Large Cell Lymphoma Transformation of Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

A Flow Cytometric Analysis of Seven Cases

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Key Words: Chronic lymphocytic leukemia; Richter syndrome; Flow cytometry; CD5

Abstract

We studied 7 cases of large cell transformation of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) immunophenotyped by multiparameter flow cytometry. The 6 women and 1 man ranged in age from 45 to 91 years. All had previous or concurrent evidence of CLL/SLL. Morphologic features and sites of involvement of the diffuse large B-cell lymphoma (DLBCL) were heterogeneous; 2 cases had paraimmunoblastic morphologic features. Six DLBCLs had an immunophenotype consistent with CLL: CD19+, CD5+, CD23+, and FMC7 negative (3 cases) or very dim (2 cases); 1 case was not studied for FMC7. CD20 was dim in 3 of these, moderate to bright in 2, and variable in 1. Surface immunoglobulin was dim in 2 cases and moderate or bright in 4. Five of 6 expressed CD38. Comparison with the immunophenotypes of the previous or coexistent CLL/SLL (4 of 6 cases) revealed minor modulations in antigen expression but no major alterations. The seventh DLBCL lacked CD5 expression, but otherwise had immunophenotypic features similar to CLL. These findings indicate that DLBCL arising in CLL/SLL tends to retain a CLL immunophenotype, in contrast with de novo CD5+ large cell lymphomas that uncommonly express such a phenotype.

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is a common B-cell lymphoproliferative disorder with a characteristically indolent clinical course. A diffuse large B-cell lymphoma (DLBCL) occurs in a small minority of patients (approximately 2% of cases). This transformation is termed Richter syndrome when associated with a constellation of clinical features that includes an abrupt change in clinical status; rapidly enlarging, asymmetric lymphadenopathy; a high serum lactate dehydrogenase level; and a poor response to aggressive therapy. Roughly two thirds of DLBCLs arising in CLL/SLL seem to represent clonal evolution of the underlying low-grade tumor, whereas in the remaining cases, they seem to be clonally unrelated, de novo neoplasms. The mechanisms underlying CLL transformation are poorly understood. Among those implicated in small numbers of cases are p53 mutations, p16 inactivation, gain of an additional chromosome 12, c-myc amplification, and Epstein-Barr virus (EBV) infection.

The immunophenotype of CLL is characteristic, permitting distinction from other small B-cell neoplasms in the large majority of cases. Specifically, nearly all cases of CLL express CD19, the T-cell–associated antigen CD5, and CD23. Most in addition lack FMC7 and show relatively dim expression of CD20 and surface immunoglobulin (SIg). Among small B-cell disorders, CD5 expression is restricted essentially to CLL and mantle cell lymphoma. However, one occasionally encounters a CD5-expressing DLBCL with no evidence of a coexisting or antecedent small B-cell neoplasm. In such cases, it is not clear whether these represent de novo processes or transformations of undetected small-cell neoplasms. While we and others have shown that de novo CD5+ DLBCL rarely has an immunophenotype resembling CLL, the literature data on the immunophenotypic
features of DLBCL transformation of CLL/SLL are sparse. For this reason, we reviewed our institutional experience with the immunophenotypic features of such cases.

Materials and Methods

Patients

The computerized flow cytometry database at the University of Texas Southwestern Medical Center, Dallas (Panorama Direct, Provue Development, Huntington Beach, CA), was searched for records entered from April 1994 through July 1999. All cases of DLBCL with a history of CLL and all CD5+ DLBCLs were retrieved. Inclusion criteria included the following: material available for morphologic review; large cell lymphoma histologic features; and evidence of previous or concurrent CLL/SLL, with or without previous flow cytometric analysis. CLL/SLL cases showing prolymphocytoid transformation in the blood or abundant proliferation centers in lymph nodes were excluded.

