Clinical and Cell Surface Marker Characterization of the Early Phase of Chronic Lymphocytic Leukemia

By Richard A. Rudders and Judith P. Howard

The immunologic surface markers on lymphocytes and clinical characteristics of 35 patients with established (stages 0–4) CLL with absolute lymphocyte counts >15,000/cu mm were compared to those of a group of 25 patients with CLL in an early or preleukemic phase (counts of <15,000/cu mm). We found a monoclonal B cell proliferation in most cases in the latter group, in spite of the paucity of clinical and laboratory findings. Furthermore, early CLL can readily be distinguished from benign lymphocytosis by surface marker criteria. In untreated CLL, surface marker characteristics are stable with time and predominantly reflect expansion of clones expressing only B cell markers; however, small increases of blood T cells are often seen. Surface markers are a simple and clinically useful tool for defining and characterizing the preleukemic phase of CLL and its ultimate progression to established CLL.

The surface marker characteristic of chronic lymphocytic leukemia (CLL) cells most often detected is membrane-bound immunoglobulin (SMIg) restricted to one heavy chain class and a single light chain type. The prevailing concept therefore is that CLL is a neoplasm derived from single clones of B cells. However, examples of T cell CLL and occasional lymphoproliferative disorders expressing mixed B and T cell marker characteristics have been reported. In addition, serial studies of individual CLL patients have shown some fluctuation in the surface receptor profile of the cell population with time, and other investigators have described alterations in marker results associated with cytotoxic treatment. In the minds of some the exact lineage of CLL clones is therefore still open to considerable question. Important clues to the cellular origin of CLL might be obtained from the study of early cases. Unfortunately, little such marker information exists, since most patients studied have had high blood counts.

We therefore report our observations using multiple surface membrane markers in contrasting a group of 35 patients with established CLL with high blood counts with a group of 25 patients with early or low-count CLL. The implications of our findings with respect to the cellular origin and evolution of CLL and the clinical distinction between benign and malignant lymphocytosis are discussed.

MATERIALS AND METHODS

Sixty consecutive patients with CLL were referred to the Tufts-New England Medical Center lymphocyte typing laboratory for study between 1974 and 1977. The major criterion for the clinical diagnosis of CLL was a sustained absolute blood and marrow lymphocytosis not at-
tributable to any other cause. Patients known to have lymphoma with a leukemic phase or lymphosarcoma cell leukemia were excluded. Patients were staged according to a modification of the clinical staging scheme of Rai et al. We took an absolute lymphocyte count > 15,000/cu mm as that level distinguishing established CLL at clinical stage 0 or greater. Of our 60 patients, 25 had absolute lymphocyte counts < 15,000/cu mm and were considered to have early CLL or be in a "preleukemic" phase. In half of these early cases, the initial impression of CLL was confirmed by associated clinical or laboratory findings. In the remainder, there were no initial confirmatory clinical or laboratory findings, but during the follow-up period there was an evolution to frank CLL in several.

At the time of initial study, all patients had a complete blood count and platelet count in addition to surface marker studies. Wright-stained blood smears were reviewed independently by two observers without prior knowledge of the surface marker studies in order to correlate the marker and morphologic findings. Smears were then classified as diagnostic or suspicious of CLL or as nondiagnostic. With certain previously reported exceptions the usual CLL cell morphology in our series was that of a small "mature" lymphocyte. The period of followup for the entire group of patients from the time of initial surface marker evaluation ranged from 2 mo to 3 yr, with 51 of 60 patients followed for more than 1 yr. In 23 patients serial surface marker determinations were performed on blood, marrow, or lymph node specimens both prior to and during cytotoxic treatment. All patients who were entered in the study were observed on a prospective basis.

In addition, we also followed a control group of five patients initially studied in order to evaluate asymptomatic lymphocytosis. In each instance the lymphocytosis was felt tentatively to be nonneoplastic in origin. Subsequent observation bore out this impression.

**Mononuclear cell isolates.** Peripheral blood or marrow drawn in preservative-free heparin was diluted four times with sterile phosphate-buffered saline (PBS) and layered on a Ficoll-Isopaque density gradient (2.4 volumes of 9% Ficoll to 1 volume 33% Isopaque). After being centrifuged at 1000 g for 15 min the cells at the interface were aspirated, washed three times in Hank's balanced salt solution (HBSS), and used in subsequent studies. The degree of monocyte contamination was assessed by morphologic appearance, glass adherence, peroxidase staining, and the ability to phagocytize neutral red dye particles after incubation with a 0.1% solution of neutral red for 10 min at 37°C.

**E rosettes.** Spontaneous sheep red blood cell rosettes were assayed according to the method of Baxley et al. In some instances sheep cells treated with vibrio cholera neuraminidase (Behring Laboratories, Sommerville, N.J.) were used as a control for assays performed with untreated red cells.

