Lymphosarcoma Cell Leukemia: The Contribution of Cell Surface Study to Diagnosis

By Alan C. Aisenberg and Barbara Wilkes

Cell surface immunoglobulin, complement receptor, and spontaneous rosette formation with sheep erythrocytes were investigated in 43 patients with malignant lymphoma, including 13 with lymphosarcoma cell leukemia, and in 59 patients with chronic lymphocytic leukemia. The quantity of immunoglobulin on the lymphocyte surface was estimated from the intensity of fluorescent staining with fluorescein-conjugated anti-immunoglobulin antisera. At least two, and probably three, B cell species could be recognized by cell surface study. Cells from chronic lymphocytic leukemia and diffuse well-differentiated lymphocytic lymphoma had sparse amounts of surface immunoglobulin, while the cells of diffuse poorly differentiated lymphocytic lymphoma had large quantities of this material. Nodular lymphoma probably represented a third B-cell subtype with intermediate amounts of surface immunoglobulin. The lymphocytes of chronic lymphosarcoma cell leukemia exhibited the intense surface staining, which was characteristic of the underlying poorly differentiated lymphocytic lymphoma (diffuse or nodular), and could be readily distinguished from the faint-staining chronic lymphocytic leukemia cells.

CRITERIA for the diagnosis of chronic lymphosarcoma cell leukemia based on the Wright’s stained blood smear and other clinical features are not uniform.1,2 While lymph node morphology in lymphosarcoma cell leukemia differs from that of chronic lymphocytic leukemia, node biopsies may not be available, and the confusion that now exists in classifying non-Hodgkin’s lymphoma3–7 diminishes the pathologist’s contribution to this differential diagnosis. In the present investigation of lymphocyte surface properties, the amount of surface immunoglobulin has been found to correlate with lymph node morphology and to provide a useful characteristic for distinguishing the two disorders.

MATERIALS AND METHODS; CASES

The methods employed to prepare lymphocytes and to study their surface characteristics have been described in detail in earlier publications.8–11 A conventional Ficoll-Hypaque gradient was used to isolate lymphocytes from defibrinated blood; in the present work, investigation of chronic lymphosarcoma cell leukemia and chronic lymphocytic leukemia was restricted to untreated patients with peripheral lymphocyte counts in excess of 10,000 per cu mm. Patients with the diagnosis of chronic lymphosarcoma cell leukemia were included only if a current or past lymph node biopsy confirmed the presence of a nodular or diffuse poorly differentiated lymphocytic lymphoma. Specimens of lymphoid tissues were obtained directly from the operating room, and cell suspensions, prepared in Medium 199 by squeezing fresh tissue through a stainless steel mesh,
were used without storing. Viability was assessed with trypan blue and monocyte contamination with latex particles. Biopsies were diagnosed in the Pathology Department on the basis of formalin-fixed sections stained with hematoxylin and eosin employing the Rappaport classification of non-Hodgkin's lymphoma. Pathologic diagnosis was made without knowledge of the cell surface findings.

Cell-surface immunoglobulin was identified with fluorescein-conjugated goat antiserums specific for the IgG heavy chain, the IgM heavy chain, the IgA heavy chain, the kappa light chain, and the lambda light chain, and with a polyvalent antiserum to both heavy and light chains. (All antiserums to human immunoglobulin were of standard fluorescence-to-protein ratio and were obtained from Meloy Laboratories, Springfield, Va.). After incubation with the fluorescein-conjugated antiserums, the washed lymphocytes were placed on a slide and examined with a Zeiss ultraviolet microscope equipped with an Osram HBD 200 mercury arc lamp and an interference primary filter. A minimum of 200 lymphocytes was examined, after which a 10 nm transmitting filter was interposed in the optical system, and 200 additional cells were counted. The percentages of cells observed to stain with the attenuated and nonattenuated light sources were used to calculate the decimal fraction of immunoglobulin-bearing cells which stained brilliantly with each antiserum. For each cell preparation, the fractions from the two antiserums giving the highest percentage of stained cells were averaged. All fluorescent antibody studies were performed by one of us (BW) without knowledge of the clinical or pathologic diagnosis.

