 cases of diffuse large-cell lymphoma (DL), three cases of diffuse mixed lymphoma (DM), two cases of diffuse small cleaved lymphoma, one case of diffuse small noncleaved non-Burkitt’s, three cases of follicular mixed lymphoma (FM), and 11 cases of follicular small cleaved lymphoma. Peripheral blood leukocytes (PBL) from patients with CLL were prepared as described. The selection of patients was not biased for patients having autoimmune pathology or Ig paraproteins with RF activity. Rather, the patients examined were those followed by the Hematology/Oncology Division of the Scripps Clinic with peripheral lymphocytosis exceeding 30,000/μL and from whom peripheral blood samples were available. The clinical stages of these patients ranged from Rai stages I to IV, the majority of patients having advanced disease. For analyses, cytocentrifuge preparations of washed lymphocytes were dispensed on poly-L-lysine–coated slides. A minimum of 1,000 cells were examined on each slide.

**Immunohistochemistry.** Enzyme immunohistochemical staining of frozen malignant lymphoid tissue sections or cytocentrifuge preparations of circulating malignant B lymphocytes was performed by using an avidin-biotin complex immunoperoxidase technique as described. Comparison staining with isotype control murine MoAbs was performed at concentrations equal to those used with the specific MoAb in each experiment. Staining intensity was graded by using a scale of (−) to (+++/−), with (−) representing nonstained and (++++) representing intense staining (Fig 1). A grade of (+/−) denotes very weak staining slightly above that of background.

**Antibodies.** MoAb G6 was the gift of Dr Roy Jefferis (University of Birmingham, England). A MoAb specific for the V<sub>κ</sub>IIb framework determinants was obtained from Dr George Abraham (University of Rochester, NY). MoAb 6B6.6 was provided by Drs Ralph E. Schrohenloher and William J. Koopman (University of Alabama, Birmingham). MoAb 17.109 was as described. Hybridomas obtained from the American Type Culture Collection (Rockville, MD) were OKT3, an IgG2a anti-CD3<sup>25</sup>; DA4-4, an IgG1 anti-human κ heavy chain<sup>26</sup>; dTEA-4-1, an IgG3 anti-human δ light chain<sup>25</sup>; and SC-1, an IgG2a anti-CD5<sup>25</sup>. Antihuman κ or λ light chain–producing hybridomas were as described. Antibodies were purified from ascites via ammonium sulfate precipitation and either absorption with QAE [(Pharmacia, Uppsala, Sweden) for IgG1 and IgG3) or protein A-Sepharose column chromatography (Bio-Rad Laboratories, Richmond, CA).

**RESULTS**

The malignant cells from 34 patients with CLL were examined by immunohistochemistry. Of the lymphocytes from each patient, fewer than 5% expressed the pan-T lymphocyte surface antigen CD3, while greater than 70% expressed CD5 and slg (data not shown). Cells were tested for reactivity with each of the panel of antiidiotypic antibodies. Many of these CLL were examined previously by using flow cytometry to detect slg reactive with phycoerythrin-conjugated 17.109 (PE-17.109) with similar results. Tissue samples from five previously examined light chain–expressing CLL cases, including one patient noted to have cells reactive with PE-17.109, were not available for immunohistochemical analyses. Greater than 80% of the PBLs from patients H.A.H., H.I.C., B.R.O., and S.M.I. reacted with 17.109 in our immunohistochemical assay (Table 1). All and only those CLL cases found to be 17.109-positive reacted with the MoAb specific for V<sub>κ</sub>IIb framework determinants (Table 1). Moreover, the cell’s staining intensity with 17.109 generally correlated with that achieved when using the anti-V<sub>κ</sub>IIb framework MoAb (Table 1). Only one κ light chain–expressing CLL, L.E.S., surveyed in the current study reacted with 6B6.6 (Table 1). Consistent with this antibody recognizing a CRI present on a subgroup of V<sub>κ</sub>IIa light chains, L.E.S. cells failed to react with the anti-V<sub>κ</sub>IIb framework MoAb. Greater than 20% (5/20 κ and 3/13 λ) of all Ig-expressing CLL reacted with the G6 monoclonal anti-CRI (Tables 1 and 2). Moreover, the staining intensity of G6 correlated with that noted for slgM for each of these CLL. Of note, half of the 17.109-positive CLL reacted with G6 (S.M.I. and B.R.O., Table 1, Fig 2), which suggests a possible biased association of the two CRIs. In CRI-positive cases where both PBL and frozen lymphoid tissue were available (H.A.H. and H.U.R.), the staining of leukemic PBL in cytocentrifuge preparations correlated with that of infiltrating leukemic cells in frozen tissue sections (Table 1).

The relative frequency of expression of these CR1 on slg of 31 cases of B-cell NHL differed significantly (Table 2). These lymphomas do not express CD5 and are considered to be of follicular center cell origin. Of the 30 κ light chain–expressing NHL, only one reacted with the 17.109 MoAb...
Fig 1. Cryostat sections of liver from CLL patient H.A.H. that demonstrate (+ + +) staining of 17.109-reactive leukemic cells infiltrating hepatic sinusoids. Sections are stained with either an IgG2b isotype control mouse MoAb of nonspecific activity (panel A) or MoAb 17.109 (panel B).