Immunophenotypic Analysis

Flow Cytometry

Fresh specimens of tissue or blood were processed according to a variety of protocols depending on the source of the specimen and the period during which it was received. All specimens were analyzed using broad 3-color (cases 1-4 and 7 and blood in case 6) or 4-color (case 5 and tissue of case 6) panels that included CD5, CD10, CD19, CD20, CD23, CD38, and 2 Ig analyses. FMC7 was analyzed in all but 1 case. The specific antibodies and antibody combinations used are detailed in Table 1 and Table 2. The specimens were run on a 3-color (FACSort) or a 4-color (FACSCalibur) flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA) and analyzed with Paint-A-Gate software (Verity House, Topsham, ME). No gating was performed, and, thus, all normal and neoplastic populations within a given sample were analyzed. Antigen expression on abnormal populations was compared with appropriate isotypic controls and negative and positive populations in a given tube. In general, antigen positivity was defined as a discrete population shift relative to the isotypic control. Assessments of intensity of antigen expression were qualitative and based on accumulated laboratory experience with individual antibodies in normal and neoplastic cell populations. Qualitative assessment of changes in antigen expression over time was made relative to internal normal populations and residual CLL populations.

Immunohistochemistry

Immunohistochemistry for cyclin D1 (AM29, 1:75; Zymed Laboratories, San Francisco, CA) expression was performed on case 2 using an automated stainer (Techmate, Ventana Medical Systems, Tucson, AZ) on a formalin-fixed,
paraffin-embedded section using a standard avidin-biotin-peroxidase method. Pretreatment consisted of 2 five-minute microwave treatments in citrate buffer at pH 6.8.

**Molecular Studies**

Polymerase chain reaction (PCR) for Epstein-Barr–encoded RNA-1 DNA sequences was performed in cases 1 and 7 according to a previously published method. Epstein-Barr–encoded RNA in situ hybridization was performed on paraffin sections in cases 1 and 7 with standard methods using a commercially available kit (Super-Sensitive ISH Detection System, Biogenex, San Ramon, CA). \( J_{\text{H}} \) PCR was performed in case 1 with framework III primers using a previously published method.

**Results**

**Clinical and Molecular Features**

Seven cases of large cell transformation of CLL/SLL were retrieved. One of these (case 1) was reported previously in abstract form. The original requisitions for 6 of these included a history of CLL/SLL; in 1 patient without a history of CLL/SLL, histologic diagnosis of concurrent CLL/SLL was made at the time of diagnosis of DLBCL. The clinical data for the 7 patients are given in Table 3. The 1 man and 6 women ranged in age from 45 to 91 years (median, 62 years) at the time of appearance of the DLBCL. The interval since the original diagnosis of CLL/SLL ranged from 0 to 93 months (median, 13 months). Two patients had undergone allogeneic bone marrow transplantation 100 days and 4 years previously (cases 1 and 7, respectively). The DLBCL in case 1 was positive for EBV by PCR and in situ hybridization, whereas the CLL/SLL was negative for EBV; \( J_{\text{H}} \) PCR revealed identically sized rearranged bands in both components. The DLBCL in case 7 was negative for EBV by both methods; material from the previous CLL/SLL was not available for heavy-chain gene analysis.

The DLBCLs involved a variety of nodal and extranodal sites; extranodal sites included blood, bone, uterus, and colon. Of 6 patients with available follow-up, 5 died of disease 2 to 14 months (median, 7 months) after diagnosis of DLBCL. One was alive with disease at 16 months.

