REVIEW

Immunologic Classification of Leukemia and Lymphoma

By Kenneth A. Foon and Robert F. Todd, III

Important insights into leukocyte differentiation and the cellular origins of leukemia and lymphoma have been gained through the use of monoclonal antibodies that define cell surface antigens and molecular probes that identify immunoglobulin and T cell receptor genes. Results of these studies have been combined with markers such as surface membrane and cytoplasmic immunoglobulin on B lymphocytes, sheep erythrocyte receptors on T lymphocytes, and cytochemical stains. Using all of the above markers, it is now clear that acute lymphoblastic leukemia (ALL) is heterogeneous. Furthermore, monoclonal antibodies that identify B cells, such as the anti-B1 and anti-B4 antibodies in combination with studies of immunoglobulin gene rearrangement, have demonstrated that virtually all cases of non-T-ALL are malignancies of B cell origin. At least six distinct subgroups of non-T-ALL can now be identified. T-ALL is subdivided by the anti-Leu-9, anti-Leu-1, and antibodies that separate T lymphocyte subsets into three primary subgroups. Monoclonal antibodies are also useful in the subclassification of non-Hodgkin's lymphoma, and certain distinct markers can be correlated with morphologic classification. The cellular origin of the malignant Reed-Sternberg cell in Hodgkin's disease remains uncertain. A substantial number of investigators favor a myelocyte/macrophage origin based on cytochemical staining; however, consistent reactivity with antimonocyte reagents has not been demonstrated. Although monoclonal antibodies are useful in distinguishing acute myeloid from acute lymphoid leukemias, they have less certain utility in the subclassification of acute myelogenous leukemia (AML). Attempts to subclassify AML by differentiation-associated antigens rather than by the French-American-British (FAB) classification are underway in order to document the potential prognostic utility of surface markers. Therapeutic trials using monoclonal antibodies in leukemia and lymphoma have been reported. Intravenous (IV) infusion of unlabeled antibodies is the most widely used method; transient responses have been demonstrated. Antibodies conjugated to radionuclides have been quite successful in localizing tumors of <1 cm in some studies. Therapy trials with antibodies conjugated to isotopes, toxins, and drugs are currently planned. Purging of autologous bone marrow with monoclonal antibodies and complement in vitro has been used in ALL and non-Hodgkin's lymphoma; preliminary data suggest that this approach may be an effective therapy and may circumvent many of the obstacles and toxicities associated with in vivo monoclonal antibody infusion.

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Supported in part by grant no. CA 39064.

Submitted Aug 26, 1985; accepted Feb 1, 1986.

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0006-4971/86/0601-0001$03.00/0
The OKT series of antibodies is available through Ortho Systems, Inc. Raritan, NJ; Leu series through Becton Dickinson Co. Mountainview, Calif; anti-T through Coulter Immunology, Hialeah, Fla; Lyt through New England Nuclear, Boston; and TiOi through Hybritech Inc. San Diego.

### Table 1. Monoclonal Antibodies Reactive with Human B Lymphocytes

<table>
<thead>
<tr>
<th>Antibody (Subclass)</th>
<th>Pattern of Reactivity</th>
<th>Mol wt of Antigen (kd)</th>
<th>Cluster Designation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA-1 (lgM)</td>
<td>B lymphocytes, granulocytes malignant B cells</td>
<td>45,55,65</td>
<td>CD24</td>
<td>4,5</td>
</tr>
<tr>
<td>FMC1 (lgM)</td>
<td>B lymphocytes, malignant B cells</td>
<td>NR</td>
<td>NA</td>
<td>6</td>
</tr>
<tr>
<td>FMC7</td>
<td>50% B lymphocytes, some malignant B cells</td>
<td>NR</td>
<td>NA</td>
<td>7</td>
</tr>
<tr>
<td>Anti-B1 (lgG2)</td>
<td>B lymphocytes, malignant B cells</td>
<td>35</td>
<td>CD20</td>
<td>8</td>
</tr>
<tr>
<td>Anti-B2 (lgM)</td>
<td>B lymphocytes, malignant B cells (receptor for Epstein-Barr virus and CD3)</td>
<td>140</td>
<td>CD21</td>
<td>9,10</td>
</tr>
<tr>
<td>Anti-B4 (lgG1)</td>
<td>B lymphocytes, malignant B cells</td>
<td>40</td>
<td>CD19</td>
<td>11</td>
</tr>
<tr>
<td>Anti-B5 (lgM)</td>
<td>Activated B lymphocytes, malignant B cells</td>
<td>75</td>
<td>NA</td>
<td>12</td>
</tr>
<tr>
<td>P1153/3</td>
<td>B lymphocytes, malignant B cells</td>
<td>NR</td>
<td>NA</td>
<td>13</td>
</tr>
<tr>
<td>OKB1 (lgG1)</td>
<td>B lymphocytes, malignant B cells</td>
<td>168</td>
<td>NA</td>
<td>14,15</td>
</tr>
<tr>
<td>OKB2 (lgG1)</td>
<td>B lymphocytes, granulocytes</td>
<td>NR</td>
<td>NA</td>
<td>14,15</td>
</tr>
<tr>
<td>OKB4 (lgM)</td>
<td>B lymphocytes, malignant B cells</td>
<td>87</td>
<td>NA</td>
<td>14,15</td>
</tr>
<tr>
<td>OKB7 (lgG1)</td>
<td>B lymphocytes, malignant B cells</td>
<td>175</td>
<td>NA</td>
<td>14,15</td>
</tr>
<tr>
<td>Anti-HLB-1 (lgG2)</td>
<td>B lymphocytes, malignant B cells</td>
<td>NR</td>
<td>NA</td>
<td>16</td>
</tr>
<tr>
<td>41H.16 (lgG2)</td>
<td>B lymphocytes, malignant B cells</td>
<td>39</td>
<td>NA</td>
<td>17</td>
</tr>
<tr>
<td>Anti-Bl1 (lgG1)</td>
<td>Subpopulation of B lymphocytes, malignant B cells, granulocytes</td>
<td>Glycolipid</td>
<td>NA</td>
<td>18</td>
</tr>
<tr>
<td>Anti-Bl2 (lgG2)</td>
<td>B lymphocytes, malignant B cells, activated T cells</td>
<td>68</td>
<td>NA</td>
<td>19,19</td>
</tr>
<tr>
<td>Anti-Bl3 (lgG2)</td>
<td>Subpopulations of B lymphocytes, some malignant B cells, plasma cells, activated T cells</td>
<td>105</td>
<td>NA</td>
<td>19</td>
</tr>
<tr>
<td>Anti-PCA-1 (lgG2)</td>
<td>Plasma cells, malignant plasma cells, weakly on monocytes and granulocytes</td>
<td>NR</td>
<td>NA</td>
<td>20</td>
</tr>
<tr>
<td>Anti-PCA-1 (lgM)</td>
<td>Plasma cells, malignant plasma cells</td>
<td>28</td>
<td>NA</td>
<td>21</td>
</tr>
<tr>
<td>LN-1 (lgM)</td>
<td>B lymphocytes, malignant B cells, epithelial tumors</td>
<td>Sialoantigen</td>
<td>NA</td>
<td>22</td>
</tr>
<tr>
<td>LN-2 (lgG)</td>
<td>B lymphocytes, malignant B cells (nuclear membrane and cytoplasm)</td>
<td>35</td>
<td>NA</td>
<td>22</td>
</tr>
<tr>
<td>HD6, HD39, 29-110</td>
<td>75% of B lymphocytes, most malignant B cells</td>
<td>135</td>
<td>CD22</td>
<td>23</td>
</tr>
<tr>
<td>SJ10-1H11, SHCL-1</td>
<td>Germinal center B cells, not on resting B cells, some malignant B cells</td>
<td>45</td>
<td>CD23</td>
<td>23</td>
</tr>
</tbody>
</table>

NR, not reported. NA, not applicable.
The anti-B series, anti-PC-1, and anti-PCA-1 are available through Coulter Immunology, Hialeah, Fla; BA-1 through Hybritech Inc, San Diego; and the OKB series through Ortho System, Inc, Raritan N.J.

### Table 2. Monoclonal Antibodies Reactive with Human T Lymphocytes

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Pattern of Reactivity</th>
<th>Mol wt of Antigen (kd)</th>
<th>Cluster Designation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKT1, anti-T1, anti-Leu-1, 10.2 (Lyt-2), SC-1, A50, T101</td>
<td>Pan-T lymphocyte, pan-thymocyte</td>
<td>65</td>
<td>CD5</td>
<td>26-34</td>
</tr>
<tr>
<td>OKT3, anti-T3, anti-Leu-4, UCHT1</td>
<td>Pan-T lymphocyte (mitogenic)</td>
<td>20,20,25</td>
<td>CD3</td>
<td>27,28,36,36</td>
</tr>
<tr>
<td>Anti-Ti</td>
<td>Anti-clonotypic (T cell antigen receptor)</td>
<td>49-51 (a)</td>
<td>NA</td>
<td>37-39</td>
</tr>
<tr>
<td>12.1, T411</td>
<td>Pan-T, subpopulation of B</td>
<td>120</td>
<td>CD6</td>
<td>40,41</td>
</tr>
<tr>
<td>OKT11, anti-T11, anti-Leu-5, 9.6 (Lyt-3)</td>
<td>Pan-T lymphocyte (sheep erythrocyte receptor)</td>
<td>40-50</td>
<td>CD2</td>
<td>42-44</td>
</tr>
<tr>
<td>3A1, anti-Leu-9 (4H9), WT1, 4A</td>
<td>Pan-T lymphocyte</td>
<td>40</td>
<td>CD7</td>
<td>48-48</td>
</tr>
<tr>
<td>OKT4, anti-T4, anti-Leu-3</td>
<td>T helper/inducer</td>
<td>55</td>
<td>CD4</td>
<td>26-28-48-52</td>
</tr>
<tr>
<td>Anti-TQ1</td>
<td>Subset of T helper cells</td>
<td>NR</td>
<td>NA</td>
<td>53</td>
</tr>
<tr>
<td>OKT5, OKT8, anti-T8, anti-Leu-2</td>
<td>T cytotoxic/suppressor</td>
<td>32-43</td>
<td>CD8</td>
<td>28,27,51,52,64,66</td>
</tr>
<tr>
<td>OKT6, NA1/34, anti-Leu-6</td>
<td>Thymocytes</td>
<td>45</td>
<td>CD1</td>
<td>27,28,58</td>
</tr>
<tr>
<td>OKT9, 5E9</td>
<td>Thymocytes, lymphoblasts, monocytes (anti-transferrin)</td>
<td>90</td>
<td>NA</td>
<td>27,28,57</td>
</tr>
<tr>
<td>OKT10</td>
<td>Thymocytes</td>
<td>45</td>
<td>NA</td>
<td>27</td>
</tr>
<tr>
<td>Anti-Ta1</td>
<td>Activated T lymphocytes</td>
<td>105</td>
<td>NA</td>
<td>58</td>
</tr>
<tr>
<td>Anti-Tac</td>
<td>Interleukin-2 receptor</td>
<td>55</td>
<td>CD25</td>
<td>59-61</td>
</tr>
</tbody>
</table>