Cells forming spontaneous rosettes were assessed by adding freshly prepared lymphoid cells to sheep erythrocytes in the presence of 9% AB serum. After incubation at 37°C, centrifugation and reincubation at 4°C, the mixture of cells was resuspended and the rosettes enumerated in a hemocytometer chamber. Complement receptors were detected with sheep erythrocytes sensitized by the addition of hemolysin amboceptor to sheep erythrocytes and mouse complement employing the method of Pincus, Bianco, and Nussenzweig.

RESULTS

Cell surface data derived from neoplastic blood and tissue lymphocytes from 43 patients with malignant lymphoma and lymphocytic leukemia (including 13 with lymphosarcoma cell leukemia) are tabulated in Tables 1 and 2, and the percentage of cells with surface immunoglobulin visible by fluorescence microscopy is plotted in Fig. 1. Figures 1–3 also contain consecutive cell surface studies from patients with chronic lymphocytic leukemia, and the range of normal values for peripheral blood lymphocytes is indicated.

Immunoglobulin was identified on the surface of neoplastic lymphocytes from 55 of 59 patients with chronic lymphocytic leukemia, 9 of 9 with diffuse well-differentiated lymphocytic lymphoma, 15 of 16 with diffuse poorly differentiated lymphocytic lymphoma (including 6 of 7 with lymphosarcoma cell leukemia), and 12 of 13 with nodular lymphoma (including all 6 with lymphosarcoma cell leukemia). A predominant heavy and light chain could be recognized in most lymphoma specimens. The heavy chain was IgM in type, except that one instance of IgG heavy chain was observed in each of the lymphoma subtypes. Approximately twice as many individuals had cells with kappa light chain predominating as with lambda light chain, and no correlation was found between light chain specie and lymphoma subtype. (The heavy and light chains that characterize the chronic lymphocytic leukemia cell surface have been described in an earlier publication.) Surface immunoglobulin could be detected on cells from only two of five specimens of histiocytic and mixed lymphocytic and histiocytic (L + H) lymphoma, and in the two positive tumors only a small proportion of cells (14% and 20%, respectively) stained.

In Fig. 2, the decimal fraction of immunoglobulin-bearing cells that stained intensely with the fluorescein conjugates (remained visible after the light source
Table 1. Surface Markers in Lymphocytic Lymphoma

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Diagnostic Tissue</th>
<th>Tissue for Surface Study</th>
<th>Leukemic Surface Immunoglobulin</th>
<th>Complement Receptors</th>
<th>E Rosettes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive (%)</td>
<td>Fraction</td>
<td>Ig Type</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Brilliant</td>
<td></td>
</tr>
</tbody>
</table>

**Nodular poorly differentiated**

1. Node Node
   - 83
   - Blood
     + 57
   + 0.4
   + Mx
   + 16
   + 25
2. Node Node
   - 4
   - No Ig
3. Node Node
   + 15
   + 0.1
   + Mx
   + 4
   + 10
4. Node Node
   - 40
   - 0.3
   + Gx
   + 70
5. Node Node
   - 40
   - 1.0
   + Ig?type
6. Node Node
   - 72
   - 0.5
   + Mx
7. Node Node
   - 84
   - 1.0
   + Mx
   + 61
   + 9
8. Node Node
   + 73
   + 0.5
   + Mx
   + 29
   + 52
   + Blood
   + 39
   + 0.5
   + Mx
9. Node Node
   - 92
   - 0.4
   + Mx
   + 84
   + 3
10. Spleen Spleen
    + 87
    + 0.9
    + Mx
    + 54
    + 9
    + Blood
    + 65
    + 1.0
    + Mx
    + 40
    + 14
11. Node Blood
    + 71
    + 0.3
    + Mx
    + 82
    + 5
12. Node Blood
    + 64
    + 1.0
    + Mx
    + 34
    + 34
13. Node Node
    - 99
    - 0.7
    + Mx
    + 72
    + 5