**Table 2**

**Three- and Four-Color Antibody Panels Used**

<table>
<thead>
<tr>
<th>Three-Color (Tissue, Blood) (FITC/PE/PerCP)</th>
<th>Four-Color (Blood) (FITC/PE/PerCP/APC)</th>
<th>Four-Color (Tissue) (FITC/PE/PerCP/APC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD30/CD14/CD45</td>
<td>CD22/CD11c/CD45/CD14</td>
<td>CD30/CD2/CD45/CD3</td>
</tr>
<tr>
<td>Monoclonal lambda/CD19</td>
<td>lambda/CD5/CD19</td>
<td>lambda/CD5/CD19</td>
</tr>
<tr>
<td>Polyclonal lambda/polyclonal</td>
<td>Polyclonal lambda/polyclonal</td>
<td>Polyclonal lambda/polyclonal</td>
</tr>
<tr>
<td>kappa/CD38</td>
<td>kappa/CD20/CD38</td>
<td>kappa/CD20/CD38</td>
</tr>
</tbody>
</table>

APC, allophycocyanin; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP, peridinin chlorophyll.

* CD22/CD11c/CD19 in case 2.
† CD23/Leu8/CD19 in case 2.

**Table 3**

**Clinical Features of Patients With Chronic Lymphocytic Leukemia (CLL)**

<table>
<thead>
<tr>
<th>Case No./Sex/Age (y)</th>
<th>Duration of CLL (mo)</th>
<th>Site(s) of Transformation</th>
<th>Other</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/57</td>
<td>93</td>
<td>Neck mass</td>
<td>100 d after BMT; EBV positive</td>
<td>DOD, 10 mo</td>
</tr>
<tr>
<td>2/M/75</td>
<td>12</td>
<td>Left tibia</td>
<td>—</td>
<td>NA</td>
</tr>
<tr>
<td>3/F/91</td>
<td>0</td>
<td>Cervical, axillary, and mesenteric LNs</td>
<td>—</td>
<td>AWD, 16 mo</td>
</tr>
<tr>
<td>4/F/54</td>
<td>13</td>
<td>Breast LN</td>
<td>—</td>
<td>DOD, 7 mo</td>
</tr>
<tr>
<td>5/F/62</td>
<td>4</td>
<td>Left inguinal, iliac, femoral LNs</td>
<td>—</td>
<td>DOD, 14 mo</td>
</tr>
<tr>
<td>6/F/77</td>
<td>45</td>
<td>Blood, massive retroperitoneal and mediastinal LNs</td>
<td>—</td>
<td>DOD, 2 mo</td>
</tr>
<tr>
<td>7/F/45</td>
<td>63</td>
<td>Uterus, colon, iliac LNs</td>
<td>4 y after BMT; EBV negative</td>
<td>DOD, 3 mo</td>
</tr>
</tbody>
</table>

AWD, alive with disease; BMT, allogeneic bone marrow transplantation; DOD, died of disease; EBV, Epstein-Barr virus; LN, lymph node; NA, not available.

* Age at transformation.
Morphologic Features

Large B-Cell Lymphomas

The morphologic features of the 7 cases were heterogeneous. Three cases (3, 4, and 7) were composed of monomorphous sheets of large cells with vesicular chromatin, prominent, often centrally located eosinophilic nucleoli, and moderate amounts of dense eosinophilic cytoplasm, resembling paraimmunoblasts.

Two cases (1 and 2) were composed of uniform proliferations of large cells with strikingly round nuclei and distinct central eosinophilic nucleoli, consistent with paraimmunoblasts. These were diffuse proliferations without a component of small lymphocytes or organization into proliferation centers. The lymph node from case 5 demonstrated a heterogeneous cellular composition with some areas showing conventional CLL/SLL, others resembling sheets of prolymphocytes, and others with the appearance of a pleomorphic large cell lymphoma. A bone marrow biopsy specimen obtained shortly after the DLBCL diagnosis in this case demonstrated a cohesive aggregate of large cells cytologically identical to the pleomorphic areas of the lymph node. A bone marrow biopsy specimen obtained shortly after the DLBCL diagnosis in this case demonstrated a cohesive aggregate of large cells cytologically identical to the pleomorphic areas of the lymph node. A bone marrow biopsy specimen obtained shortly after the DLBCL diagnosis in this case demonstrated a cohesive aggregate of large cells cytologically identical to the pleomorphic areas of the lymph node. A bone marrow biopsy specimen obtained shortly after the DLBCL diagnosis in this case demonstrated a cohesive aggregate of large cells cytologically identical to the pleomorphic areas of the lymph node.