**19S EAC rosettes.** The C'3 receptor was determined according to the method of Jaffe et al. A 19SEA reagent was always used as a control, and in those cases where the control was greater than 5% the test was considered invalid.

**Fluorescent conjugated antisera.** Fluorescein or rhodamine isothiocyanate conjugated antisera specific for human immunoglobulins, μ, γ, δ, α, κ, and λ chains, were prepared in rabbits and rendered monospecific as described previously. All antisera were routinely ultracentrifuged prior to use in order to remove aggregated material.

**Detection of SMIg.** Cells (10⁶) suspended in HBSS were incubated for 30 min at 4°C with 50 μl of the appropriate dilution of fluorescent reagent. The cell pellet was washed three times in PBS and resuspended in glycerine-PBS on a glass slide; at least 200 cells were observed immediately in a Leitz Ortholux microscope with an Opaq-Fluor vertical illuminator. If SMIg results were inconclusive, cells were cultured overnight in minimal essential medium (MEM) and 10% fetal calf serum (FCS) at 37°C under 10% CO₂ to rid them of cytophilic antibody. Repeat determinations of SMIg were performed at 24-hr intervals. A separate aliquot of cells was also stripped of its SMIg by incubation for 30 min at 37°C with 2.5% crystalline trypsin in MEM and 10% FCS. SMIg was determined following stripping and 6 hr later.

**Other marker assays.** In those instances where the above receptors were not detected on 25% or more of the cell population, we considered the number of E⁻ SMIg⁻ C'3⁻ "null" cells to be significant, and we examined the cell population for the Fc receptor (7SEA) according to the method of Jaffe et al. and for the receptor for mouse red blood cells utilizing a rosetting assay.

Normal blood values in our laboratory for absolute blood lymphocytes, E⁺ cells, SMIg⁺ cells, and the percentage of C'3⁺ cells are listed in the footnotes to Table 3. It should be noted that
calculations of absolute blood E+ and SM1g+ cells derived from the study of Ficoll-Hypaque gradient mononuclear cell populations require a correction for contaminating monocytes. The mean values listed are corrected values. In contrast to normals there is negligible monocyte contamination of gradients obtained from CLL patients, particularly those with high leukocyte counts.

RESULTS

We divided our series of 60 patients into two groups based on the level of the absolute lymphocyte count. Thus patients with absolute lymphocyte counts >15,000/cu mm were designated established or high-count CLL and those <15,000/cu mm were termed early or low-count CLL.

High-Count CLL

This group contained 35 patients, 23 of whom had received no prior treatment. The remaining 12 had either received prior treatment but were untreated at the time of study or were in florid relapse on therapy such that the lymphocyte count was a true reflection of the stage of their disease. Absolute lymphocyte counts in this group were 15,400–190,000/cu mm (median 32,670). Of 35 patients, 34 had SM1g+ cells that were restricted to a single heavy chain class and light chain type and could therefore be characterized as monoclonal. The SM1g detected in these instances was predominantly IgM κ. However, in 7 instances the δ chain was found associated with the μ chain on the same cell. The percentage of Ig+ cells in this group was uniformly high, accounting for 85% or more of the entire cell population in 31 or 35 cases. This preponderance of monoclonal SM1g+ cells bore no relationship to the level of the initial leukocyte count or to the absolute lymphocyte count. For instance, all of the SM1g+ patients with the lower absolute lymphocyte counts (15,000–20,000/cu mm) had more than 90% SM1g+ cells present. In each instance the sum of E+ rosetting cells and SM1g+ cells was less than 100%, suggesting that double marked cell populations were not present. We saw a fluctuation in the expression of surface markers in serial studies in only one patient prior to therapy. This individual was SM1g+ but on several occasions expressed light Ig chains exclusively on the membrane that were variably associated with a single class of heavy chain. A single patient in this group also had a high proportion (80%) of cells of the phenotype E- SM1g- C3-; when tested for, other markers such as mouse RBC rosettes and the Fc receptor were not found. We tentatively designated this patient as an example of null cell CLL.

In each of these subjects the peripheral blood smear was considered to be absolutely diagnostic of CLL. Likewise, all patients had clinical histories and physical findings consistent with a diagnosis of CLL. Thus confirmatory clinical or laboratory evidence of CLL was uniformly present if one selects an absolute lymphocyte count of 15,000/cu mm as a baseline and was apparently independent of the height of the lymphocyte count.

