Relevance of Surface Markers in Chronic Lymphocytic Leukemia to Acute Lymphocytic Leukemia

Alan C. Aisenberg and Barbara M. Wilkes

Abstract

The surface membrane of the B-lymphocyte of chronic lymphocytic leukemia (CLL) has been subject to detailed investigation over the past decade. Surface immunoglobulin of low density, punctate in distribution, without the tendency to polar cap formation, and clonal with respect to light and heavy chains, is characteristic. Other B-cell properties include the presence of the la antigen and the receptor for the C3d portion of complement. The CLL surface membrane lacks such T-cell attributes as the ability to form rosettes with sheep erythrocytes and reactivity with anti-T-cell antisera, although T-cells may be increased early in the disorder. CLL is believed to be a proliferation of a B-lymphocyte of the medullary cord of the lymph node, although the exact place of this cell in lymphocyte development remains to be clarified. Surface markers are useful in distinguishing classical B-cell CLL from other proliferations of small lymphocytes (lymphosarcoma cell leukemia of follicle-center B-cells, T-cell CLL, Sézary syndrome, and reactive lymphocytosis).

Introduction

CLL has little in common (Table 1) with ALL. The former is an indolent disorder of the elderly in which overzealous treatment is apt to bring disaster, while the latter is a fulminant condition of the young, fatal within a few months to a year if untreated, but curable by aggressive chemotherapy in about one-half of the cases.

Nonetheless, the disease of the elderly does have a considerable amount to teach those interested in the disorder of the young. CLL and ALL raise the same fundamental problem; the inadequacy of traditional definitions of hematological neoplasms based upon the morphology of the Wright's-stained blood smear. In CLL, the problem revolves about the small "mature" lymphocyte; while in ALL, the cell concerned is the "immature" lymphocyte of intermediate size. For a variety of reasons, the cell in CLL has been the easier of the 2 to study, and its surface phenotype was defined first (1, 18, 28). Surface marker analysis of CLL proceeded more rapidly because cells are readily available. Since the disorder is relatively common and indolent, and since conventional medical wisdom leaves these patients untreated, CLL cells represent the most convenient source of neoplastic human lymphocytes. A further convenience of the CLL cell is the presence of Slg, among the first lymphocyte markers identified, and still among the most reliable markers for the B-lymphocyte. For these reasons, more is known about the cell surface in CLL than is known in ALL, and this knowledge can clarify thinking about the childhood disorder.

It may be remarked in passing that identification and purification of neoplastic lymphocytes resemble the similar identification and purification of chemical compounds that played an integral role in the development of chemistry. If this parallel is accepted, then the CLL cell is at present among the purest and best-defined reagents in cellular immunology.

B-Cell Markers in CLL (4, 6, 11, 12, 25)

Table 2 presents surface marker findings in 77 patients with the clinical diagnosis of CLL (6). The series is consecutive with the exclusion of patients under the age of 40, individuals treated within the preceding 2 months, and those with lymphocyte counts below 10,000/cu mm (contaminating nonneoplastic cells complicate the interpretation of surface marker results). These and other properties of the CLL cell are summarized in Table 3.

The findings are consistent. Of the 77 patients studied, the cells of 73 (94%) exhibited Slg, whose clonal nature was demonstrable in each individual by the preponderance of a single light chain. Patients with κ-type light chain exceeded those with λ-type in a ratio of 2:1, a ratio similar to the proportion of the 2 light chains in human circulating immunoglobulin. Individuals with cells bearing IgM heavy chain were 6 times more frequent than those with IgG heavy chain, and approximately one-half of the surface IgM tumors were accompanied by surface IgD.