**Diffuse poorly differentiated**

1. Tonsil Blood
   + 96
   + 0.9
   + Mx
   + 2
2. Node Blood
   + 91
   + 0.8
   + Mx
   + 2
3. Spleen Spleen
   + 99
   + 1.0
   + Mx
   + Blood
   + 94
   + 0.9
   + Mx
   + 11
4. Node Node
   - 55
   - 0.7
   + Mx
   + 5
   + Node Node
   - 96
   - 0.9
   + Mx
   + 20
   + Node Node
   - 53
   - 0.9
   + Mx
   + 2
   + Node Node
   - 89
   - 0.9
   + Mx
   + 5
   + Node Node
   - 68
   - 0.6
   + Mx
   + 8
   + Node Node
   - 96
   - 0.9
   + Mx
   + 5
   + Thyroid Thyroid
   - 81
   - 0.9
   + Mx
   + 37
11. Node Blood
    + <10
    - No Ig
    + 0
    + 2
12. Node Node
    - 59
    - 1.0
    + Gx
    + 23
    + 22
13. Node Blood
    + 100
    + 1.0
    + Mx
    + 83
    + 0
14. Spleen Spleen
    + 75
    + 0.9
    + Mx
    + Blood
    + 95
    + 0.8
    + Mx
    + 0
    + 8
15. Node Blood
    + 89
    + 0.0
    + Mx
    + 58
    + 12

**Diffuse well differentiated**

1. Node Node
   + 90
   + 0.3
   + Mx
   + Blood
   + 58
   + 0.3
   + Mx
2. Node Node
   - 22
   - 0.0
   + Ig?type
   + Blood
   + 87
   + 0.0
   + Mx
   + 6
   + Spleen
   + 60
   + 0.0
   + Mx
3. Node Blood
   + 96
   + Igx
   + 1
4. Node Blood
   + 83
   + Igx
   + 12
5. Node Blood
   + 83
   + Igx
   + 12
6. Orbit Orbit
   - 75
   - 0.1
   + Mx
   + 16
   + 47
7. Node Blood
   + 90
   + 0.0
   + Mx
   + 68
   + 11
8. Node Node
   - 56
   - 0.0
   + Igx
   + 58
   + 15
9. Node Node
   + 84
   + 0.0
   + Gx
   + 24
   + 10
   + Blood
   + 74
   + 0.0
   + Gx
   + 85
   + 13
was reduced by 90% is plotted. The fraction of intensely staining cells exceeded 0.2 in only 3 of 41 patients with chronic lymphocytic leukemia, and 1 of 7 with diffuse well differentiated lymphocytic lymphoma. However, of 14 individuals with diffuse poorly differentiated lymphocytic lymphoma and immunoglobulin-bearing cells, in 13 the intensely staining fraction was 0.6 or greater; in 9 it was 0.9 or 1.0. The average fraction of intensely staining cells in nodular lymphoma was greater than in chronic lymphocytic lymphoma and well-differentiated tumors. Thus, in four nodular lymphoma patients, all cells stained intensely (fraction of 1.0), in seven the fraction ranged from 0.3 to 0.7, in one it was 0.1, and one specimen did not stain. The intensity of cell surface staining in patients with lymphosarcoma cell leukemia was the same as the underlying lymphoma, i.e., the quantity of surface immunoglobulin was not altered by the presence of leukemia. The striking difference in appearance of faintly fluorescent-staining chronic lymphocytic leukemia cells and brilliantly staining chronic lymphosarcoma cell leukemia cells (in a patient with the lymph node morphology of diffuse poorly differentiated lymphocytic lymphoma) is illustrated in Fig. 4.