Low-Count or Early CLL

Clinical characteristics. The clinical features of this group of 25 patients are listed in Table 1 with stratification according to the level of absolute lymphocyte count. There were 16 females and 9 males; the median age was 66 yr (range 25–82). In 11 patients the history and physical examination were negative and
Table 1. Initial Clinical Characteristics of Patients With Early CLL
According to Absolute Lymphocyte Count

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)/Sex</th>
<th>Symptoms</th>
<th>Organomegaly</th>
<th>Marrow</th>
<th>Associated Phenomena</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>73/F</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>56/F</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>66/F</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>60/M</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>5*</td>
<td>72/M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>62/F</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>Hepatitis B</td>
</tr>
<tr>
<td>7</td>
<td>77/F</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>66/F</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>82/M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Coombs'-positive AHA</td>
</tr>
<tr>
<td>10</td>
<td>77/M</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>50/F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>62/F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Bullous pemphigoid</td>
</tr>
<tr>
<td>13</td>
<td>73/F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Evan syndrome</td>
</tr>
<tr>
<td>14*</td>
<td>74/F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>15*</td>
<td>76/F</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>IgM monoclonal protein; Coombs'-positive AHA</td>
</tr>
<tr>
<td>16</td>
<td>74/M</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>17</td>
<td>49/F</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>18*</td>
<td>67/M</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>Hypogammaglobulinemia</td>
</tr>
<tr>
<td>19</td>
<td>65/F</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>20*</td>
<td>72/M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Coombs'-positive AHA</td>
</tr>
<tr>
<td>21</td>
<td>47/F</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>22</td>
<td>58/F</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>Coombs'-positive AHA</td>
</tr>
<tr>
<td>23</td>
<td>62/F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>60/F</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>25/M</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>Hepatitis A</td>
</tr>
</tbody>
</table>

AHA, autoimmune hemolytic anemia; ND, not done.
*Received some prior treatment.

the diagnosis of CLL was based on the presence of blood and marrow lymphocytosis alone. Total leukocyte counts in the group varied between 5,000 and 23,100/cu mm (median 14,000). Fourteen patients had a history consistent with the diagnosis of CLL and/or physical findings suggesting the diagnosis such as adenopathy or splenomegaly. It is of interest that 6 patients manifested some form of aberrant immunologic behavior consisting of Coombs'-positive hemolytic anemia (or a positive Coombs' test without overt hemolysis), Evan syndrome, a serum monoclonal protein, or severe hypogammaglobulinemia.

Of the 25 patients, 20 had never received prior treatment of any kind when initially studied, and 5 had received prior therapy consisting of low-dose chlorambucil or corticosteroids usually given for control of hemolysis. The leukocyte counts at the time of study in these 5 patients were in the pretreatment range, and 3 of the 5 had received no treatment for several months prior to study. It is of note that if one divides this group of patients into three subgroups according to the level of the absolute lymphocyte count, there is no striking correlation between the presence or absence of symptoms or organo-
megaly in the two higher subgroups. However, in the group with the lowest counts (1500–5000/cu mm) no aberrant immune phenomena occurred during the period of followup.

Surface marker characteristics. The results of morphologic and surface marker studies in these patients are listed in Table 2 according to absolute lymphocyte count. In 23 of 25 patients there was an increase in the SMIg+ cell compartment of at least two to three times normal. As absolute lymphocyte counts rose, the SMIg+ compartment increased as well, such that at lymphocyte levels 10,000–15,000/cu mm the increase was, with one exception, 15–25 times normal. At higher lymphocyte levels all patients had an increase in SMIg+ cells. A single patient with a normal number of SMIg+ cells had a marked increase in the E−SMIg− cell compartment, suggesting a diagnosis of null cell CLL.

There was a much wider variation in the size of the E+ cell compartment. In 12 patients it was normal or decreased and in each instance was associated with a concomitant increase in the SMIg+ cell compartment. Thirteen patients had an increase in the E+ cell compartment. In two the increase was marked, and they were tentatively designated as examples of T cell CLL. One such patient (No. 12) had an associated dermatosis labeled as bullous pemphigoid. The remaining 11 increases in E+ compartments were more modest, but with only a single exception they were associated with an increase in the SMIg+ cell compartment.

As can be seen in Table 2, five patients in the low-count group had significant increases in E−SMIg− cells of 25%–65%. In each instance there was an associated increase in SMIg+ and/or E+ compartments as well. In one of these cases (No. 8) the number of C3-bearing cells was increased in parallel fashion, while the SMIg+ cell pool was normal (8%), suggesting that the increase in null cells was in reality an increase in cells with the phenotype E−SMIg− C3+. Although in general the number of C3-bearing cells paralleled the SMIg+ cell population, the possibility exists that CLL cell populations expressing the phenotype E−SMIg− C3+ occur in a small percentage of cases and should not be included in what has been referred to as null cell CLL. Increases in null or C3+ null cell compartments were seen only in the two lower absolute lymphocyte subgroups (1,500–10,000/cu mm).