In contrast to normal B-lymphocytes and a variety of other B-cell neoplasms, the Slg of the CLL cell is sparse in amount (Fig. 1). This can be demonstrated semiquantitatively with a fluorescent microscope equipped with appropriate neutral filters (6), or more quantitatively with the fluorescence-activated cell sorter (7, 33), by immunoperoxidase staining (34), or by radioimmunoassay (13). With the fluorescence microscope, the Slg in this condition is seen as a punctate, finely stippled fluorescence, sometimes barely visible, and never accompanied by the capping observed with other B-lymphocytes (normal or neoplastic). A rare CLL variant exhibits crystals of immunoglobulin in the cytoplasm in addition to the Slg. The significance of these crystals is unknown, but the phenomenon is restricted to individuals whose cells bear surface IgM of λ light chain type (14). The intracytoplasmic immunoglobulin crystals of CLL should be distinguished from the cytoplasmic immunoglobulin of immunoglobulin-secreting plasma cells.

Complement receptor (erythrocyte-amebocyte-complement rosettes) is another marker present on CLL cells (Tables 2 and 3). The reagent used (27) is complement, which is bound to...
sheep erythrocytes via amboceptor. Mouse complement must be used to detect the receptor for C3d which is present on CLL cells; the receptor for C3b is absent. It should be noted that 4 Slg-negative specimens in Table 2 were complement receptor positive. While complement receptor does not enjoy the exclusive B-cell specificity of Slg, the presence of this marker on a small lymphocyte with neither T- nor macrophage surface markers is circumstantial evidence for B-cell lineage.

CLL cells have several other properties which are characteristic of B-lymphocytes. Thus, they adhere to nylon fibers, express the la-like antigen, and form rosettes with mouse erythrocytes.

**T-Cell Markers in CLL**

With the exception of the rare cases of T-cell CLL, to be discussed in a later section, CLL cells are uniformly devoid of most T-lymphocyte markers (Tables 2 and 3). Thus, they do not form rosettes with unsensitized sheep erythrocytes (E-rosettes), and are unreactive with anti-thymocyte heteroantisera.

**Macrophage and Other Markers in CLL**

CLL cells are not phagocytic and lack the receptor for the Fc portion of IgG (determined with sheep erythrocytes as IgG-erythrocyte-amoceptor rosettes). The latter surface marker, once considered specific for macrophages, is present on many

### Table 1

**CLL versus ALL**

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Lineage</th>
<th>Age distribution</th>
<th>Course</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small “mature” lymphocyte</td>
<td>B-cell (medullary cord lymphocyte)</td>
<td>Elderly</td>
<td>None cured, symptoms relieved</td>
<td></td>
</tr>
<tr>
<td>Intermediate size “immature” lymphocyte</td>
<td>Most “null” (stem), 20–25% T-cell, rare B- or pre-B-cell</td>
<td>Indolent</td>
<td>Acute</td>
<td></td>
</tr>
</tbody>
</table>

* Unless otherwise specified, the term CLL excludes the rare T-cell variant.