For case 1, a peripheral blood smear from 7 months before the DLBCL diagnosis revealed an absolute lymphocytosis of 14,300/µL (14.3 · 10^9/L) consisting of a monomorphous proliferation of small lymphocytes with round nuclei, clumped chromatin, inconspicuous nucleoli, and a narrow rim of lightly basophilic cytoplasm (“typical CLL”). Prolymphocytes were rare.

Case 3 had no history of CLL, and the peripheral blood lacked an absolute lymphocytosis. However, focal areas of the DLBCL biopsy specimen contained typical CLL/SLL, with a monomorphous proliferation of small round cells with clumped chromatin and occasional admixed prolymphocytes and paraimmunoblasts.

Peripheral blood smears were available for review for case 5 at initial diagnosis (5 months before) and concurrent with the DLBCL diagnosis. These had absolute lymphocytoses of 13,600/µL (13.6 · 10^9/L) and 14,400/µL (14.4 · 10^9/L), respectively. Both showed typical CLL, as described. There was no appreciable morphologic difference between these 2 peripheral blood specimens; in particular, only occasional prolymphocytes were seen at both time points. A bone marrow biopsy at the time of DLBCL diagnosis...
demonstrated, in addition to the large cell infiltrate described in the previous section, several small to medium-sized random focal and interstitial infiltrates of monotonous small, round lymphocytes.

Seven peripheral smears ranging from 12 to 45 months before the transformation diagnosis were reviewed for case 6. Absolute lymphocyte counts during this period ranged from 16,500 to 25,800/µL (16.5-25.8 × 10^9/L). The lymphocytes were predominantly typical CLL cells, although from the time of diagnosis, a small population of folded or clefted lymphocytes was also present. These did not increase in relative number during the observation period. Prolymphocytes were found easily in all samples but always represented fewer than 5% of the lymphocytes.

A peripheral blood smear from case 7 obtained 17 months before DLBCL diagnosis revealed an absolute lymphocyte count of 6,700/µL (6.7 × 10^9/L) that consisted of typical CLL with occasional prolymphocytes. Morphologic material demonstrating the preexisting CLL/SLL was not available for cases 2 and 4.

**Immunophenotypic Features**

Six cases (1-6) were CD5+ and 1 case (7) was CD5–.

**Table 4**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>CD5</th>
<th>CD23</th>
<th>FMC7</th>
<th>CD20</th>
<th>CD38</th>
<th>Slg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>Dim</td>
<td>+</td>
<td>Dim</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>Dim +</td>
<td>ND</td>
<td>Variable</td>
<td>+</td>
<td>Dim</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>Dim +</td>
<td>Moderate/bright</td>
<td>Variable +</td>
<td>Dim</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>Dim</td>
<td>+</td>
<td>Bright</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>Dim +</td>
<td>Moderate</td>
<td>+</td>
<td>Moderate</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>Moderate/bright</td>
<td>Dim, partial +</td>
<td>Dim</td>
</tr>
<tr>
<td>7</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Moderate/bright</td>
<td>+</td>
<td>Dim</td>
</tr>
</tbody>
</table>

CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; ND, not done; SIg, surface immunoglobulin; +, positive; –, negative.