The SMIg characteristics of the group are also listed in Table 2. In 18 of 25 cases a monoclonal Ig was clearly identified. As in the high-count group, IgM was most often found (15 instances), predominantly κ type (12 of 15). The δ chain was also found to be associated with the μ chain on single cells in 4 of 15 patients. Monoclonal IgG was found on the cell surface in 3 patients, reflecting the fact that some degree of differentiation does occur in CLL clones. Two additional patients had less clearcut abnormalities, since the predominant Ig chain was found in less than 50% of the total Ig+ population (patients 6 and 24). In one, there was an increase in the γ chain without an associated light chain increase; in the other both γ and κ chain percentages were elevated. A polyclonal distribution of SMIg was detected in 5 instances, but in each case this was associated with an increase in E−SMIg− or E+ cell compartments.

The peripheral blood smears of these patients were examined by several observers, and excellent agreement in interpretation was achieved. Smears diag-
Table 2. Initial Immunologic and Morphologic Characteristics of Early CLL

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>WBC/cu mm (x 10^3)</th>
<th>Lymphs (%)</th>
<th>Abs. Lymphs (/cu mm x 10^3)</th>
<th>E^+(%) (Abs. E^+ / cu mm x 10^3)</th>
<th>SMig^+ (%) (Abs. SMig^+ / cu mm x 10^3)</th>
<th>E^- SMig^- (%)</th>
<th>C^-3^+ (%)</th>
<th>Marker Abnormality</th>
<th>Blood Smear</th>
</tr>
</thead>
<tbody>
<tr>
<td>1500-5000/cu mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5.0</td>
<td>73</td>
<td>3.60</td>
<td>45 (1.62)</td>
<td>50 (1.80)</td>
<td>0</td>
<td>—</td>
<td>Mono IgM(x)</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>10.1</td>
<td>47</td>
<td>4.74</td>
<td>9 (0.43)</td>
<td>74 (3.51)</td>
<td>0</td>
<td>—</td>
<td>Mono IgM(x)</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>6.8</td>
<td>71</td>
<td>4.82</td>
<td>45 (2.17)</td>
<td>9 (1.73)</td>
<td>0</td>
<td>—</td>
<td>Mono IgM(x)</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>5.5</td>
<td>55</td>
<td>3.02</td>
<td>48 (1.45)</td>
<td>20 (0.65)</td>
<td>30</td>
<td>23</td>
<td>Mono IgM(x)</td>
<td>S</td>
</tr>
<tr>
<td>5</td>
<td>8.3</td>
<td>43</td>
<td>3.56</td>
<td>18 (0.64)</td>
<td>75 (2.67)</td>
<td>0</td>
<td>71</td>
<td>Mono IgM(x)</td>
<td>S</td>
</tr>
<tr>
<td>6</td>
<td>5.8</td>
<td>62</td>
<td>3.59</td>
<td>51 (1.83)</td>
<td>48 (1.73)</td>
<td>0</td>
<td>—</td>
<td>Possible increase γ</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>7.9</td>
<td>46</td>
<td>3.63</td>
<td>54 (1.96)</td>
<td>40 (1.45)</td>
<td>0</td>
<td>7</td>
<td>Mono IgM(x) and δ</td>
<td>S</td>
</tr>
<tr>
<td>5,000-10,000/cu mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>11.9</td>
<td>49</td>
<td>5.83</td>
<td>56 (3.26)</td>
<td>8 (0.46)</td>