### Table 2

**Cell surface markers in CLL and malignant lymphoma (modified from Ref. 6)**

<table>
<thead>
<tr>
<th></th>
<th>CLL</th>
<th>Nodular poorly differentiated lymphocytic lymphoma</th>
<th>Diffuse histiocytic lymphoma</th>
<th>Diffuse poorly differentiated lymphocytic lymphoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Slg positive</td>
<td>73/77</td>
<td>94</td>
<td>28/28</td>
<td>100</td>
</tr>
<tr>
<td>Slg negative, EAC&lt;sup&gt;a&lt;/sup&gt; positive</td>
<td>4/77</td>
<td>6</td>
<td>0/28</td>
<td>0</td>
</tr>
<tr>
<td>Total B-cell positive</td>
<td>77/77</td>
<td>100</td>
<td>28/28</td>
<td>100</td>
</tr>
<tr>
<td>Light chain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>κ</td>
<td>49/73</td>
<td>67</td>
<td>17/28</td>
<td>61</td>
</tr>
<tr>
<td>λ</td>
<td>21/73</td>
<td>29</td>
<td>9/28</td>
<td>32</td>
</tr>
<tr>
<td>Unresolved</td>
<td>2/73</td>
<td>4</td>
<td>2/28</td>
<td>8</td>
</tr>
<tr>
<td>Heavy chain, IgM vs. IgG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM</td>
<td>61/73</td>
<td>83</td>
<td>17/28</td>
<td>61</td>
</tr>
<tr>
<td>IgG</td>
<td>10/73</td>
<td>14</td>
<td>5/28</td>
<td>29</td>
</tr>
<tr>
<td>Unresolved</td>
<td>2/73</td>
<td>3</td>
<td>3/28</td>
<td>11</td>
</tr>
<tr>
<td>Heavy chain, IgM vs. IgM + IgD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM only</td>
<td>32/49</td>
<td>65</td>
<td>12/14</td>
<td>86</td>
</tr>
<tr>
<td>IgM + IgD</td>
<td>17/49</td>
<td>35</td>
<td>2/14</td>
<td>14</td>
</tr>
<tr>
<td>EAC positive&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20%</td>
<td>2/34</td>
<td>4</td>
<td>11/20</td>
<td>55</td>
</tr>
<tr>
<td>20–40%</td>
<td>2/34</td>
<td>4</td>
<td>4/20</td>
<td>20</td>
</tr>
<tr>
<td>&gt;40%</td>
<td>30/34</td>
<td>88</td>
<td>5/20</td>
<td>25</td>
</tr>
<tr>
<td>E-rosette positive&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20%</td>
<td>55/56</td>
<td>98</td>
<td>17/25</td>
<td>68</td>
</tr>
<tr>
<td>20–40%</td>
<td>1/56</td>
<td>2</td>
<td>6/25</td>
<td>32</td>
</tr>
<tr>
<td>&gt;40%</td>
<td>0/56</td>
<td>0</td>
<td>0/25</td>
<td>0</td>
</tr>
<tr>
<td>Anti-thymocyte globulin positive&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20%</td>
<td>53/56</td>
<td>94</td>
<td>10/26</td>
<td>38</td>
</tr>
<tr>
<td>20–40%</td>
<td>1/56</td>
<td>2</td>
<td>5/26</td>
<td>19</td>
</tr>
<tr>
<td>&gt;40%</td>
<td>2/56</td>
<td>4</td>
<td>11/26</td>
<td>42</td>
</tr>
</tbody>
</table>

<sup>a</sup> The denominator is always the number tested.<br><sup>b</sup> EAC, complement receptor.<br><sup>c</sup> These figures apply only to Slg-positive specimens. No cases of T-cell CLL were encountered.
Fig. 1. Slg stained with fluorescein-conjugated anti-IgM heavy chain antiserum. Left to right, CLL, nodular poorly differentiated lymphocytic lymphoma, and diffuse poorly differentiated lymphocytic lymphoma (modified from Ref. 6). Initial magnification, × 380.

Attempts have been made to induce CLL cells to differentiate in vitro into antibody-secreting plasma cells. This has been accomplished by the addition of normal helper T-cells, but only in the uncommon instances of CLL where the disorder is accompanied by a monoclonal immunoglobulin in the serum (17). The considerable literature (11, 30) which has accumulated about the delayed or abnormal response of the CLL cell to in vitro stimulation with phytohemagglutinin will not be discussed here. One may conclude that much remains to be clarified about the mechanism of hypogammaglobulinemia in CLL, and the place of the CLL cell in B-cell development.

Disorders of Small Lymphocytes Distinguishable from CLL by Surface Markers

Three neoplasms of small T-lymphocytes can be distinguished from B-cell CLL. All are E-rosette positive, Slg negative, and terminal transferase negative, and the cells from all react with anti-thymus heteroantisera. Both T-cell CLL (11) and the Sézary syndrome appear to be neoplasms of the helper subset of T-cells (9, 29). T-cell CLL is a rare disease, reported more frequently from Europe and Japan than from the United States; the incidence is only about 1% that of B-cell CLL, and the condition is characterized by skin involvement, unresponsiveness to treatment, and short survival (35). The Sézary syndrome, a disorder with T-cell lymphocytosis (10) and erythroderma, is related to mycosis fungoides. Only 2 cases of the syndrome of T-cell lymphocytosis associated with thymoma have been reported (19).