NR, not reported. NA, not applicable.
The OKT series of antibodies is available through Ortho Systems, Inc. Raritan, N.J; Leu series through Becton Dickinson Co, Mountainview, Calif; anti-T through Coulter Immunology, Hialeah, Fla; Lyt through New England Nuclear, Boston; and T101 through Hybritech Inc, San Diego.
SmIg but have Clg. Unlike the Clg found in pre-B lymphocytes, Clg in plasma cells includes both heavy and light chains.

A number of heteroantisera and, more recently, monoclonal antibodies that identify B cell-associated antigens have been described (Table 1). Where applicable, the nomenclature and clusters of differentiation (CD) defined by the Second International Workshop on Human Leukocyte Differentiation Antigens are shown.23-25

**T lymphocytes.** T lymphocytes were initially identified by their ability to bind sheep erythrocytes spontaneously. T lymphocytes also react with T cell-specific antiserum and anti-T cell monoclonal antibodies, which may also be used to identify T lymphocytes, and have proven to be more sensitive and discriminatory (Table 2).26-41 Many of these antibodies react with immature T cells; others react with more mature T cells. Some of these antibodies identify antigens found on all T cells, whereas others occur only on T cell subsets.

**Myeloid cells.** Monoclonal antibodies to cell surface markers on peripheral blood myeloid cells and their bone marrow progenitors have been extensively investigated.42-125 Some of these monoclonal antibodies detect antigens expressed by either peripheral blood monocytes or neutrophils. Other reagents identify surface markers common to monocytes and neutrophils; monocytes, neutrophils, and large granular lymphoid cells (LGL); monocytes and platelets; or neutrophils and LGLs (Table 3).

The expression of several monoclonal antibody-defined myeloid antigens corresponds to pathways of normal differentiation within the myeloid lineage. These antibodies are

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**Table 3. Representative Murine Monoclonal Antibodies That Identify Human Myeloid Cell Surface Antigens:**

<table>
<thead>
<tr>
<th>Antibody/antigen (cluster designation [CD], antigen mol wt, kd, reducing conditions), key references (superscript).</th>
<th>Antibody immunoprecipitates broad band of 28 to 65 kd on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).</th>
<th>Antibody immunoprecipitates two broad bands of 155 to 288 and 75 to 125 kd on SDS-PAGE.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Table 3. Representative Murine Monoclonal Antibodies That Identify Human Myeloid Cell Surface Antigens:</strong> Distribution of Antigen Expression Among Peripheral Blood Cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Monocytess</strong></td>
<td><strong>Neutrophils</strong></td>
<td><strong>Neutrophils and Monocytes</strong></td>
</tr>
<tr>
<td>Mo2 (CDw14,55)82-84*</td>
<td>B40.977</td>
<td>MY7 (CDw13,160)89</td>
</tr>
<tr>
<td>Mo3*</td>
<td>R1819 (145,105)77</td>
<td>MY89</td>
</tr>
<tr>
<td>UC45 (45)86</td>
<td>B2H5 (CD15)88</td>
<td>Mo5 (CD11,94)86</td>
</tr>
<tr>
<td>UCHM1 (CDw14)86</td>
<td>80H.54</td>
<td>B13.47,96</td>
</tr>
<tr>
<td>UCHALF56</td>
<td>TG-1 (CDw15)88</td>
<td>B9.87,96</td>
</tr>
<tr>
<td>S16-14487,88</td>
<td>VIM-DS (CD15,145,105)86</td>
<td>B34.377</td>
</tr>
<tr>
<td>MY3 (55)89</td>
<td>FMC10 (CDw15)56</td>
<td>AML-2-2381</td>
</tr>
<tr>
<td>MY4 (CDw14)89</td>
<td>FMC12 (CDw15)87</td>
<td>PM-8197</td>
</tr>
<tr>
<td>MY570</td>
<td>FMC13 (CDw15)87</td>
<td>G10 (CD15)56</td>
</tr>
<tr>
<td>D50E71</td>
<td>AHN-1 (145,105)56,88</td>
<td>M206 (180)89</td>
</tr>
<tr>
<td>C10H571</td>
<td>MY-1580</td>
<td>MMA or anti-Leu M1100</td>
</tr>
<tr>
<td>63D3 or antimonocytic (2 (202)72,73</td>
<td>PMN681</td>
<td>S4-7 (150)87,88</td>
</tr>
<tr>
<td>61D3 or antimonocytic (2 (75)74</td>
<td>PMN2981</td>
<td>AHN-7121</td>
</tr>
<tr>
<td></td>
<td>PMN7C382</td>
<td>80H.184</td>
</tr>
<tr>
<td></td>
<td>3G8 (60-70)83</td>
<td>80H.3 (CDw15)84</td>
</tr>
<tr>
<td></td>
<td>18S84</td>
<td>DUHL60.1 (CDw15)102</td>
</tr>
<tr>
<td></td>
<td>4D1 (69)84</td>
<td>DUHL60.3 (CDw15)102</td>
</tr>
<tr>
<td>MOP-15 (CDw14)78</td>
<td>DUHL60.4 (CDw13)102</td>
<td></td>
</tr>
<tr>
<td>MOP-9 or anti-Leu-M3 (CDw14)78</td>
<td>DUH60.3 (CDw15)102</td>
<td></td>
</tr>
<tr>
<td>Mac-120 or anti-Leu-M2 (120)78</td>
<td>DUH60.3 (CDw13)102</td>
<td></td>
</tr>
<tr>
<td></td>
<td>120F (CDw12)102</td>
<td></td>
</tr>
<tr>
<td>B44.1 (55)77</td>
<td>T5A7 (CDw17)103</td>
<td></td>
</tr>
<tr>
<td>1D578</td>
<td>20.2 (CDw12)104</td>
<td></td>
</tr>
<tr>
<td>PHM3 (50)78</td>
<td>VIM-2106</td>
<td></td>
</tr>
<tr>
<td>4F2 (40,80)80,81</td>
<td>FMC17 (CDw14)102</td>
<td></td>
</tr>
</tbody>
</table>

*Antibody/antigen (cluster designation [CD], antigen mol wt, kd, reducing conditions), key references (superscript).
†Anti-C3bi receptor antibodies.
‡Anti-Fc receptor antibody.
§Antibodies bind to X-hapten, lacto-N-fucose-pentaosyl III.
@@Antibody immunoprecipitates broad band of 28 to 65 kd on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).
††Antibody immunoprecipitates two broad bands of 155 to 288 and 75 to 125 kd on SDS-PAGE.
therefore useful as tools for identifying hematopoietic cells at various stages of maturation and proliferative potential (Table 4 and Fig 1). Using either complement-dependent monoclonal antibody-mediated lysis (negative selection), or techniques such as fluorescence-activated cell sorting or immune rosetting (positive selection), it is possible to determine patterns of antigen expression by multipotent stem cells (CFU-GEMM) and by stem cells committed to the myeloid (CFU-GM), erythroid (BFU-E, CFU-E), or megakaryocyte/platelet (CFU-Mega) pathways of differentiation. In several reports, myeloid progenitor cells have been purified 50- to 100-fold from bone marrow mononuclear cells\textsuperscript{5,26} enabling studies of morphologic and functional characteristics. Certain determinants are uniquely expressed by progenitor cells (eg, MY-10); other antigens are detectable on myeloid, erythroid, or platelet precursors corresponding to morphologically and histochemically distinct stages of maturation within the bone marrow. In the case of neutrophil differentiation, some antigens are either lost (Ia, MY-10) or acquired (Mo1, MY8, 80H.3, or B34.3) as cells progress from myeloblasts to mature neutrophils. Expression of other determinants (B2H5, R1B9, S4–7) are maintained on all recognizable myeloid cells.

Although many of these antigenic determinants operationally serve as differentiation markers, some represent epitopes on functionally significant plasma membrane proteins, glycoproteins, and glycolipids. The glycoprotein heterodimer identified by the murine monoclonal antibodies Mo1 and OKM1 is a receptor for the binding of particles opsonized with C3 cleavage product C3bi (CR3 activity).\textsuperscript{17,128} Antibodies B73.1 and 3G8 identify an Fc receptor for IgG that is expressed by LGL and/or neutrophils.\textsuperscript{9,112} Data from immu-
nophosphatase or immunoblotting analyses indicate that several of these antibodies are specific for epitopes on the same parent structure or even the same epitope.