<td>36</td>
<td>45</td>
<td>† E^- SMig^- C^-3^+</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>15.3</td>
<td>44</td>
<td>6.73</td>
<td>5 (0.33)</td>
<td>70 (4.71)</td>
<td>25</td>
<td>—</td>
<td>Mono IgM(x) and δ</td>
<td>S</td>
</tr>
<tr>
<td>10</td>
<td>15.2</td>
<td>58</td>
<td>8.81</td>
<td>15 (1.28)</td>
<td>20 (1.76)</td>
<td>65</td>
<td>—</td>
<td>† E^- SMig^-</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>15.0</td>
<td>65</td>
<td>9.75</td>
<td>10 (9.75)</td>
<td>90 (8.77)</td>
<td>0</td>
<td>—</td>
<td>Mono IgM(x)</td>
<td>S</td>
</tr>
<tr>
<td>12</td>
<td>9.7</td>
<td>74</td>
<td>7.17</td>
<td>80 (3.74)</td>
<td>29 (2.43)</td>
<td>32</td>
<td>—</td>
<td>† E^- SMig^-</td>
<td>ND</td>
</tr>
<tr>
<td>13</td>
<td>14.0</td>
<td>60</td>
<td>8.40</td>
<td>36 (3.02)</td>
<td>8 (0.57)</td>
<td>0</td>
<td>7</td>
<td>† E^- SMig^-</td>
<td>ND</td>
</tr>
<tr>
<td>14</td>
<td>16.6</td>
<td>60</td>
<td>9.96</td>
<td>1 (0.10)</td>
<td>97 (9.66)</td>
<td>0</td>
<td>—</td>
<td>Mono IgM(λ)</td>
<td>S</td>
</tr>
<tr>
<td>15</td>
<td>8.8</td>
<td>92</td>
<td>8.09</td>
<td>0 (0.0)</td>
<td>98 (7.93)</td>
<td>0</td>
<td>—</td>
<td>Mono IgM(x)</td>
<td>D</td>
</tr>
<tr>
<td>16</td>
<td>14.3</td>
<td>56</td>
<td>8.00</td>
<td>40 (3.2)</td>
<td>43 (3.44)</td>
<td>0</td>
<td>49</td>
<td>Mono IgM(λ) and δ</td>
<td>ND</td>
</tr>
<tr>
<td>17</td>
<td>10.2</td>
<td>67</td>
<td>6.83</td>
<td>35 (2.4)</td>
<td>59 (4.03)</td>
<td>0</td>
<td>—</td>
<td>Mono IgG(x)</td>
<td>ND</td>
</tr>
<tr>
<td>10,000-15,000/cu mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>16.9</td>
<td>87</td>
<td>14.7</td>
<td>&lt;1 (0.0)</td>
<td>98 (14.4)</td>
<td>0</td>
<td>—</td>
<td>Mono IgM(x)</td>
<td>D</td>
</tr>
<tr>
<td>19</td>
<td>14.7</td>
<td>72</td>
<td>10.5</td>
<td>10 (2.11)</td>
<td>75 (7.93)</td>
<td>0</td>
<td>—</td>
<td>Mono IgM(x)</td>
<td>D</td>
</tr>
<tr>
<td>20</td>
<td>15.0</td>
<td>70</td>
<td>10.5</td>
<td>10 (1.05)</td>
<td>85 (8.92)</td>
<td>0</td>
<td>—</td>
<td>Mono IgM(x)</td>
<td>D</td>
</tr>
<tr>
<td>21</td>
<td>16.5</td>
<td>75</td>
<td>12.3</td>
<td>16 (1.98)</td>
<td>64 (7.92)</td>
<td>0</td>
<td>—</td>
<td>Mono IgM(x)</td>
<td>D</td>
</tr>
<tr>
<td>22</td>
<td>12.5</td>
<td>86</td>
<td>10.7</td>
<td>&lt;1 (0.0)</td>
<td>90 (9.65)</td>
<td>0</td>
<td>—</td>
<td>Mono IgM(λ)</td>
<td>D</td>
</tr>
<tr>
<td>23</td>
<td>23.0</td>
<td>53</td>
<td>12.1</td>
<td>68 (8.28)</td>
<td>17 (2.07)</td>
<td>0</td>
<td>—</td>
<td>† E^- SMig^-</td>
<td>D</td>
</tr>
<tr>
<td>24</td>
<td>23.1</td>
<td>50</td>
<td>11.6</td>
<td>66 (7.62)</td>
<td>32 (3.70)</td>
<td>0</td>
<td>—</td>
<td>Possible mono IgG(x)</td>
<td>D</td>
</tr>
<tr>
<td>25</td>
<td>16.2</td>
<td>79</td>
<td>12.8</td>
<td>10 (1.28)</td>
<td>85 (10.98)</td>
<td>0</td>
<td>10</td>
<td>Mono IgM(x) and δ</td>
<td>S</td>
</tr>
</tbody>
</table>