The percentage of neoplastic cells with complement receptor is depicted in Fig. 3. This B lymphocyte marker was present on a significant fraction of cells

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**Table 2. Surface Markers in Histiocytic and Mixed Lymphomas**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Diagnostic Tissue</th>
<th>Tissue for Surface Study</th>
<th>Leukemic</th>
<th>Surface Immunoglobulin</th>
<th>Complement Receptors</th>
<th>E Rosettes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nodular (mixed lymphocytic and histiocytic)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Node</td>
<td>Node</td>
<td>-</td>
<td>14</td>
<td>? No Ig</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse (histiocytic)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Spleen</td>
<td>Spleen</td>
<td>-</td>
<td>&lt;10</td>
<td>No Ig</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Spleen</td>
<td>Spleen</td>
<td>-</td>
<td>&lt;10</td>
<td>No Ig</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Node</td>
<td>Node</td>
<td>-</td>
<td>&lt;10</td>
<td>No Ig</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse (mixed lymphocytic and histiocytic)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Node</td>
<td>Node</td>
<td>-</td>
<td>20</td>
<td>? Ig</td>
<td>45</td>
<td>34</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Cell surface immunoglobulin in various lymphocytic neoplasms. Open symbols (o, O) represent determinations made on circulating leukemic cells, and solid symbols (○) represent determinations made on tumor lymphocytes. A half-open symbol (a) indicates that both leukemic and tumor lymphocytes were analyzed separately and the mean value plotted. The open square symbols permit the four immunoglobulin-negative chronic lymphocytic leukemia patients to be identified in Figure 3.
from most patients with all varieties of lymphocytic lymphoma and lymphocytic leukemia. It is of particular interest that complement receptor was present on the cells from the four chronic lymphocytic leukemia patients whose cells lacked detectable surface immunoglobulin (identified by the open squares).

**DISCUSSION**

A distinction between chronic lymphosarcoma cell leukemia and chronic lymphocytic leukemia based on the quantity of cell surface immunoglobulin appears to be reliable, and the fluorescent antibody technique employed in the present study is technically undemanding. Brilliant fluorescent staining of lymphosarcoma cells with anti-immunoglobulin antisera and faint staining of chronic lymphocytic leukemia cells have been noted in earlier work, and the difference in surface immunoglobulin has been confirmed by a more elaborate quantitative method. The latter finding makes it less likely that the degree of aggregation or specificity of the anti-immunoglobulin antiserum, or the Fc receptor state of the cell surface antigen, is responsible for the observed differences in fluorescent staining. While there is still need for a simple technique
that accurately measures surface immunoglobulin, the difference between the two cell types is gross and can be assessed with the crudely quantitative fluorescent antibody procedure employed here. Quantitation of other B-lymphocyte surface markers, complement receptor for example, may reveal other differential features between the neoplastic cells of the two disorders.

In chronic lymphocytic leukemia, the lymph node morphology by the Rappaport classification is a diffuse well-differentiated lymphocytic lymphoma, while chronic lymphosarcoma cell leukemia is a poorly differentiated lymphocytic lymphoma, either diffuse or nodular. Lymph node biopsy is infrequently required for the diagnosis of these leukemias in clinical practice, but the above formulation has been substantiated where adequate pathologic material is available\textsuperscript{6,15,18} and is supported by our cell surface findings. The present work was restricted to lymphosarcoma cell leukemia patients in whom the diagnosis was confirmed by a current or past lymph node biopsy, though data from lymphocytic lymphoma patients without leukemia have been included. The inclusion of nonleukemic patients in the present study is appropriate, since cell
surface properties of the various lymphoma subtypes are not altered by the presence or absence of leukemia (Table I, Figs. 1–3).