**Leukemia-associated antigens.** The common acute lymphoblastic leukemia-associated antigen (CALLA) was originally defined by antiserum produced in rabbits by immunization with SmIg-negative, sheep erythrocyte rosette-negative acute lymphoblastic leukemia (ALL) cells. This antiserum reacted with a 100 kd glycoprotein antigen. Monoclonal antibodies that recognize CALLA (CD10) have recently been described (Table 5). Although CALLA is absent on normal peripheral blood lymphocytes, it is not leukemia specific, and is present on normal terminal deoxynucleotidyl transferase (TdT) and Ia antigen–positive bone marrow cells that are thought to be lymphohematopoietic precursor cells. CALLA has also been identified on renal tubular and glomerular cells, mammary epithelium, fetal small intestine epithelial cells, granulocytes, fibroblasts, and melanoma cell lines. CALLA is also present on Burkitt lymphoma cells, follicular lymphoma cells, and cells from 40% of patients with lymphoblastic lymphoma.

P24/BA-2 is a second leukemia-associated antigen with a mol wt of 24 kd defined by the BA-2, SJ-9A4, and DU-ALL-1 monoclonal antibodies (CD9). These antibodies do not react with any normal circulating hematopoietic cells except for platelets. They react with most non-T ALL cells and, like CALLA, the P24/BA-2 antigen is present on normal peripheral blood lymphocytes and T-ALL, rare normal cells.

### Table 5. Monoclonal Antibodies Leukemia-Associated Antigens

<table>
<thead>
<tr>
<th>Antibody (Subclass)</th>
<th>Pattern of Reactivity</th>
<th>Molecular wt of Antigen (kd)</th>
<th>Cluster Designation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>J5, BA-3, anti-CALLA</td>
<td>Most non-T-ALL, Burkitt's lymphoma, follicular lymphoma, some lymphoblastic lymphoma and T-ALL, rare normal cells</td>
<td>100</td>
<td>CD10</td>
<td>130-136</td>
</tr>
<tr>
<td>RFB-1</td>
<td>Myeloid progenitor cells, immature lymphoid bone marrow cells</td>
<td>NR</td>
<td>NA</td>
<td>141</td>
</tr>
<tr>
<td>BA-2, SJ-9A4 Du-ALL-1</td>
<td>Lymphohematopoietic bone marrow progenitor cells, most non-T-ALL, platelets</td>
<td>24</td>
<td>CD9</td>
<td>137-140,142</td>
</tr>
<tr>
<td>Anti-3-3</td>
<td>T-ALL</td>
<td>35-40</td>
<td>NA</td>
<td>143</td>
</tr>
<tr>
<td>Anti-3-40</td>
<td>T-ALL, some non-T-ALL, rare AML, vimentin and keratin intermediate filaments in normal cells</td>
<td>35-40</td>
<td>NA</td>
<td>143,144</td>
</tr>
<tr>
<td>SN1</td>
<td>T-ALL</td>
<td>NR</td>
<td>NA</td>
<td>148</td>
</tr>
<tr>
<td>CALL2</td>
<td>T-ALL</td>
<td>NR</td>
<td>NA</td>
<td>148</td>
</tr>
</tbody>
</table>

NR, not reported; NA, not applicable.

J5 is available through Coulter Immunology, Hialeah, Fl; BA-2 and BA-3 through Hybritech Inc, San Diego.
(VDJ), which are then linked to the constant region locus. Immunoglobulin gene rearrangements are hierarchical; μ heavy chain rearrangements precede light chain rearrangements, and κ light chain rearrangement precedes λ light chain rearrangements.156-158 These rearrangements can be detected by Southern blot analyses of DNA from B cells using appropriately radiolabeled heavy or light chain probes.

Heavy chain rearrangements have been identified in non-B cells, but light chain rearrangements appear to be restricted to B cells.157,159,160 Clonal rearrangements of light chain genes are therefore an extremely sensitive tool to identify B cell malignancies.

The antigen-specific T cell receptor is a heterodimer formed by a 40- to 50-kd α subunit (Tα), and a 40- to 45-kd β subunit (Tβ).161 It is associated with three 20- to 25-kd peptide chains identified by the T3 monoclonal antibody.161 Recently, cDNA clones to the Tβ and Tα receptors have been isolated.162-166 The human Tβ receptor gene has been localized to chromosome 7167 and the human Tα receptor gene maps to chromosome 14.168 The less well-defined Tγ receptor gene is currently under investigation. The T cell receptor genes undergo rearrangements in a fashion analogous to that of immunoglobulin genes. The Tγ receptor gene has been shown to be rearranged in murine cytotoxic T lymphocyte cell lines but not myeloma cell lines.169 Similar to the Tβ gene chain, the Tγ gene rearrangement appears to occur early in T cell development, whereas Tα chain expression occurs later in thymic ontogeny.170

Tγ gene rearrangements have been detected in malignant human T cells by Southern blotting.171-173 This technique can detect as few as 1% tumor cells in a mixed cell population174; it is a sensitive diagnostic marker for T cell diseases. Rearrangements of the Tβ antigen receptor are reported in 25% of patients with non-T-ALL,175 and in a small proportion of patients with B cell leukemia.176 This is similar to the rearrangement of immunoglobulin heavy chain genes in 10% of patients with T cell leukemia.159,160

Intracellular enzymes and biochemical markers. TdT is present in thymocytes and in a small percentage of bone marrow cells, but not in mature lymphocytes.176,177 TdT is identified in all subtypes of ALL and is, therefore, not discriminative. TdT has also been demonstrated in a small proportion of acute myelogenous leukemia (AML) cells.178 Other intracellular enzymes reported useful in identifying subsets of ALL include hexosaminidase, adenosine deaminase,160 5'-nucleotidase,161 purine nucleoside phosphorylase,162,163 and acid phosphatase.164 Acid phosphatase is present in T-ALL cells but not in non-T-ALL cells. Cytotoxic reactions are useful in the subclassification of AML.183,184 The M1 through M3 myeloid subtypes contain myeloperoxidase and sometimes nonspecific esterase. Myelomonocytic leukemia cells (M4) also contain myeloperoxidase and nonspecific esterase; the latter is variably inhibited by sodium fluoride. Acute monocytic leukemia (M5) cells contain myeloperoxidase and nonspecific esterase which is completely inhibited by sodium fluoride.

Several surface membrane-associated biochemical markers of leukemia cells have also been described. The glycolipid asialo GM1 is found on cells from patients with ALL (non-T-ALL and T-ALL) but not on cells from patients with other forms of leukemia.185 Alterations in membrane carbohydrates, such as decreased complex gangliosides,186 carbohydrate-containing antigens,187 and receptors for cholera toxin188 have been reported on leukemia cells.

CLASSIFICATION OF THE LYMPHOID LEUKEMIAS AND LYMPHOMAS

Acute lymphoblastic leukemia. ALL is heterogeneous. The first surface markers used to differentiate subclasses of ALL were receptors for sheep erythrocytes,191-193 which identify a T cell subset (15% to 20% of cases), and SmIg, which identifies a B cell subset (<5% of cases). Both T and B cell subgroups have an unfavorable prognosis.194,195 The next important advance in identifying ALL was the development of an antiserum to CALLA.196 CALLA reactivity identified a non-B, non-T subclass of ALL patients (~70% of cases) with a more favorable prognosis than T-ALL, B-ALL, or non-B, non-T-ALL without CALLA.197 Other markers such as Ia antigen were commonly found on non-T-ALL and could help differentiate non-T-ALL from T-ALL.198 By testing for Cμ heavy chain, a subset designated pre-B ALL has been identified.197-199 Except for the presence of Cμ, this subset expresses the same surface markers as the CALLA form of non-T-ALL; it appears, however, to have a less favorable prognosis.200

With the development of monoclonal antibodies, it became evident that the T cell subset of ALL was heterogeneous.201-205 More recently, studies employing immunoglobulin gene rearrangements and monoclonal antibodies that identify B cell-associated antigens have demonstrated that most cases of non-T-ALL are derived from the B cell lineage.156,206-208 We review these data and present a new classification for ALL based on these recent observations.

Non-T-ALL. Two important areas of research have prompted a reassessment of non-T-ALL. First, monoclonal antibodies that recognize B cell-associated antigens have been identified; many are present on non-T-ALL cells. The most specific of these antibodies is probably anti-B4, which react with 95% of cases of non-T-ALL.11,209 Second, clonal rearrangements of immunoglobulin genes provide strong evidence for the B cell lineage of most cases of non-T-ALL.156,206,208

Although Ia antigen is present on most non-T-ALL cells, and CALLA is present in 75% of cases of non-T-ALL, these antigens are also identified on ~10% of cases of T-ALL. Therefore, B cell-associated antigens (Table 1), which are not identified on T-ALL cells, are the most useful in distinguishing non-T-ALL. The B1 and B4 antigens are model antigens for this discussion.

Less than 5% of cases of ALL express SmIg (usually IgM); these cells are typically classified as B-ALL. These cells generally express other B cell antigens, including B1 (CD20), B4 (CD19), and Ia. B-ALL in children is probably a leukemic phase of non-Hodgkin’s or Burkitt’s lymphoma.193,194 Another marker that identifies a subset of non-T-ALL is Cμ heavy chain; κ and λ light chains and SmIg are typically absent.197 These cells are considered pre-B cells. As
indicated, most cases of non-T-ALL cells of B lineage; thus Cμ is useful in determining the level of differentiation. Pre-B cells that synthesize μ heavy chain are more mature than those pre-B cells that do not synthesize μ heavy chain, but are less mature than those with SmIg.