ND, nondiagnostic; S, suspicious; D, diagnostic. Normal blood values: absolute lymphocytes, 2.10 ± 0.90/cu mm x 10^3; E^+ cells, 1.66 ± 0.19/cu mm ± 10^3; SMig^+ cells, 0.35 ± 0.15/cu mm x 10^3; C^-3^+ cells, 13% ± 3%.
nostic of CLL were seen in eight patients and correlated with an increase in absolute lymphocyte count. With one exception all patients with counts of 10,000–15,000/cu mm had diagnostic smears. No patients in the 1500–5000/cu mm group had a diagnostic smear. Suspicious smears were seen in six additional patients in the lower-count subgroups. The patients with diagnostic and suspicious smears all had diagnostic abnormalities in surface marker studies as well, either a monoclonal SMIg or, in one case, a marked increase in E+ cells. Ten patients had nondiagnostic smears and were in the two lower-count categories. It is of note that each of these ten patients had a significant abnormality in their surface marker profile, consisting of a monoclonal increase in SMIg+ cells in six. E− SMIg− or E− SMIg− C3+ cell increases in three, and a marked E+ cell increase in one patient.

Serial observations in early CLL. Serial clinical and/or immunologic studies are available for 23 of 25 patients and are listed in Table 3. Twenty-one patients were followed for 6 mo or more from the initial surface marker evaluation, with a median for the group of 1 yr. Eleven patients pursued a stable course during followup with no change in clinical parameters and stable blood counts. In view of the lack of clinical disease progression, surface marker studies were repeated in only four patients. In two of four the monoclonal IgM(κ) population remained constant or increased on multiple determinations. In a third (No. 1) the monoclonal IgM(κ) population declined and was replaced by E+ cells. Two additional patients had essentially stable disease except for the appearance of asymptomatic adenopathy or a rising leukocyte count. Surface marker studies in these two patients again showed a stable monoclonal SMIg+ population. Five patients displayed progressive clinical disease during followup that necessitated the institution of some form of therapy. Two of these patients had repeat marker studies; in one the IgM(κ) monoclonal population was stable; and the other patient required splenectomy, and remarkably, following this, the monoclonal IgM(λ) population decreased to 17% and was replaced by E+ cells.

Two patients are particularly noteworthy (Nos. 6, 25). Both individuals were first seen because of acute hepatitis and were evaluated subsequently for persistent lymphocytosis. In each a monoclonal SMIg+ population was either suspected or definitely present initially. As the hepatitis subsided or became chronic, the monoclonal population increased dramatically in association with persistent lymphocytosis.

Three patients died subsequently of causes unrelated to CLL. It is of interest that one (No. 12) developed nonlymphocytic leukemia and died rapidly without achieving remission. The coexisting CLL was stable at the time of death.

Nonneoplastic lymphocytosis. We include surface marker data on five additional patients with nonneoplastic lymphocytosis for comparison (Table 4). Three were young individuals with typical subacute viral syndromes, a single individual had chronic active hepatitis, and another had long-standing lymphocytosis thought due to a chronic hypersensitivity reaction. Characteristically, in this group both E+ and SMIg+ cell compartments were increased simultaneously and usually symmetrically. The one exception was patient No. 24, who had an increase in her E+ cell compartment alone. In each instance the
Table 3. Serial Clinical and Immunologic Observations in Early CLL

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Duration of Followup (yr)*</th>
<th>Clinical Parameters</th>
<th>Surface Marker Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1500–5000/cu mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.0</td>
<td>Asymptomatic, negative exam, blood counts stable</td>
<td>Mono IgM(x) declines, E⁺ increases</td>
</tr>
<tr>
<td>2</td>
<td>1.2</td>
<td>Asymptomatic, negative exam, blood counts stable</td>
<td>Stable mono IgM(x) population</td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
<td>Died breast cancer, CLL stable</td>
<td>Not repeated</td>
</tr>
<tr>
<td>4</td>
<td>0.9</td>
<td>Died heart attack, CLL stable</td>
<td>Not repeated</td>
</tr>
<tr>
<td>5</td>
<td>1.3</td>
<td>Chronic persistent hepatitis, splenomegaly, blood counts stable</td>
<td>Emergence of mono IgG(x) in high percentage</td>
</tr>
<tr>
<td>6</td>
<td>0.3</td>
<td>Asymptomatic, negative exam, blood counts stable</td>
<td>Increase in mono IgM(x) and δ population</td>
</tr>
<tr>
<td>5,000–10,000/cu mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1.2</td>
<td>Asymptomatic, negative exam, blood counts stable</td>
<td>Increase in E⁻ SmIg⁻ C⁺δ population</td>
</tr>
<tr>
<td>8</td>
<td>0.9</td>
<td>Prednisone for AHA, nocardial pneumonia</td>
<td>Not repeated</td>
</tr>
<tr>
<td>9</td>
<td>1.4</td>
<td>Asymptomatic, adenopathy appeared, blood counts stable</td>
<td>Stable mono IgM(x) population</td>
</tr>
<tr>
<td>10</td>
<td>0.9</td>
<td>CLL stable, died acute myelomonocytic leukemia</td>
<td>Masked by acute leukemia</td>
</tr>
<tr>
<td>11</td>
<td>1.0</td>
<td>Asymptomatic, negative exam, blood counts stable</td>
<td>Not repeated</td>
</tr>
<tr>
<td>12</td>
<td>1.2</td>
<td>Asymptomatic, negative exam, blood counts stable</td>
<td>Not repeated</td>
</tr>
<tr>
<td>13</td>
<td>1.0</td>
<td>Asymptomatic, negative exam, blood counts stable</td>
<td>Not repeated</td>
</tr>
<tr>
<td>14</td>
<td>1.5</td>
<td>Asymptomatic, negative exam, blood counts stable</td>
<td>Not repeated</td>
</tr>
<tr>
<td>15</td>
<td>0.3</td>
<td>Asymptomatic, negative exam, blood counts stable</td>
<td>Not repeated</td>
</tr>
<tr>
<td>16</td>
<td>1.2</td>
<td>Asymptomatic, negative exam, blood counts stable</td>
<td>Not repeated</td>
</tr>
<tr>
<td>17</td>
<td>3.0</td>
<td>Asymptomatic, negative exam, blood counts stable</td>
<td>Not repeated</td>
</tr>
<tr>
<td>18</td>
<td>3.0</td>
<td>Symptomatic, progressive adenopathy, WBC increased requiring chemotherapy</td>
<td>Not repeated</td>
</tr>
<tr>
<td>19</td>
<td>1.0</td>
<td>Asymptomatic, negative exam, blood counts stable</td>
<td>Not repeated</td>
</tr>
<tr>
<td>20</td>
<td>3.0</td>
<td>Died accelerated disease, thrombocytopenia, infection</td>
<td>Stable mono IgM(x) population</td>
</tr>
<tr>
<td>21</td>
<td>1.8</td>
<td>Asymptomatic, negative exam, blood counts stable</td>
<td>Not repeated</td>
</tr>
<tr>
<td>22</td>
<td>2.0</td>
<td>Splenectomy, WBC stabilized at 12,000/cu mm, asymptomatic, negative exam</td>
<td>Following splenectomy mono IgM(λ) declined to 17%, E⁺ increased</td>
</tr>
<tr>
<td>23</td>
<td>1.5</td>
<td>Symptomatic, progressive adenopathy, WBC rose requiring chemotherapy</td>
<td>Not repeated</td>
</tr>
<tr>
<td>24</td>
<td>0.3</td>
<td>Asymptomatic, negative exam, blood counts stable</td>
<td>Not repeated</td>
</tr>
<tr>
<td>25</td>
<td>0.7</td>
<td>Hepatitis remitted, asymptomatic, negative exam, persistent lymphocytosis</td>
<td>Emergence of mono IgM(x) and δ in high percentage</td>
</tr>
</tbody>
</table>