**Table 5**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Source of CLL/SLL Component</th>
<th>Immunophenotype of DLBCL Compared With CLL/SLL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blood, 7 mo earlier</td>
<td>&gt;CD5, &gt;CD38</td>
</tr>
<tr>
<td>2</td>
<td>Concurrent CLL component in tissue</td>
<td>More variable CD20, &gt;CD38</td>
</tr>
<tr>
<td>5</td>
<td>Blood, 4 mo earlier</td>
<td>Loss of dim FMC7, more variable CD20</td>
</tr>
<tr>
<td>6</td>
<td>Blood, 45 mo earlier</td>
<td>&gt;CD5, &gt;CD20, &gt;FMC7, fl CD19</td>
</tr>
<tr>
<td>6</td>
<td>Concurrent CLL in blood at transformation</td>
<td>&gt;CD5, &gt;CD20, &gt;CD38, &gt;FMC7, &gt;SIg*</td>
</tr>
<tr>
<td>7</td>
<td>Blood, 17 mo earlier</td>
<td>Loss of CD5, &gt;CD20, &gt;CD38</td>
</tr>
</tbody>
</table>

CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; DLBCL, diffuse large B-cell lymphoma; SIg, surface immunoglobulin; >, increased; fl, decreased.

* CLL component with dimmer CD19 and more variable CD23 and Slg compared with previous CLL/SLL.

Immunophenotyping of the previous CLL/SLL was performed in our laboratory for cases 1, 5, 6, and 7. In addition, a residual CLL/SLL population was present in the flow cytometry specimens of cases 2 and 6; in both of these cases, the CLL/SLL and large cell populations were distinct, based on light-scatter properties. Overall, therefore, the immunophenotypic features of the antecedent CLL/SLL population were available for comparison with the large cell population in 5 of 7 cases.

In all 5 cases, the same light-chain type was expressed by both components. The alteration of immunophenotype of the large cell population compared with the CLL/SLL population is detailed in **Table 5** and illustrated in **Figure 1** through **Figure 4**.

Cases 1, 2, 5, and 6 showed no gross changes in antigen expression, although minor modulations in some antigens were noted. Two cases showed increased CD5 intensity, 1 showed increased CD20 expression, and 2 showed more variability in CD20 expression on the tumor cell population. CD38 intensity was increased in 2 cases compared with the previous CLL/SLL, although CD38 was expressed on the CLL/SLL population in both cases. One case showed a mild...
**Figure 1** (Case 1) Side-by-side flow cytometry dot plots of diffuse large B-cell lymphoma (DLBCL) and preceding chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). Compared with the CLL/SLL, the DLBCL has increased intensity of CD38 and CD5. Red, DLBCL cells; yellow, CLL/SLL cells; green, T cells.

**Figure 2** (Case 5) The diffuse large B-cell lymphoma (DLBCL) demonstrates more variable CD20 and loss of dim partial FMC7 expression compared with preexisting chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). Red, DLBCL cells; yellow, CLL/SLL cells; green, T cells; blue, polytypic B cells.
Figure 3 (Case 6) In this case, previous and concurrent immunophenotypes are available for the chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) component. Note that the CLL/SLL has altered its immunophenotype between these 2 analyses, which are 45 months apart. In the later sample, the cells have dimmer CD19 and more variable CD23 and surface lambda. The diffuse large B-cell lymphoma (DLBCL) component more closely resembles the original CLL/SLL, although it has increased intensity of CD5 and CD20 and has gained FMC7 compared with both. In addition, the CD19 intensity is similar to the concurrent CLL/SLL. Red, DLBCL cells; yellow, CLL/SLL cells; blue, polytypic B cells.

Figure 4 (Case 7) While it seems that the diffuse large B-cell lymphoma (DLBCL) is positive for CD5 and FMC7, in fact, there is no increased fluorescence for either of these markers compared with the same population in the isotypic control tube. The DLBCL does, however, show increased intensity of both CD20 and CD38. Red, DLBCL cells; yellow, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) cells; green, T cells.
increase in FMC7 expression, and another lost the partial dim expression of FMC7 that had been present on the preexisting CLL/SLL. The CLL/SLL population in case 6 showed an alteration in immunophenotype between the initial study and the time of transformation in the peripheral blood 45 months later. Specifically, at the time of transformation, the expression of CD23 and SIg was more variable than it had been previously. As a result, the large cell tumor in this case showed greater expression of these antigens compared with the coexistent CLL/SLL population. Case 7, in addition to the loss of CD5 expression, also showed increased CD20 intensity in the large cell population.