Nadler and co-workers recently classified 138 patients with non-T-ALL. They divided these cases into four major subgroups. The first subgroup was Ia antigen positive, representing 5% of cases. Another subgroup expressed the Ia and B antigens, representing 15% of cases. The third subgroup expressed the Ia, B4, and CALLA antigens, comprising one third of the cases. Finally, one half of the cases of non-T-ALL were Ia, B4, CALLA, and B1 positive. The fourth group was further subdivided into cases with and without Cμ. We propose that cases with Cμ be placed in a separate group (group V). The final and most differentiated group, group VI, represents SmIg-positive B-ALL (Table 7).

Nadler and co-workers also studied immunoglobulin gene rearrangements in cells from patients in groups II, III, and IV. Patients in group II (Ia and B4 positive) demonstrated rearranged heavy chain genes, with germ lines for both κ and λ light chain genes. Based on these data, they hypothesized that the group II phenotype represents the earliest stage in B cell maturation. Patients in group III had rearranged heavy chain genes and most had κ light chain gene recombination, either rearrangement or deletion. Patients in group IV had rearranged heavy chain genes; two of four had deletions of κ.

Virtually all non-T ALL cells have immunoglobulin heavy chain rearrangement; not all of them, however, demonstrate light chain rearrangements. Because heavy chain rearrangements also occur in non-B cells, immunoglobulin heavy chain rearrangements are insufficient to assign non-T-ALL to the B cell lineage. Nadler and co-workers have proposed that the B4 antigen provides the most important independent parameter with which to identify B cell-derived non-T cell ALL. More recently, up to 5% of non-T ALL have been reported to have rearrangements of the Tβ receptor gene in cases of T-ALL.

Clinical features associated with T-ALL include a high blast cell count, predominance of older male patients, and mediastinal masses. T-ALL was originally identified by rosetting with sheep erythrocytes. The most sensitive marker for T-ALL is probably the pan-T 40 kd antigen identified by the anti-Leu-9 antibody (CD7). This antigen is present on most thymocytes and T cells but not on non-T-ALL or B cell lymphomas or leukemias. In a study of 23 patients with T-ALL, all cases expressed the Leu-9 antigen.

T-ALL represents 15% to 25% of cases of ALL. Table 7. Classification of Non-T-ALL

<table>
<thead>
<tr>
<th>Group</th>
<th>Ia</th>
<th>B4</th>
<th>CALLA</th>
<th>B1</th>
<th>Cytoplasmic μ</th>
<th>Surface Membrane Immunoglobulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
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<tr>
<td>IV</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>V</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VI</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

In Table 8, we summarize these data and propose a scheme for the classification of T-ALL. The common marker for all of the subgroups is Leu-9. Nearly all cells also express Leu-1 and most express T11/Leu-5 (CD2) that identifies the receptor for sheep erythrocytes. Cells in subgroup I also express T9 and/or T10 antibodies and account for ~10% of the thymic cells. In their study, Reinherz and co-workers reported that most T-ALL cells express antigens found on early thymocytes. The next level of thymic differentiation, which includes the majority of thymocytes, is referred to as common or stage II. These cells lose T9, retain T10, and acquire T6 (CD1), T4/Leu-3 (CD4), and T8/Leu-2 (CD8) antigens. Approximately 20% of cases of T-ALL express this phenotype. Mature stage III thymocytes no longer express T6 but segregate into T4/Leu-3 or T8/Leu-2 subsets similar to peripheral blood T lymphocytes. Only rarely did Reinherz and colleagues find T-ALL cells with the phenotype of mature thymocytes or circulating T lymphocytes. In a more recent study, Roper and coworkers confirmed many of the findings reported by Reinherz and colleagues, but reported some major differences. In this study, only one third of the T-ALL patients had the phenotype of early or stage I thymocytes; most had the phenotype of either intermediate or late stage thymocytes.

In Table 8, we summarize these data and propose a scheme for the classification of T-ALL. The common marker for all of the subgroups is Leu-9. Nearly all cells also express Leu-1 and most express T11/Leu-5 (CD2) that identifies the receptor for sheep erythrocytes. Cells in subgroup I also express T9 and/or T10. The pan T antigen, identified by T3/Leu-4 (CD3), represents a mature antigen and is not found on group I cells. The T4/Leu-3 helper-associated antigen, the T8/Leu-2 suppressor-associated antigen, and the T6 antigen are not expressed on group I cells.

The next level of differentiation is group II. T9 is found on some cells, however, the T6 antigen as well as the simulta-
neous expression of T4/Leu-3 and T8/Leu-2 antigens clearly distinguish group II from group I. Some cells in group II may also express T3/Leu-4. Group III T-ALL cells lose the T6 antigen and segregate into cells that have the phenotype of mature thymocytes and T lymphocytes (T3/Leu-4, T4/Leu-3 or T3/Leu-4, T8/Leu-2).

Although Roper and co-workers searched for clinical correlations among these three groups of T-ALL, they found no unique clinical features among the subgroups and no differences in remission duration or survival. However, the groups were too small for statistically valid conclusions. Presently, we believe it useful to subclassify T-ALL using this system, so that data from a number of institutions can be analyzed for clinical correlations between the subgroups of T-ALL (Table 8).

Non-Hodgkin’s lymphoma. The non-Hodgkin’s lymphomas are a diverse group of neoplasms whose pathologic classification is controversial. It is even more difficult to correlate pathologic classification with immunologic classification. Several immunologic patterns emerge, however, and we will attempt to place them within the non-Hodgkin’s lymphoma working classification as well as the Rappaport classification.

Follicular or nodular lymphomas. The follicular or nodular lymphomas most likely represent neoplastic proliferation of lymph node-derived follicular center B lymphocytes. The cell type may be a small cleaved cell (nodular lymphocytic poorly differentiated lymphoma by the Rappaport classification), mixed small cleaved and large cleaved or noncleaved cells (nodular mixed), or predominantly large cell (nodular histiocytic). The first two cell types fall within the working classification as low-grade lymphoma, whereas the latter cell type is classified as an intermediate-grade lymphoma. Although the predominantly small cleaved cell will almost always express high-density monoclonal SmIg, larger cells may be SmIg negative. However, the small cleaved and large cells will routinely express Ia, B4, and B1 antigens and will often express the B2 antigen. More than half of these cases will also express CALLA. Follicular lymphoma cells may be found in the peripheral blood as a “leukemic” phase of the disease (formerly referred to as lymphosarcoma cell leukemia). These cells can usually be differentiated from chronic lymphocytic leukemia (CLL) cells because they may express CALLA, which is not expressed on CLL cells; they do not express the T1/Leu-1 pan-T antigen found on CLL cells; and they generally will have a low percentage of mouse erythrocyte rosette formation (see below). Malignant lymphoma, small lymphocytic. Malignant lymphoma, small lymphocytic (diffuse lymphocytic well-differentiated lymphoma in the Rappaport classification) is a low-grade malignancy, and some cases may be identical to CLL. Also included within this subclassification are the plasmacytoid lymphocytic subgroups with and without an IgM monoclonal gammopathy; some of these cases are similar to Waldenström’s macroglobulinemia (described below). Surface markers on these small lymphocytic cells include low-intensity SmIg, mouse erythrocyte receptors, C3 and receptors for the Fc portion of IgG, and Ia, B1, B2, B4, Bα1, and other B cell antigens. These features are similar to CLL, and the cells also express the T1/Leu-1 pan-T antigen.

Malignant lymphoma, diffuse small cleaved cell and diffuse mixed small and large cell. Malignant lymphoma, diffuse small cleaved cell (diffuse lymphocytic poorly differentiated lymphoma in the Rappaport classification) is an intermediate prognostic group. The cells are B lymphocytes that (similar to follicular lymphoma cells) usually display large amounts of monoclonal SmIg. Unlike follicular lymphoma cells, however, they do not usually express CALLA. Similar to follicular lymphoma cells, they do not express the T1/Leu-1 antigen as do cells from most small lymphocytic lymphomas and CLL. However, all these cell types have in common the expression of Ia, B4, B1, B2, and other B cell antigens.

The diffuse mixed small and large cell (diffuse mixed lymphocytic-histiocytic) lymphomas have not been extensively studied but are most likely predominantly B cell diseases. They are also considered an intermediate-grade prognostic group.