*From initial surface marker studies.
Table 4. Initial Immunologic and Morphologic Characteristics of Benign Lymphocytosis

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>WBC/cu mm × 10^3</th>
<th>Lymphs (%)</th>
<th>Abs. Lymphs (1 cu mm × 10^3) E⁺ (%)</th>
<th>Abs. E⁺/cu mm × 10^3</th>
<th>SMIg⁺ (%)</th>
<th>Abs. SMIg⁺/cu mm × 10^3</th>
<th>E⁻ SMIg⁻ (%)</th>
<th>Clinical Diagnosis³</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>75/F</td>
<td>7.2</td>
<td>55</td>
<td>3.96</td>
<td>64 (2.53)</td>
<td>20 (0.79)</td>
<td>0</td>
<td></td>
<td></td>
<td>CHR</td>
</tr>
<tr>
<td>27</td>
<td>27/F</td>
<td>7.7</td>
<td>56</td>
<td>4.32</td>
<td>64 (2.76)</td>
<td>20 (0.86)</td>
<td>0</td>
<td></td>
<td></td>
<td>SVS</td>
</tr>
<tr>
<td>28</td>
<td>52/F</td>
<td>6.8</td>
<td>58</td>
<td>3.88</td>
<td>70 (2.72)</td>
<td>16 (0.63)</td>
<td>0</td>
<td></td>
<td></td>
<td>SVS</td>
</tr>
<tr>
<td>29</td>
<td>62/F</td>
<td>7.8</td>
<td>62</td>
<td>3.59</td>
<td>51 (1.83)</td>
<td>48 (1.72)</td>
<td>0</td>
<td></td>
<td></td>
<td>CAH</td>
</tr>
<tr>
<td>30</td>
<td>61/F</td>
<td>7.7</td>
<td>53</td>
<td>4.08</td>
<td>61 (2.49)</td>
<td>23 (0.94)</td>
<td>0</td>
<td></td>
<td></td>
<td>SVS</td>
</tr>
</tbody>
</table>

There were no marker abnormalities; all blood smears were nondiagnostic.

*CHR, chronic hypersensitivity reaction; SVS, subacute viral syndrome; CAH, chronic active hepatitis.

distribution of SM Ig chains was clearly polyclonal and no increases in E⁻ SM Ig⁻ or E⁻ SM Ig⁻ C⁺ were found. Peripheral blood smear morphology was uniformly classified as nondiagnostic.

**DISCUSSION**

Our data in established (stages 0–4) CLL are consistent with the view that E⁻ SM Ig⁺ cells, which presumably originate from the B cell compartment, comprise the vast majority of monoclonal CLL proliferations. In our group of established cases we saw little evidence for simultaneous symmetrical expansions of both Ig⁺ and E⁺ compartments, although some elevation of E⁺ cells was seen. With respect to double marking of cells, this would also appear to be a rare phenomenon, since in all cases the sum of percentages of E⁺ and SM Ig⁺ cells did not exceed 100. With a single exception, marker patterns in the untreated and often the treated state tended to remain remarkably stable with time. This might have been anticipated, since CLL is essentially a neoplasm with a slow growth rate and most cells are out of the proliferative cell cycle.¹⁶ Cytotoxic treatment can be expected to modify marker expression through the mechanism of cloning; we observed this in some of our patients.