**Discussion**

We have presented the immunophenotypic features of 7 DLBCLs arising in the setting of CLL/SLL. All but one patient in this series had a previous diagnosis of CLL/SLL; 1 case was diagnosed at the time of discovery of the DLBCL. For all patients, the median interval between diagnoses of CLL/SLL and DLBCL was 13 months. This is shorter than in other published series in which the median interval to diagnosis of DLBCL ranged from 47 to 49 months. Unexpectedly, all but 1 of our patients were women, whereas a mild male predominance has been reported previously. Similar to findings in other studies, the survival of the patients in our series was poor; 5 of 6 with available follow-up died of disease after a median interval of 7 months (range, 2-14 months); 1 was alive with disease at 16 months after diagnosis.

Our results indicate that the majority of DLBCL transformations of CLL/SLL (6 of 7) retain the major immunophenotypic features of CLL/SLL, specifically expression of CD5 and CD23, with no or dim expression of FMC7. Furthermore, when the immunophenotype of the previous or coexisting CLL/SLL was available, we noted only minor modulations. These findings lend support to the notion that de novo CD5+ DLBCLs, which uncommonly express CD23, are not related biologically to CLL/SLL. The only DLBCL that showed a significant deviation from a CLL/SLL-like immunophenotype was case 7, which lacked expression of CD5. However, this tumor expressed CD23 in the absence of FMC7 and had dim SIg expression. Dim expression of CD20 and SIg were each noted in only 3 of 7 DLBCLs. Bright expression of each of these markers has been reported in up to approximately one fourth of CLL/SLL cases; thus, the importance of this finding in our small series is unknown. Substantial CD38 expression has been reported in approximately one half of cases of CLL/SLL. All but one of the DLBCLs in the present study expressed substantial amounts of CD38, which also was seen on the preexisting CLL/SLL; CD38 was increased in intensity in the DLBCLs in 3 of the 6 cases.

The morphologic features of the cases in the present series were variable. Of note, the tumors in 2 cases exhibited diffuse, uniform proliferations of cells cytologically similar to paraimmunoblasts seen in proliferation centers of CLL/SLL. The concept of paraimmunoblastic transformation of CLL/SLL is poorly recognized in the literature. Pugh et al reported 16 cases of a tumor that they described as a paraimmunoblastic variant of CLL/SLL. Of 7 of these assessed for CD5 expression in frozen sections, 6 were positive. Several in addition had associated small cell proliferations that were thought to represent typical CLL/SLL, although immunophenotypic data on the small cell processes was not provided. The median survival of the patients in the series of Pugh et al was 28 months, indicating a less aggressive course than classic Richter syndrome. Interestingly, a t(11;14) was detected in 2 of 3 cases in which cytogenetic analysis was performed. A report of 4 additional cases of this tumor documented bel-1 rearrangement in 2 and established an immunophenotype similar to mantle cell lymphoma (CD5+, CD23–). Based on these findings, it seems likely that these tumors are more closely related to mantle cell lymphoma than to CLL/SLL and are not relevant to our study.

Only about two thirds of DLBCL transformations of CLL/SLL have been reported to be related clonally to the preexisting CLL/SLL, and the remainder apparently represent de novo DLBCLs. With the exception of case 1, which had identically sized rearranged bands on JH PCR, we have not strictly proven clonal identity of these DLBCLs with the preexisting CLL/SLLs. However, the immunophenotypic features in the other 5 CD5+ cases provide evidence for clonal identity. De novo CD5+ large B-cell lymphomas constitute only 5% to 8% of DLBCLs. Furthermore, these usually express FMC7 and uncommonly express CD23. Therefore, on an immunophenotypic basis, we believe it is likely that the CD5+ DLBCLs in the present study are related clonally to the preexisting CLL/SLLs. The 1 CD5– case expressed CD23 in the absence of FMC7. In our experience, this is an extremely unusual finding among de novo CD5– DLBCLs. Therefore, we are presumptively considering this case to be related clonally as well. These findings support the concept that most DLBCLs in CLL/SLL are derived from the same clone as the associated small B-cell neoplasm. In addition, our data suggest that immunophenotypic analysis provides an alternative method of establishing clonal identity in such cases when molecular analysis is unavailable or unsatisfactory, although this hypothesis requires confirmation by molecular genetic methods.