Malignant lymphoma, diffuse large cell and large cell immunoblastic. In the working classification, the diffuse large cell lymphomas are considered within the intermediate prognostic group, whereas large cell immunoblastic lymphoma is a high-grade malignancy. By the Rappaport classification, both of these cell types would be described as histiocytic. This is clearly a misdesignation since 80% to 90% of cases represent clonal expansions of malignant B cells. A high percentage of these cells express T9 and T10 antigens. Fifty-seven cases of diffuse large cell lymphoma were recently studied and divided into the following subgroups: (a) B1, B4, and SmIg positive; B2 negative (50%); (b) B1, B4, SmIg, and B2 positive (30%); (c) B1 and B4 positive; SmIg and B2 negative (10%); and (4) B1 and SmIg positive, and B2 negative (10%). These data suggest that most of these lymphomas represent the malignant

<table>
<thead>
<tr>
<th>Group</th>
<th>Leu-9*</th>
<th>Leu-1</th>
<th>T11/Leu-5</th>
<th>T3/Leu-4</th>
<th>T3/Leu-6</th>
<th>T8/Leu-2</th>
<th>T6</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>+</td>
<td>+</td>
<td>+ (75%)</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>II</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+ (25%)</td>
<td>+ (90%)</td>
<td>+ (90%)</td>
<td>+</td>
</tr>
<tr>
<td>III</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/- f</td>
<td>+/- f</td>
<td>-</td>
</tr>
</tbody>
</table>

*Found on virtually all T-ALL cells.
†No longer simultaneous expression of T4/Leu-3 and T8/Leu-2 as found in group II.
counterpart of B cells at the midstage of differentiation. Ten to 20 percent of cases are T cell lineage; 2% are derived from the monocyte-myeloid lineage. Recently, clonal rearrangement of the Tβ receptor has been described in patients with T-derived non-Hodgkin’s lymphoma.172,173

**Malignant lymphoma, lymphoblastic.** Malignant lymphoma, lymphoblastic, or lymphoblastic lymphoma, is a high-grade malignancy. The nuclear membrane is characteristically deeply subdivided, exhibiting either a lobulated (convoluted) appearance or a fine linear (nonconvoluted) subdivision in a round nucleus. Lymphoblastic lymphoma represents approximately one-third of the cases of non-Hodgkin’s lymphomas in children and 5% of cases in adults. The disease is more prevalent in males; these patients often have a mediastinal mass. In some cases, the disease may evolve into a leukemic phase morphologically indistinguishable from T-ALL. The malignant cells are T cells, form E rosettes, react with T cell antisera,223-225 and have rearrangements of the Tβ receptor.226 Studies with monoclonal antibodies have demonstrated marked heterogeneity. Lymphoblastic lymphoma cells differ from T-ALL in that the cells rarely express the surface markers common to immature thymocytes (group I).227 Phenotypes are equally divided between group II and group III T-ALL. In 40% of cases, the cells are reported to express CALLA; CALLA expression is less common in T-ALL (10%).218

**Malignant lymphoma, small noncleaved cell.** Malignant lymphoma, small noncleaved cell includes Burkitt’s lymphoma and other lymphomas previously designated undifferentiated non-Burkitt type (high grade). Burkitt cells from peripheral blood and bone marrow are usually classified as L3 by the FAB criteria.185,186 Most cases of Burkitt’s lymphoma from Africa are endemic and are associated with the Epstein-Barr virus (EBV). Most non-African cases (nonendemic) are EBV negative.228 Chromosomal abnormalities involving chromosome 8 (carrying the oncogene c-myc) and either 2, 14, or 22 occur in virtually all cases of endemic and nonendemic Burkitt’s lymphoma.229 These are designated t(2;8), t(8;14) and t(8;22), respectively. Usually the light chain class expressed on these cells is correlated with the translocation, ie, κ in t(2;8) and λ in t(8;22). African Burkitt’s lymphoma cells have receptors for C3 and for the Fc portion of IgG in addition to the EBV receptor. American Burkitt’s lymphoma cells do not express these receptors.228 Phenotyping of cell lines derived from patients with undifferentiated lymphoma of the Burkitt’s and non-Burkitt’s type have demonstrated heterogeneity.230 These studies suggest that Burkitt cells follow a divergent pathway of B cell evolution because they are all TdT negative (unlike early B cell non-T-ALL). The most primitive of the Burkitt cell lines are la and b1 positive and may or may not express CALLA. Maturation was evident in other Burkitt cell lines by the expression of C4, surface membrane IgM, and/or IgM secretion. Some of these Burkitt cell lines also expressed the Tac antigen.

**Peripheral T cell lymphoma.** Peripheral T cell lymphoma would usually be classified as malignant lymphoma, large cell immunoblastic (high grade) under the working formulation. However, this tumor has unique features and will be described separately. The term “peripheral T cell lymphoma” is used to distinguish it from lymphoblastic lymphoma of presumed thymic origin. Peripheral T cell lymphomas are thought to derive from peripheral T lymphocytes in lymph nodes and other nonlymphoid sites. These lymphomas comprise a broad spectrum of morphologic types of lymphocytes. In all instances, the cells have T cell markers admixed with epithelioid histiocytes, plasma cells, eosinophils, and vascular hypertrophy. Clinically, peripheral T cell lymphoma is characterized by generalized lymphadenopathy, weight loss, and a high incidence of pulmonary involvement.219 Surface markers are usually but not always characteristic of mature TH helper cells,222 including the T4/Leu-3 helper-associated antigen and the T3/Leu-4, T11/Leu-5, and T1/Leu-1 pan-T antigens. Rearrangement of the Tβ receptor has been reported.226

**Tγ lymphoproliferative disease.** Tγ lymphocytes are a subset of T lymphocytes with receptors for the Fc portion of IgG. A high proportion of normal Tγ lymphocytes are LGL. These cells are thought to be responsible for natural killer (NK) and antibody-dependent cell-mediated cytotoxicity in humans233 and rodents.234 A lymphoproliferative disorder made up of predominantly Tγ lymphocytes has been described; we refer to this as chronic Tγ lymphoproliferative disease.235 Typically, patients are elderly males with increased Tγ lymphocytes infiltrating the bone marrow and spleen.235,236 Although the disease is not rapidly progressive, neutropenia and recurrent infections are common. Most patients do not require chemotherapy. Variants of this disease, including a more aggressive form, have been described.237 Clonal chromosomal abnormalities,238 as well as clonal rearrangement of the Tγ receptor, have been reported.175,239 Cells from chronic Tγ lymphoproliferative disease usually contain acid phosphatase and β-glucuronidase and express the Tα antigens T3/Leu-4, T11/Leu-5, the suppressor-associated antigens T8/Leu-2, and the NK-associated antigen Leu-7 (HNK-1). Other monoclonal antibodies that react with LGL may also prove to be useful.

**Cutaneous T cell lymphoma (mycosis fungoides, Sézary cell leukemia).** Skin lesions are the most prominent feature of patients with cutaneous T cell lymphoma.242 Lesions vary from limited plaques to diffuse generalized plaques, tumors, and generalized erythroderma. Rare patients with limited plaque disease and <50% with generalized plaques and tumors have extracutaneous disease detected by light microscopy. Cutaneous T cell lymphomas comprise a broad spectrum of morphologic types of lymphocytes. In all instances, the cells have T cell markers admixed with epithelioid histiocytes, plasma cells, eosinophils, and vascular hypertrophy. Clinically, peripheral T cell lymphoma is characterized by generalized lymphadenopathy, weight loss, and a high incidence of pulmonary involvement.219 Surface markers are usually but not always characteristic of mature T helper cells,222 including the T4/Leu-3 helper-associated antigen and the T3/Leu-4, T11/Leu-5, and T1/Leu-1 pan-T antigens. Rearrangement of the Tβ receptor has been reported.226

The malignant cells in this disorder are characterized by a cerebriform nucleus. In the skin, the cells are referred to as mycosis fungoides cells and in the peripheral blood as Sézary cells. Sézary and mycosis cells form E rosettes, react with T
antisera and anti-T monoclonal antibodies, and have clonal rearrangements of the Tβ receptor. In most cases, the cells express the phenotype associated with normal helper/inducer T lymphocytes (T-1/Leu-1, T3/Leu-4, T4/Leu-3 positive) and function as helper T lymphocytes in vitro assays. The 3A1 antibody (CD7), which reacts with $>85\%$ of normal circulating T lymphocytes and with mycosis cells in the skin, does not generally react with Sézary cells in the blood.

**Adult T cell leukemia/lymphoma.** Adult T cell leukemia/lymphoma is associated with a human retrovirus designated human T cell leukemia/lymphoma virus-1 (HTLV-1). Virtually all patients tested have antibodies to HTLV-1. Patients with this disease have been identified primarily in Japan, the United States, and the Caribbean. In the United States, the patients are young (median age 33 years), predominantly black, and born in the southeast. Common clinical features include a rapid onset of symptoms with rapidly progressive cutaneous lesions and hypercalcemia. Skin lesions are variable and include small and large discrete or confluent nodules, or nonspecific plaques, papules, or patches. Patients have increased bony turnover with abnormal bone scans and elevated alkaline phosphatase and may have lytic bone lesions. Lymphocytosis is common, and circulating malignant cells are present in low numbers in most patients. Peripheral lymphadenopathy is common, with retroperitoneal and hilar involvement in approximately 50% of cases. Bone marrow, gastrointestinal, pulmonary, leptomeningeal, and hepatic involvement are somewhat less common (20% to 50%). Response to combination chemotherapy is prompt and often complete, but duration of response is short (median 13 months). Opportunistic infections are extremely common in these patients.

The typical malignant circulating cells have moderately condensed nuclear chromatin, inconspicuous nucleoli, and a markedly irregular nuclear contour in which the nucleus is divided into several lobes. These cells typically express the phenotype of helper/inducer T lymphocytes and the Tac antigen (CD25) that identifies the IL-2 receptor. Variability in the expression of T3, T11, and T12 have been reported. Clonal rearrangements of the Tβ receptor are identified in cells from patients with adult T cell leukemia/lymphoma. The leukemic cells are reported to suppress B cell Ig secretion by a complex mechanism involving induction of suppressor cells following activation of normal suppressor cell precursors.

**CLL and prolymphocytic leukemia.** CLL is a monoclonal proliferation of SmIg-positive B lymphocytes. Clonality of CLL has been demonstrated by expression of a single Ig light chain, either κ or λ, on the cell surface membrane. More sophisticated techniques have confirmed clonality by showing unique immunoglobulin idiotype specificities, a single pattern of glucose-6-phosphate dehydrogenase activity, clonal chromosome abnormalities, or immunoglobulin gene rearrangement. The malignant B cell involved in CLL is an intermediatedly differentiated cell. The cell appears frozen in differentiation and does not mature to the final stage of B cell development, the mature plasma cell. However, recent data have demonstrated that in vitro treatment of these cells with phorbol esters or pokeweed mitogen can induce differentiation into mature immunoglobulin-secreting plasma cells. In another study, this immunoglobulin secretion was preceded by a rapid increase in the level of mRNA coding for IgM, a predominantly secretory form of mRNA rather than a membrane form of mRNA. This selection is similar to that seen in plasma cells, and the study clearly demonstrated at the molecular level that CLL cells consistently retain the capacity to differentiate to plasma cells and secrete immunoglobulin. Under certain circumstances, CLL cells stimulated in vitro with phorbol esters differentiate into cells with cytoplasmic protrusions and other characteristics of hairy cell leukemia.