The group of diseases that go under the rubric chronic lymphosarcoma cell leukemia must also be distinguished from CLL. The former are B-cell proliferations of the follicle center cells of the lymph node, and the lymph node morphology of patients with lymphosarcoma cell leukemia is either a nodular or diffuse poorly differentiated lymphocytic lymphoma. The

T-cells, and on some B-cells as well (36).] Terminal transferase (15), a marker of lymphoid stem cells, is absent, and specific and nonspecific esterases, acid and alkaline phosphatases, and peroxidase cannot be detected (25).

Function of the CLL Cell and Its Place in B-Cell Development

The pathologist can not distinguish the lymph node morphology of 3 clinical variants of CLL: (a) conventional CLL with circulating small lymphocytes; (b) CLL with leukemic cells and monoclonal spikes of IgG or IgM in the serum; and (c) a malignant lymphoma confined to the lymph nodes with neither circulating cells nor abnormal immunoglobulin in the serum (26). All 3 are classified as diffuse well-differentiated lymphocytic lymphoma (a nonnodular lymphoma of small lymphocytes) under the microscope, and the 3 have the same cell surface phenotype (3). Pathologists believe that the CLL lineage is derived from a B-lymphocyte of the medullary cord of the lymph node, a conviction based on cell morphology (25). The medullary cord lymphocyte must be distinguished from the B-lymphocytes of the follicle center (21, 23). The latter cells are responsible for the majority of adult non-Hodgkin's lymphoma [almost all of nodular and diffuse poorly differentiated lymphocytic (tumors of intermediate size lymphocytes) lymphoma, and about one-half of histiocytic (tumors of large lymphocytes) lymphoma] (8, 16, 22, 24). Follicle center cell tumors contain dendritic reticulum cells with desmosomes visible with the electron microscope (20) and exhibit a surface phenotype which contrasts with that of CLL by the presence of large amounts of Slg (3).

When CLL cells are placed in culture, either with or without pokeweed mitogen, immunoglobulin is not secreted into the medium, as is the case with normal B-lymphocytes (13). This in vitro phenomenon is thought to be the basis of the clinical hypogammaglobulinemia which is a hallmark of advanced CLL.
blood smear usually reveals cells which are somewhat larger than the cells in CLL and possess cleaved nuclei, but the conditions cannot always be distinguished by cell morphology. In contrast to the CLL cell, the lymphosarcoma cell is characterized by brightly staining Slg, frequently associated with capping (5). Like B-cell CLL, the lymphosarcoma cells are E-rosette negative, but they differ in that complement receptor may be absent.

Cell surface markers can also distinguish reactive lymphocytosis, which is usually of T-cell lineage in the adult, from clonal B-cell CLL. Finally, cases are being recognized which resemble B-cell CLL (faint Slg, E-rosette negative), but with other surface markers which are atypical. We have observed several such young individuals with clinical features unusual for CLL, and for that reason we exclude patients under 40 from our consecutive CLL series. Such cases may represent a variant of B-cell CLL, or a distinct B-cell subset.

**Conclusion**

Surface markers (the presence of clonal Slg of low density, complement receptor, and the failure to form rosettes with sheep erythrocytes) allow a more accurate delineation of CLL than is possible from the blood smear alone. T-cell disorders (T-cell CLL, Sézary syndrome, thymoma with lymphocytosis, and reactive lymphocytosis) and B-cell proliferations of follicle center cells (chronic lymphosarcoma cell leukemia) can be readily distinguished from true B-cell CLL. Surface markers also permit the detection of a smaller burden of CLL cells than is possible with the usual clinical techniques. Unfortunately, since the treatment of CLL and its "optical isomers" does not conditions cannot always be distinguished by cell morphology.

**References**


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