There are occasional exceptions to these general findings. Several patients appeared to have proliferations of E⁺ SM Ig⁻ or E⁻ SM Ig⁻ cells; we designated these cases tentatively as originating from a T or null cell compartment. To date we have observed skin manifestations of disease in cases of T cell proliferation but cannot attribute any unusual clinical characteristics of SM Ig⁻ E⁻ cases. Within this group with SM Ig⁻ E⁻ null cell proliferation there was a subset in which the cells apparently bore only C⁺3 receptors. These patients should be segregated from the null cell group, since it is probable that the proliferation in these patients also originated from the B cell compartment. To date no distinctive clinical features characterize these patients.

An absolute lymphocyte count of 15,000/cu mm defines a workable transition zone above which the diagnosis of CLL can be made with reasonable certainty on either clinical or surface marker criteria. Below this level, as was suggested in the staging system of Rai et al.,¹⁶ the diagnosis cannot be made with uniformity on clinical grounds alone. However, with time, many patients in this early or preleukemic phase will develop more convincing evidence of CLL. If one stratifies patients in our low-count group with respect to absolute lymphocyte counts, a difference in clinical and surface marker characteristics
can still be detected. Patients with the lowest counts remained asymptomatic with few clinical findings and had nondiagnostic or suspicious peripheral blood smear morphology. As counts increased towards 15,000/cu mm the clinical findings increased and smear morphology became more typical. In contrast, surface marker studies in the lowest-count patients were distinctly abnormal and in most cases suggested a monoclonal SM Ig+ expansion. At higher lymphocyte counts within this low-count group it was possible to define a monoclonal expansion with certainty in virtually every patient.

Thus in CLL with relatively low lymphocyte counts it is still possible to detect a monoclonal expansion in the majority of individuals with minimal interference from contaminating normal cell populations. It may therefore be possible to make the diagnosis of CLL on the basis of surface marker studies in patients with lymphocytosis without resorting to marrow or node biopsy. The importance of performing multiple tests for surface markers is illustrated by the occasional patients with E- SM Ig- C3+ cells who might otherwise be overlooked or be characterized as having an increase in the null cell compartment.

Other investigators have commented on an apparent increase in the E+ compartment in SM Ig+ CLL. We noted this increase in T cells in 13 of our 25 patients with early CLL. This increase was usually small but clearly abnormal and may thus be an integral feature of the disease. It is not necessarily due to neoplastic proliferation. We may have observed a nonneoplastic T cell hyperplasia in the blood analogous to the lymphoid hyperplasia seen in lymph nodes partially involved or contiguous to those involved by malignant lymphomas. In the absence of a clonal T cell marker the best evidence to date seems to favor the T cell expansion in B cell–predominant CLL as being a nonneoplastic concomitant.

Our serial observations in early CLL patients provided several important insights into the natural history of the disease. Clinical progression of CLL is indolent and marker studies are stable in many patients. Our data suggest, however, that more instability may exist in the monoclonal cell population at this stage when compared to later stages of the disease. Five patients showed significant fluctuations in the size of the monoclonal cell population that were not associated with cytotoxic treatment. It may well be that expanding monoclonal B cell populations are to some degree under the influence of control mechanisms in the early stages, which is lost as CLL progresses. Particularly noteworthy are the two patients whose abnormal clones became dramatically expanded in the peripheral blood in the wake of an episode of acute hepatitis. The influence of this event on the triggering or perpetuation of the associated lymphoproliferation remains conjectural.

Finally, the surface marker distinction between early or preleukemic CLL and benign lymphocytosis should be commented upon. All of the patients with nonneoplastic lymphocytosis studied had distinctive surface marker features. These lymphoid proliferations were most often characterized by a symmetric increase in both B and T cell compartments. Furthermore, the increase in B cells showed polyclonal SM Ig chain ratios and was thus similar to what we found in similar analyses of histologically benign hyperplastic lymph nodes. In no instance did we find increased numbers of E- SM Ig- C3+ or E- SM Ig-
C'3+ cells, suggesting that in the untreated state these findings can be considered suspicious but not diagnostic for neoplastic clonal transformation. This difference in marker characteristics between benign lymphocytosis and the asymptomatic preleukemic phase of CLL has important diagnostic implications. In many of the cases cited in this paper the surface marker findings were very useful in distinguishing these two entities and influencing important therapeutic decisions.

ACKNOWLEDGMENT

The authors would like to thank Dr. Jane Desforges, Dr. Robert Schwartz, Dr. Peter Levine, and Dr. Steven Davis, who kindly referred their patients for study.

REFERENCES

15. Stathopoulos A, Elliott EV: Formation of mouse or sheep red blood cell rosettes by lymphocytes from normal and leukaemic individuals. Lancet 1:600, 1974
18. Rudders RA, DeLellis R: Unpublished observations
Clinical and cell surface marker characterization of the early phase of chronic lymphocytic leukemia

RA Rudders and JP Howard