The B lymphocyte characteristic of CLL displays a relatively small amount of SmIg, estimated to be ~9,000 molecules per cell. Relatively weak fluorescence of SmIg has been used to distinguish CLL from the leukemic phase of nodular and diffuse lymphocytic lymphomas and from prolymphocytic leukemia in which the cells generally display considerably more SmIg. Immunoglobulin isotype analyses indicate that most CLL display a single heavy chain class; typically, μ or μ and δ. Less commonly, γ, α, or no heavy chain determinant is found. CLL cells display either κ or λ light chains but never both. Some data suggest that heavy chain switching can occur in B-CLL, which may indicate increasing maturity of the malignant cell. Other studies indicate that CLL cells contain only μ or μ and δ and that γ is extrinsic and not synthesized by the leukemic cell. Although there has been controversy as to whether CLL B cells contain C1g, the presence of cytoplasmic heavy chains (μ and δ) has been reported in most patients with CLL; no γ or α chains were detected. B-CLL cells display receptors for mouse erythrocytes, a feature characteristic of immature B lymphocytes. The cells also have the receptor for the Fc portion of IgG and complement with a relative increase of C3d receptors (CR2) over C3b receptors (CR1); this is typical of immature B cells. B-CLL cells display several antigens, including Ia and human B cell antigens such as BA1, B1, B2, and B4. One unanticipated finding was that B-CLL cells display a 65-kd glycoprotein antigen previously thought to be restricted to T lymphocytes. This antigen was first recognized by using heteroantisera, and later, it was recognized with the T101 and equivalent monoclonal antibodies. The precise meaning of this anomalous expression of a T cell antigen is unclear, although a normal B cell counterpart has been reported in human tonsil lymph nodes, and stimulation in vitro of normal B cells with phorbol ester may induce expression of this antigen. Recently, the TQ1 antigen, reported to define the inducer of suppression within the T helper subset, was identified on 60 to 75 B-CLL patients' cells.

Rearrangement of immunoglobulin heavy and light chains has been reported as expected in B-CLL cells; however, rearrangement of the Tβ receptor has also been reported in ~10% of cases of B-CLL. This is analogous to the reported Tβ rearrangement in 25% of non-T (pre-B) ALL, and again emphasizes that immunoglobulin and Tβ receptor rearrangement alone are not adequate to assign lineage.

In 3% to 10% of patients with CLL, the disease may evolve
into a diffuse histiocytic lymphoma (Richter's syndrome). This is associated with loss of the TQ1 antigen. Most data suggest that this evolution involves transformed follicular center B cells rather than histiocytes or macrophages. Some transformations represent evolution of the malignant clone with expression of the same monoclonal immunoglobulin and karyotypic abnormality present in the original CLL clone. In other cases, the lymphoma cells have different markers and immunoglobulin gene rearrangements than those of the original CLL cells; these cases probably represent the concomitant development of a B cell lymphoma or a histiocytic malignancy in patients with CLL.

Prolymphocytic leukemia (PL) is related to CLL and is also likely to be derived from cells from the medullary cords of the lymph node. Immunoglobulin gene rearrangements of heavy and light chains have been reported. Patients with PL generally have extremely high blast counts and spenomegalgy but lack significant lymphadenopathy. Prolymphoblasts likely are activated cells and appear morphologically immature, with a fine lacy nuclear chromatin and one to two nucleoli; they may contain intracytoplasmic granules. These cells generally have higher density SmIg than do CLL cells; they have Ia and B4 antigens and may form rosettes with mouse erythrocytes. PL cells from 14 consecutive patients reacted with the FMC7 monoclonal antibody that recognizes an antigen found on one half of normal B lymphocytes, whereas cells from only 5 of 20 patients with CLL reacted with this antibody.

Approximately 5% of cases of CLL and PL result in a malignant proliferation of T rather than B cells. These cells react with T antisera and anti-T monoclonal antibodies reflecting the phenotypes of mature T lymphocytes; they lack SmIg and other B cell markers. Many of these patients have diffuse organ and skin involvement. T-CLL cells have been reported to have either helper or suppressor surface markers. One patient's cells rosetted with sheep erythrocytes expressed the Leu-2 (suppressor-associated) antigen and also had SmIg and Clg (IgM). Other instances in which the leukemia/lymphoma cells expressed characteristic features of both B and T lymphocytes have also been reported.

Hairy cell leukemia. Hairy cell leukemia (leukemic reticuloendotheliosis) is characterized by invasion of the bone marrow and spleen by morphologically distinct monocellular cells with "hairy" cytoplasmic projections. These cells usually contain an isoenzyme of acid phosphatase (isoenzyme 5) that is resistant to tartrate; this isoenzyme is not unique to hairy cells. Surface markers of hairy cells are most consistent with a monoclonal proliferation of B lymphocytes. SmIg with a single light chain is frequently identified, as are B cell-associated antigens including Ia, B1, FMC-1, FMC-7, and sometimes BA-1. The PCA-1 antigen (but not the PC-1 antigen) typically on plasma cells is identified on hairy cells; these data suggest that hairy cells may be pre-plasma cells. Perhaps the most convincing evidence for the B cell origin of hairy cells comes from studies of immunoglobulin genes which indicate clonal rearrangement of heavy chain genes and at least one light chain gene. Most cases of hairy cell leukemia demonstrate the
Fig 2. Schematic representation of human lymphoid differentiation and related lymphoid malignancies. ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; SL, malignant lymphoma, small lymphocytic; FSC, malignant lymphoma, follicular small cleaved cell; FLg, malignant lymphoma, follicular large cell; DSC, malignant lymphoma, diffuse small cleaved cell; DLg, malignant lymphoma, diffuse large cell; PL, prolymphocytic leukemia; LL, lymphoblastic lymphoma; Tγ-LPD, Tγ-lymphoproliferative disease; ATL, adult T cell leukemia/lymphoma; CTCL, cutaneous T cell lymphoma; PTCL, peripheral T cell lymphoma; TdT, terminal deoxynucleotidy transferase; H, heavy chain; L, light chain; O, germline configuration; R, rearranged gene; Tp, clonal rearrangement of the Tp receptor; MR, mouse rosette; CR, complement receptor.
most malignant lymphoid cells reflect the phenotype of a normal lymphocyte. The proposed phenotype of the progenitor B lymphocyte probably has the same surface markers as the group I non-T-ALL and represents the earliest identifiable B cell. This cell expresses the Ia antigen, but no other B cell-associated antigens. The next level of B cell differentiation coincides with the group II non-T-ALL; heavy but not light chain immunoglobulin genes are rearranged. At the next level of B cell differentiation, the cells express CALLA and light chain gene rearrangements occur; this coincides with group III non-T-ALL. With sequential steps in B cell differentiation, the B1 antigen is expressed, followed by C4 and then SmIg. At the next level of B cell differentiation, the B cell acquires the B2 antigen and the receptor for mouse erythrocytes; both SmIg and Clg are present. Most CLL cells—and malignant lymphoma, small lymphocytic type cells—express the phenotype of intermediate B lymphocytes. The cells express receptors for complement and the Fe portion of IgG, Leu-1, in addition to the surface markers identified on more primitive B cells. At this level of differentiation, there is low-density SmIg. The maturing B cells express high-density SmIg (IgM, IgG, or IgA) without B2 or mouse erythrocyte receptors. The malignant counterparts of the mature B cell are the follicular small cleaved and large cell lymphomas and the diffuse small cleaved and large cell lymphomas and PL cells. At the next step of maturation, the plasmacytoid B cell secretes Ig, usually of the IgM subclass, and expresses new surface membrane antigens including OKT10, PCA-i, and PC-1. It has recently been demonstrated that hairy cell leukemia falls somewhere between the mature B cell and plasma B cell; they also express the PCA-1 antigen. The plasma cell, the most differentiated B lymphocyte, expresses the same phenotype as myeloma cells. Although these cells have Clg, produce immunoglobulin, and express OKT10, PC-1, and PCA-1, they lose other surface membrane markers, including SmIg, Ia, and B cell antigens.

T cell differentiation follows a distinct pathway. Early thymocytes (stage I) express Leu-9, Leu-1, T9, T10, and often T11/Leu-5 (sheep erythrocyte receptor); this phenotype probably represents the malignant counterpart of group I T-ALL cells. A case has been made that the stage I thymocyte has not yet rearranged the Tβ receptor gene; this remains to be confirmed. The common thymocyte (stage II) no longer expresses T9; it gains T6 antigen and simultaneously expresses the helper-associated (T4/Leu-3) and suppressor-associated antigens (T8/Leu-2). This cell clearly rearranges the Tβ gene, confirming that Tβ rearrangement precedes surface membrane expression of the T3–Ti complex. This cell corresponds to the phenotype of group II T-ALL and some lymphoblastic lymphomas. Subsequently, the cells lose either the helper-associated or suppressor-associated antigens. This is equivalent to group III T-ALL or some cases of lymphoblastic lymphoma. In the final stage of maturation, the suppressor-associated cell (T8/Leu-2) may express the receptor for the Fe portion of IgG as well as the surface markers previously attributed to the mature thymocyte (including the T3–Ti complex), coinciding with the phenotype of some T-CLL cells and chronic Tγ lymphoproliferative disease. The helper-associated mature T lymphocyte (T4/Leu-3), on the other hand, may express the receptor for the Fc portion of IgM and coincides with the phenotypes of some T-CLL, adult T cell leukemia/lymphoma, cutaneous T cell lymphoma, and peripheral T cell lymphoma.