Table 1. CLL and NHL Positive for CRIs

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Source</th>
<th>CDS</th>
<th>sigM</th>
<th>sigD</th>
<th>κ</th>
<th>λ</th>
<th>17.109</th>
<th>Vκllb</th>
<th>G6</th>
<th>886.6</th>
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<tr>
<td>A.N.D.</td>
<td>CLL</td>
<td>PBL</td>
<td>++</td>
<td>++++</td>
<td>+/+</td>
<td>-</td>
<td>+</td>
<td>++++</td>
<td>-</td>
<td>-</td>
<td>++++</td>
</tr>
<tr>
<td>B.R.O.</td>
<td>CLL</td>
<td>PBL</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>++++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F.U.H.</td>
<td>CLL</td>
<td>PBL</td>
<td>+++</td>
<td>++++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
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<td>CLL</td>
<td>PBL</td>
<td>+++</td>
<td>++++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>H.A.H.</td>
<td>CLL</td>
<td>Liver</td>
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<td>++++</td>
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<td>+</td>
<td>-</td>
<td>++++</td>
<td>++++</td>
<td>-</td>
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<td>Spleen</td>
<td>+++</td>
<td>++++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++++</td>
<td>++++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>H.I.C.</td>
<td>CLL</td>
<td>PBL</td>
<td>+++</td>
<td>++++</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>H.U.R.</td>
<td>CLL</td>
<td>PBL</td>
<td>+++</td>
<td>++++</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
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<tr>
<td>H.U.R.</td>
<td>CLL</td>
<td>LN</td>
<td>+++</td>
<td>++++</td>
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<td>-</td>
<td>-</td>
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<td>N.E.I.</td>
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<td>PBL</td>
<td>+++</td>
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<tr>
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<td>+++</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>S.A.L.</td>
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<td>LN</td>
<td>-</td>
<td>++++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>D.E.R.</td>
<td>DM</td>
<td>LN</td>
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<td>++++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<td>B.R.I.</td>
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<td>LN</td>
<td>-</td>
<td>++++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
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</tr>
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Abbreviation: LN, lymph node.
gene(s) or of a rebated VH gene with similar first- and second-framework subgroup. As such it might be expected to recognize a subset of the $\kappa$ light chain–expressing CLL. The $\kappa$ light chain of the 6B6.6-CRI–positive CLL L.E.S. had been analyzed in an earlier study because of the RF activity of the L.E.S. CLL paraprotein. The light chain encoded by the expressed L.E.S. $\kappa$ gene belongs to the V\text{k}III sub-subgroup and shares extensive homology with the $\kappa$ light chains of the RF paraprotein P0. We have recently isolated a nonrearranged V\text{k}III gene from the granulocyte DNA of patient L.E.S., designated humkv328, from which the expressed gene apparently is derived.

Finding that only one of the tested CLL samples reacts with this antibody is consistent with the notion that the humkv328 gene is utilized less frequently than is humkv325 in CLL or that the CRI recognized by 6B6.6 is lost because of somatic mutation in the expressed $\kappa$ gene.

In addition to the anti-CRI antibodies, we used a MoAb specific for V\text{k}IIIb framework determinants to determine the expression frequency of this variable region sub-subgroup in CLL. Only CLL samples that also reacted with the 17.109 antibody were recognized by this antiframework MoAb. Estimates of the number of distinct $\kappa$ genes in humans have been noted to undergo Ig V gene somatic mutation that introduces heterogeneity in the expressed antigen have been used in this study. Consistent with this notion is the lack of concordance between the reactivities of the 17.109 and the anti-V\text{k}IIIb framework antibodies in CLL cases studied. Furthermore, the inability to detect V\text{k}IIIb-positive CLL cells that do not react with the 17.109 MoAb is consistent with the notion that this V\text{k} gene is expressed with little or no somatic mutation in CLL.

Although expression of autoantibody-associated CRIs has been detected in NHL, our study indicates that expression of such CRIs is significantly less frequent in NHL of follicular center cell origin than in CLL (Tables 1 and 2). The divergent frequencies of these CRIs is significantly less frequent in NHL of follicular center cell origin than in CLL. The divergent frequencies of these CRIs is significantly less frequent in NHL of follicular center cell origin than in CLL. The divergent frequencies of these CRIs is significantly less frequent in NHL of follicular center cell origin than in CLL.