In 2 of the patients in this series (cases 1 and 7), DLBCL developed after allogeneic bone marrow transplantation, including the one with a CD5– tumor, raising the
differential diagnosis of posttransplantation lymphoproliferative disorder (PTLPD). Indeed, the tumor in case 1 occurred 100 days after transplantation and was found to contain EBV by PCR and in situ hybridization. In addition, the tumor regressed after donor lymphocyte infusion, although it rapidly recurred, resulting in the patient’s death. Interestingly, this DLBCL had identical immunoglobulin heavy-chain gene rearrangements, in addition to a very similar immunophenotype. Thus, it would seem that this neoplasm represented an EBV-driven PTLPD derived from the preexisting CLL/SLL clone. This PTLPD would be classified as monomorphic after the morphologic criteria of Knowles et al.39 PTLPDs after allogeneic marrow transplantation have been reported to occur at short intervals (generally less than 1 year after transplantation), are usually positive for EBV, and generally pursue a fulminant course.50-52 The DLBCL in case 7 occurred 4 years after transplantation and was negative for EBV. A minority of PTLPDs are negative for EBV, and these have been reported to occur later than EBV-positive cases.53 Therefore, whether this case represented a PTLPD or transplantation-unrelated transformation is uncertain.

Previous data on the immunophenotypic features of DLBCL transformation of CLL/SLL are limited and include mostly studies using frozen section immunoperoxidase or immunofluorescence methods. Interpretation is complicated further by cases not truly representing CLL/SLL or in which there is no clonal relationship between the tumors. If one restricts analysis to documented CLL/SLL cases with proven clonal identity to the subsequent DLBCL, there seem to be only 11 cases in the literature.5,6,10,11,13,18,20,22,23,25 Of these, 8 seemed to have lost expression of CD5 in the process of transformation. In contrast, 6 of our 7 cases retained the CD5 surface. It seems improbable, therefore, that we failed to identify CD5– cases. Additional immunophenotypic changes in the 11 cases from the literature are given in Table 6.

We have demonstrated that DLBCL transformation of CLL/SLL usually exhibits an immunophenotype similar to CLL/SLL, in contrast to cases of de novo CD5+ DLBCL. In addition, only minor immunophenotypic changes occur in the process of transformation.

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Table 6
Immunophenotypic Alteration in Reported Cases of Richter Syndrome With Documented Clonal Identity to Preceding CLL

<table>
<thead>
<tr>
<th>Antigen/No. of Cases Assessed</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD5/11</td>
<td>Lost in 8 cases 5,6,10,11,13,18,20,22,23,25</td>
</tr>
<tr>
<td>CD19/7</td>
<td>Lost in 1,10; fl in 1 20</td>
</tr>
<tr>
<td>CD20/9</td>
<td>&gt; in 1 22; fl in 1 22</td>
</tr>
<tr>
<td>CD22/3</td>
<td>Lost in 1 10</td>
</tr>
<tr>
<td>CD23/1</td>
<td>Gained 10</td>
</tr>
<tr>
<td>CD38/1</td>
<td>Gained 10</td>
</tr>
<tr>
<td>Surface Immunoglobulin/9</td>
<td>Lost in 1 10; gained in 1 20; &gt; in 1 22; light-chain switch (lambda to kappa) in 1 11</td>
</tr>
</tbody>
</table>

CLL, chronic lymphocytic leukemia; >, increased; fl, decreased.

References