CLASSIFICATION OF NONLYMPHOID LEUKEMIAS AND LYMPHOMAS

Hodgkin’s disease. Hodgkin’s disease (HD) is a malignant neoplasm of uncertain cellular origin characterized by the appearance of distinctive binucleate or multinucleate giant cells (Reed-Sternberg cells, RSCs) and their mononuclear variants (Hodgkin’s cells, HCs). The malignant nature of this disease is suggested by cytogenetic studies that have shown a clonal distribution of chromosomal aneuploidy. Considerable debate has arisen as to what constitutes the malignant cell of HD. However, most investigators now agree that the RSCs or HCs (a subset constituting a minute fraction of the tumor mass) represent the neoplastic cell population. The normal cellular counterpart from which RSCs and HCs arise has not yet been identified.

Investigators have used morphology (light and electron microscopy), cell culture, and immunohistochemistry in an attempt to characterize the nature of the RSCs and HCs. Based on these observations, it has been argued that HD arises from the T lymphoid, B lymphoid, or myeloid-macrophage lineages. Although this controversy is unresolved, the application of immunologic marker analysis has contributed to our further understanding of the disease, and several general statements can be made. First, with few exceptions, most observers have failed to detect the uniform expression of T cell surface markers (as defined by polyclonal and monoclonal reagents) by RSCs or HCs, suggesting that these cells are not of T lymphocyte origin. RSCs and HCs have been shown to express the Tac antigen (IL-2 receptor), in two reports, these cells were found to be T9 positive (transferrin receptor). Neither receptor-associated marker is restricted to the T cell lineage. Second, although the detection of SmIg or Clg in RSCs and HCs favors a B cell origin, the expression of these determinants is often polyclonal, which suggests that immunoglobin is adsorbed onto RSC and HC cells rather than being synthesized by the malignant cell. There are no convincing data that RSCs or HCs produce immunoglobin. Immunologic staining of RSCs and HCs for the expression of B cell differentiation antigens has produced conflicting results. In an interesting case of B cell HD, a patient with nodular sclerosing HD developed a terminal leukemic phase. The circulating HCs expressed the B1 and B4 antigens and had cytoplasmic μ heavy chains and a clonal rearrangement of heavy and light chains (consistent with a B cell origin). Substantial data, however, favor a myeloid-macrophage origin for HD. This conclusion is based on the demonstration of nonspecific esterase (NSE) and acid phosphatase, α-1-antitrypsin and α-1-antichymotrypsin, muramidase, lectin-binding properties, and the variable expression of Fc and C3 receptors on...
RSC and HC cells. Although short-term cell lines believed to be derived from RSCs demonstrate weak phagocytic activity, and one line was reported to synthesize IL-1, other established cell lines have not uniformly shown these activities. In most instances, RSCs and HCs do not react with antibodies to monocytes, although in one report a substantial number of biopsy specimens contained RSC and HC positive for markers characteristic of late granulocytic maturation (TU5, TU6, TU9). Based on Ia expression and characteristic cytotoxic features, other authors have suggested that the cell of origin for HD is a "reticulum cell" (either a dendritic cell or an interdigitating reticulum cell). Finally, some data suggest that the RSCs and HCs represent a subset of activated lymphoid cells of either T or B lymphoid origin. This conclusion is based on an immunologic analysis in which RSC and HC uniformly expressed the Ki-1 marker (35 of 35 biopsy specimens of all histological subtypes) as defined by a monoclonal antibody raised by immunization against an established HD cell line. Among normal cells, Ki-1 is expressed by T and B lymphocytes activated in vitro by various stimuli that also induce interleukin-2 (IL-2) receptor expression. In situ staining of biopsy specimens from nonneoplastic and reactive tissues demonstrated Ki-1 expression by a population of normal perifollicular lymphoid cells (lymph node and spleen) and variable degrees of expression by abnormal lymphoid cells in cases of angioimmunoblastic lymphadenopathy and lymphoid papulosis. Among 290 cases of non-Hodgkin’s lymphoma, Ki-1 expression was observed in 19 cases of peripheral T cell lymphoma and in 45 cases of diffuse large cell lymphoma (including 35 specimens expressing T cell surface markers and 7 bearing B cell antigens). These results suggest that Ki-1 is a lymphoid activation antigen that identifies a group of large lymphoid cells in normal and neoplastic tissues (including RSCs) that remains poorly characterized. Another monoclonal reagent, HeFi-1, is similar if not identical to Ki-1.

In summary, the cellular origin for HD remains unclear. Although Ia and T9 antigen staining of RSC and HC cells have been reported, Ki-1 antigen expression may prove to be the most useful immunologic marker for this disease. RSCs and HCs constitute only a small portion of cells within the tissue of Hodgkin’s disease. Recent efforts are directed toward characterizing the remaining cells and have been recently reviewed. Use of in situ techniques has demonstrated that lymphoid tissues involved with HD appear to be heterogeneous in immunohistologic make-up; some cases demonstrate numerous T lymphocytes with few B cells, whereas others exhibit prominent follicles of polyclonal B lymphocytes and only small numbers of T cells within these follicles. In two studies, these B cell-rich cases were of the lymphocyte-predominant type. In specimens containing T lymphocytes, RSCs and HCs tend to appear in areas of heaviest T cell infiltration, suggesting a relationship. Many cells within areas of T cell infiltration are Ia or T10 positive, suggesting that these T lymphocytes are activated. Several investigators have demonstrated that most HD-associated T lymphocytes are of the helper cell subset. Genotyping for immunoglobulin and T cell receptor rearrangements may lead to a better understanding of the cellular origin of this disease.

Malignant disorders of macrophages. Several malignant diseases of macrophages (or histiocytes) have been described. The term histiocytic lymphoma, used in the Rappaport classification, encompasses a heterogeneous group of neoplasms of large transformed lymphocytes and, rarely, of macrophages.

The malignancies of macrophages (histiocytosis X) are heterogeneous. Clinical presentations include solitary benign eosinophilic granuloma, Hand-Schuller-Christian disease, and histiocytic medullary reticulosis; the latter is a generalized systemic disorder characterized by fever, wasting, hepatosplenomegaly, variable lymphadenopathy, and progressive pancytopenia due to diffuse tissue invasion by malignant macrophages. The equivalent disease in children is sometimes referred to as Letterer-Siwe disease. Because of morphologic and ultrastructural similarities between malignant macrophages and epidermal Langerhans cells, it has been proposed that these diseases represent a proliferative disorder of Langerhans cells. Both cell types possess receptors for C3 and Fc portion of IgG and express the T6 and Ia antigens. The malignant macrophages also express the OKM1 and other macrophage surface markers. An unexpected and unexplained finding was the presence of the T4 antigen in this disorder.

AML. AML is a clonal malignancy of myeloid progenitor cells resulting in excessive proliferation and accumulation of immature hematopoietic elements. The subtypes of this disease are generally classified according to the morphologic similarity of the leukemic cell population to normal myeloid precursors, eg, acute myeloblastic leukemia, acute promyelocytic leukemia, acute monoblastic leukemia, and acute erythroleukemia.

Monoclonal antibodies have been evaluated for reactivity against AML cells (Tables 9 and 10). In all cases, these antibodies identify determinants expressed by either normal circulating myeloid cells or bone marrow progenitors. None of these reagents recognizes a leukemia-specific determinant and, with few possible exceptions, attempts to generate leukemia-specific antisera have been unsuccessful. Studies of the reactivity of antilymphoid cell antibodies for AML cells have raised several issues: (a) whether these reagents are specific in their reactivity for myeloid v lymphoid leukemia cells; (b) whether antibody reactivity correlates with classification using the FAB nomenclature; (c) whether surface marker expression by myeloid leukemia cells corresponds to stages of normal myeloid differentiation and, if so, whether this is of prognostic significance; (d) whether myeloid leukemia cells in a patient are homogeneous in their expression of surface markers; (e) whether leukemia progenitor cells defined by their ability to form leukemic colonies in vitro exhibit the same antigenic phenotype as their progeny in bone marrow and blood; and (f) whether monoclonal antibodies that identify antigens expressed by myeloid leukemia cells can be used for immunotherapy.

AML v the lymphoid leukemias. Because of differences in prognosis and therapy, it is important to distinguish between AML and the lymphoid leukemias. Although differ-
CLASSIFICATION OF LEUKEMIA AND LYMPHOMA

Table 9. Use of Myeloid Surface Markers to Discriminate Between Acute Myeloid and Lymphoid Leukemia

<table>
<thead>
<tr>
<th>Monoclonal Antibody</th>
<th>AML</th>
<th>CML (MBC)</th>
<th>Total Myeloid</th>
<th>ALL</th>
<th>CML (LBC)</th>
<th>Total Lymphoid</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo1/OKM1</td>
<td>62 (228)*</td>
<td>57 (23)</td>
<td>62 (251)</td>
<td>0 (82)</td>
<td>0 (11)</td>
<td>0 (82)</td>
<td>66,106,118,124,389,410</td>
</tr>
<tr>
<td>MY7</td>
<td>76 (97)</td>
<td>92 (13)</td>
<td>78 (110)</td>
<td>1 (109)</td>
<td>0 (13)</td>
<td>1 (122)</td>
<td>118,119,419</td>
</tr>
<tr>
<td>MY8</td>
<td>53 (73)</td>
<td>7 (15)</td>
<td>45 (88)</td>
<td>0 (82)</td>
<td>0 (11)</td>
<td>0 (83)</td>
<td>118,119,419</td>
</tr>
<tr>
<td>MY9</td>
<td>85 (97)</td>
<td>92 (13)</td>
<td>85 (110)</td>
<td>2 (109)</td>
<td>0 (13)</td>
<td>2 (122)</td>
<td>70</td>
</tr>
<tr>
<td>VIM-2</td>
<td>91 (66)</td>
<td>93 (30)</td>
<td>92 (96)</td>
<td>5 (60)</td>
<td>0 (11)</td>
<td>4 (71)</td>
<td>105</td>
</tr>
<tr>
<td>VIM-D5</td>
<td>68 (116)</td>
<td>88 (8)</td>
<td>69 (174)</td>
<td>2 (88)</td>
<td>NR</td>
<td>2 (88)</td>
<td>389,418</td>
</tr>
</tbody>
</table>

MBC, myeloid blast crisis; LBC, lymphoid blast crisis.