The present study extends this analysis by examining CLL for expression of other CRIs that also may be phenotypic markers for conserved Ig V genes. Greater than 20% of all CLL cells tested reacted with MoAb G6, which identifies an antibody heavy chain–associated CRI present on several RF paraproteins. Protein sequence data of four paraproteins reactive with G6 suggest that the G6-CRI may be a marker for the expression of heavy-chain V gene(s) of the V\text{H}I subgroup. All four paraproteins share extensive amino acid sequence homology in the first- and second-framework regions and apparently utilize the J\text{H}4 gene segment. Whether the repeated appearance of the G6-CRI in CLL is secondary to high-frequency utilization of a particular V\text{H}I gene(s) or of a related V\text{H}I gene with similar first- and second-framework determinants is currently under investigation. Another RF-associated CRI, identified by MoAb 6B6.6, was detected on only one of the 20 CLL samples expressing $\kappa$ light chains. This antibody reacts with a V\text{k}IIIa-encoded determinant(s) that is present on several IgM-RF paraproteins. As such it might be expected to recognize a subset of the $\kappa$ light chain–expressing CLL. The $\kappa$ light chain of the 6B6.6-CRI–positive CLL L.E.S. had been analyzed in an earlier study because of the RF activity of the L.E.S. CLL paraprotein. The light chain encoded by the expressed L.E.S. $\kappa$ gene belongs to the V\text{k}III sub-subgroup and shares extensive homology with the $\kappa$ light chains of the RF paraprotein P0. We have recently isolated a nonrearranged V\text{k}III gene from the granulocyte DNA of patient L.E.S., designated humkv328, from which the expressed gene apparently is derived.

Finding that only one of the tested CLL samples reacts with this antibody is consistent with the notion that the humkv328 gene is utilized less frequently than is humkv325 in CLL or that the CRI recognized by 6B6.6 is lost because of somatic mutation in the expressed $\kappa$ gene.

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Although expression of autoantibody-associated CRIs has been detected in NHL, our study indicates that expression of such CRIs is significantly less frequent in NHL of follicular center cell origin than in CLL (Tables 1 and 2). The divergent frequencies of these CRIs in NHL v CLL may be secondary to differences in antibody V gene utilization by the malignant B cells in these two diseases. Alternatively, the divergence may relate to differences in the rates at which the expressed antibody V genes undergo somatic mutation. Human B-cell follicular lymphomas that lack the CD5 antigen have been noted to undergo Ig V gene somatic mutation that introduces heterogeneity in the expressed antibody idiotypes. Such a V gene somatic mutation may permutate and distort CRI determinants that are recognized by the murine MoAbs used in this study. Consistent with this notion is the lack of concordance between the reactivities of the 17.109 and the anti-V\text{k}IIIb framework antibodies in NHL. The latter MoAb detected two additional lymphomas in addition to the single case that expressed the 17.109-CRI. Thus, in this limited survey, the expression frequency of V\text{k}IIIb framework determinants in NHL does not differ significantly from that noted in CLL.

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**Table 2. Expression Frequencies of Autoantibody-Associated CRIs in B-Cell Neoplasms**

<table>
<thead>
<tr>
<th>Major CRI</th>
<th>$\kappa$ Light Chain Expressing (%)</th>
<th>$\lambda$ Light Chain Expressing (%)</th>
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<tr>
<td>17.109</td>
<td>20 (±9)*</td>
<td>3 (±4)</td>
</tr>
<tr>
<td>V\text{k}IIb</td>
<td>20 (±9)</td>
<td>10 (±6)</td>
</tr>
<tr>
<td>686.6</td>
<td>5 (±5)</td>
<td>0</td>
</tr>
<tr>
<td>G6</td>
<td>25 (±10)</td>
<td>21 (±11)</td>
</tr>
</tbody>
</table>

* $\pm$ SE.
Fig 2. Cytocentrifuge preparations of PBL from patient S.M.1. that demonstrate (+ +) and (+ + +) staining of peripheral leukemic cells. Cells have (−) staining with an IgG2b isotype control mouse MoAb of nonspecific activity (panel A), (+ + +) staining with MoAb 17.109 (panel B), or (+ +) staining with MoAb G6 (panel C).
CROSS-REACTIVE IDIOTYPES

These findings may have important implications for immunotherapy for CLL (and perhaps Waldenström’s macroglobulinemia). A disease derived from a minor B-cell subset that expresses slg with a restricted set of Ig V genes with minimal somatic diversification should be particularly amenable to passive immunotherapy with antidiotypic antibodies. Furthermore, as shown here in these studies, MoAbs can be identified that detect CRIs that frequently are expressed in CLL. Batteries of such monoclonal antidiotypic reagents may be useful for the early diagnosis and immunotherapy for large numbers of patients with this malignancy.

ACKNOWLEDGMENT

We thank Drs Roy Jefferis (University of Birmingham, England), George Abraham (University of Rochester, NY), and Ralph E. Schrohlohe and William J. Koopman (University of Alabama, Birmingham) for providing us with some of the MoAbs used in these studies as listed in Materials and Methods. We appreciate the cooperation of other physicians in the Hematology/Oncology Division of the Scripps Clinic Medical Group, in particular, Drs Lawrence Piro and William Miller, in providing clinical samples from their patients.

REFERENCES

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TJ Kipps, BA Robbins, P Kuster and DA Carson