It should be emphasized that the Rappaport class of diffuse poorly differentiated lymphocytic lymphoma is heterogeneous, a central point in the recent pathologic classifications of Lennert et al.,16 and Lukes and Collins.4 Thus, patients with chronic lymphosarcoma cell leukemia and the lymph node morphology of diffuse poorly differentiated lymphocytic lymphoma by the Rappaport classification are usually placed by the Lennert system under tumors of low grade malignancy of follicle center cell origin (composed either of small centrocytes with cleaved nuclei, or centrocytes mixed with larger centroblasts with uncleaved nuclei). Other pathologists have attempted to separate this group of diffuse lymphomas of lesser malignancy under the term intermediate cell lymphoma.17 However, the majority of nonleukemic patients with the Rappaport classification of diffuse poorly differentiated lymphocytic lymphoma have a highly malignant process which Lennert et al.16 and Lukes and Collins4 classify differently from the lymphosarcoma cell leukemia patients.

Similar considerations do not apply to chronic lymphosarcoma leukemia with nodular lymph node morphology, since both the Rappaport3 and the newer pathologic systems separate nodular lymphoma into a distinct and more homogeneous group. (Lennert et al.16 and Lukes and Collins4 ascribe the origin of nodular lymphomas to follicle center cells [mixed tumors of centrocytes and centroblasts]). Nonetheless, it is apparent that our nodular lymphoma series is not representative of this disorder because of the excess of patients with leukemia, an occurrence that can be attributed to the availability of peripheral blood for cell surface study and the comparative difficulty in finding viable lymph node material for investigation.

It appears that two, and probably three, subtypes of B lymphocytes can be recognized by cell surface study. One cell type with sparse surface immunoglobulin is of B lymphocyte origin and proliferates as chronic lymphocytic leukemia and diffuse well-differentiated lymphocytic lymphoma. A second cell, recognized by the abundance of surface immunoglobulin, proliferates as chronic lymphosarcoma cell leukemia and poorly differentiated lymphocytic lymphoma, either diffuse or nodular. According to newer pathologic classifications, this second B lymphocyte is of follicle center cell origin, while the first is not.4,16 Certainly the large group of neoplasms arising form the second cell type, which constitute the major fraction of non-Hodgkin’s lymphoma, is not uniform. Cell surface study suggests that nodular lymphomas may differ from diffuse poorly differentiated lymphocytic lymphoma by being composed of a lower percentage of bright cells (lymphocytes with large amounts of surface immunoglobulin) or of cells with a lesser degree of brightness (though still considerably brighter than the lymphocytes of chronic lymphocytic leukemia). Thus, it is probable that two subclasses of follicle center cells could be recognized by surface immunoglobulin study if accurate quantitative methods were available. Another point of difference between nodular and diffuse poorly differentiated lymphocytic lymphomas is the association of circulating IgM M components with the latter but not with the former.18

There is need for comment about the three chronic lymphocytic leukemia
patients with bright cells (Fig. 2) and the lymphosarcoma leukemia patient whose cells lacked surface immunoglobulin. On the basis of cell surface study, one would predict that these chronic lymphocytic leukemia patients have, in fact, chronic lymphosarcoma cell leukemia, and that lymph node biopsy would reveal a poorly differentiated lymphocytic lymphoma (diffuse or nodular) rather than a well differentiated lymphocytic tumor. Chronic lymphocytic leukemia patients are infrequently subjected to lymph node biopsy, and lymph node morphology is not available in these individuals. The single lymphosarcoma leukemia patient whose cells lacked surface immunoglobulin (or other surface markers) presented an unusual clinical picture with a lymphocyte count in excess of one-half million per cu mm and a fulminant course. The presentation had some features of prolymphocytic leukemia. However, regardless of the exact nature of the proliferation in the last patient, his disorder appeared distinct from the usual case of chronic lymphosarcoma cell leukemia. This occurrence does not detract from the view that cell surface study is of use in classifying lymphoid neoplasms.

At the present time, the distinction between chronic lymphocytic leukemia and chronic lymphosarcoma cell leukemia is not of critical importance to the patient, since treatment and prognosis of the two disorders are similar. However, when more effective therapeutic regimens are available, it may become essential to identify accurately these conditions. Should that occur, quantitation of the immunoglobulin on the lymphocyte surface with the fluorescent antibody method will prove a useful diagnostic tool.

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REFERENCES

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