*Percentage of patients positive (total number of patients tested). A patient is considered positive for a given marker if >20% of malignant cells bind the monoclonal antibody.

ences in morphology and histochemistry often lead to the correct diagnosis, the distinction between immature variants of AML and ALL is not always evident. Monoclonal reagents that identify antigens expressed by myeloid but not lymphoid leukemias (or vice versa) would therefore be important. Six monoclonal antibodies have been extensively tested for reactivity to myeloid and lymphoid leukemia (Table 9). Each of the six antigenic determinants defined by these antibodies (Mo1/OKM1, MY7, MY8, MY9, VIM-2, and VIM-D5) is expressed by more than one half of patients with AML (53% to 91%), defined as antibody binding by >10% to 20% of malignant cells in each patient. The myeloblasts of patients with myeloid blast crisis of CML demonstrate similar frequencies of expression for these determinants except for MY8. Conversely, expression of these antigens on acute lymphoid leukemia cells (including the T and B cell variants of ALL and chronic myelogenous leukemia (CML) lymphoid blast crisis) is rare. Clearly, these monoclonal reagents can complement other tests in the differential diagnosis of AML versus ALL. The accuracy of immunologic diagnosis can be extended by using more than one antimyeloid reagent in conjunction with antibodies that detect antigenic determinants uniquely expressed by B or T lymphoid leukemias (anti-CALLA, B1, B4, OKT3, Leu-4, etc.) (see above). Occasionally, however, this approach has produced seemingly disparate results, with the detection of leukemia cells with myeloid and lymphoid differentiation markers.374,376 These rare situations may reflect the existence of biopotential clones of malignant cells expressing features of more than one lineage.377

Correlation between surface marker phenotype and FAB classification. There are several types of AML differ in morphology, histochemistry, and surface marker expression. Classifications have been proposed to identify these types, based on the hypothesis that this information may be of prognostic and therapeutic significance. The FAB group classification, which relates the morphologic appearance of leukemic cells to presumed normal hematopoietic counterparts, is widely used.65,186,379 Seven subtypes of AML (M1 through M7) are identified: M1 and M2 represent undiff-

Table 10. Correlation Between Myeloid Surface Marker Expression and FAB Classification System

<table>
<thead>
<tr>
<th>Monoclonal Antibody</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
<th>References</th>
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<tbody>
<tr>
<td>VIM-2</td>
<td>73 (15)†</td>
<td>96 (23)</td>
<td>83 (6)</td>
<td>100 (16)</td>
<td>100 (6)</td>
<td>106</td>
</tr>
<tr>
<td>R1B19</td>
<td>40 (10)</td>
<td>44 (8)</td>
<td>13 (8)</td>
<td>48 (27)</td>
<td>38 (8)</td>
<td>67,86</td>
</tr>
<tr>
<td>S4-7</td>
<td>50 (10)</td>
<td>56 (9)</td>
<td>25 (8)</td>
<td>70 (27)</td>
<td>78 (9)</td>
<td>67,88</td>
</tr>
<tr>
<td>PM 81</td>
<td>90 (10)</td>
<td>50 (2)</td>
<td>86 (7)</td>
<td>100 (3)</td>
<td>97,122</td>
<td></td>
</tr>
<tr>
<td>MY9</td>
<td>85 (54)‡</td>
<td>100 (6)</td>
<td>81 (31)</td>
<td>83 (6)</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>MY7</td>
<td>78 (54)</td>
<td>67 (6)</td>
<td>81 (31)</td>
<td>50 (6)</td>
<td>118,119</td>
<td></td>
</tr>
<tr>
<td>Mo5</td>
<td>50 (38)</td>
<td>74 (27)‡</td>
<td>96</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOP9</td>
<td>17 (6)</td>
<td>13 (8)</td>
<td>0 (3)</td>
<td>100 (7)</td>
<td>100 (10)</td>
<td>389</td>
</tr>
<tr>
<td>AML-2-23</td>
<td>0 (12)</td>
<td>0 (2)</td>
<td>0 (3)</td>
<td>100 (7)</td>
<td>100 (10)</td>
<td>121,122</td>
</tr>
<tr>
<td>MY4</td>
<td>25 (36)</td>
<td>0 (3)</td>
<td>52 (25)</td>
<td>100 (6)</td>
<td>119</td>
<td></td>
</tr>
<tr>
<td>UCHM1</td>
<td>6 (17)</td>
<td>92 (24)</td>
<td>100 (16)</td>
<td>66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MY8</td>
<td>36 (39)</td>
<td>33 (3)</td>
<td>76 (25)</td>
<td>83 (6)</td>
<td>118,119</td>
<td></td>
</tr>
<tr>
<td>Mo1/OKM1</td>
<td>39 (124)</td>
<td>100 (3)</td>
<td>91 (101)</td>
<td>66,106,118,124,389</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mo2</td>
<td>14 (65)</td>
<td>45 (31)</td>
<td>124</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIM-D5</td>
<td>29 (38)</td>
<td>71 (52)</td>
<td>67 (12)</td>
<td>88 (40)</td>
<td>92 (24)</td>
<td>389,418</td>
</tr>
<tr>
<td>82H5</td>
<td>0 (5)</td>
<td>69 (13)</td>
<td>100 (4)</td>
<td>100 (6)</td>
<td>100 (7)</td>
<td>83</td>
</tr>
</tbody>
</table>

*Antigen expression by peripheral blood monocytes (M) or neutrophils (N).
†Percentage of patients positive (total number of patients tested).
‡Patients with M1 and M2 or M4 and M5 leukemia combined.
entiated and differentiated myeloblastic leukemia; M3, promyelocytic leukemia; M4 and M5, myelomonocytic and monocytic variants; M6, erythroleukemia; and M7, megakaryocytic leukemia. Although some investigators have reported brief remissions or lower response and survival rates in patients with the M5 variant, fewer remissions in erythroleukemia (M6), or longer remissions in promyelocytic leukemia (M3) these observations are controversial. With the development of immunologic reagents that detect antigenic markers expressed by normal and leukemic myeloid cells, analyses have been undertaken to compare the FAB system with patterns of surface marker expression. Table 10 indicates 16 monoclonal reagents whose relative reactivity against FAB-classified AML variants can be critically assessed. Within the first group of seven antibodies (VIM-2, R1B19, S4-7, PM81, MY9, MY7, and Mo5), no clear distinction exists in antigen expression by cells in each of the five FAB variants (M1-M5; too few patients with the M6 and M7 variants were examined to draw conclusions). The frequency of expression by patients in each subclass is generally 50%. In the second group of seven antibodies (MOP9, AML-2-23, MY4, UCMH1, MY8, Mo1/OKM1, and Mo2), there is a trend toward higher frequency of antigen expression among individuals whose leukemia cells display monocytic differentiation (M4 + M5). In the case of VIM-D5 and 82H5, only undifferentiated M1 cells have a lower frequency of expression. None of these reagents demonstrates preferential binding frequency to M1 and/or M2. One antibody, VIE-64, which binds to glycoporphin A, displays relative binding specificity toward M6 variant cells. Monoclonal antibody SFL 23.6 has a well-defined reactivity restricted to the erythroid lineage including erythroleukemia cell lines and should be useful in distinguishing M6. Monoclonal antibodies against platelet glycoproteins Ib, IIb/IIIa, and aIIa, for factor VIII-related antigen can be used to identify megakaryoblasts. With these possible exceptions, the degree of correlation between surface marker expression and the criteria for FAB classification is not convincing.

Subclassification of AML according to differentiation-associated phenotypes as identified by monoclonal antibodies. Because there is controversy over whether the FAB classification system provides prognostic information, alternative classifications have been proposed. In a surface marker analysis of 70 patients with AML, Griffin and co-workers identified four phenotypes based on patterns of surface antigen expression that correlated with phenotypes displayed by myeloid cells during normal differentiation. Group I AML cells (21% of patients) expressed the antigenic phenotype of the CFU-C-committed myeloid progenitor cell (Ia and MY7-positive); group II cells (26%) displayed the phenotypic characteristic of normal myeloblasts (MY7, Ia, and Mo1/OKM1 and My8-positive); group III cells (8%) had a phenotype featured by normal promyelocytes (MY7 and Mo1/MY8-positive; Ia-negative); and group IV cells (45%) with the phenotype of promonocytes and monocytes (MY4, MY7, MY8/Mo1, and Ia-positive). Within these four differentiation-related groups there was considerable morphologic heterogeneity: although all three of the pro-

myelocytic leukemia (M3) patients were in group III and all six monocytic leukemia (M5) patients were in group IV, the myeloid leukemia (M1 and M2) and myelomonocytic leukemia (M4) patients were dispersed throughout all four groups, with a tendency for myeloid patients to be in groups I, II, and III, and myelomonocytic patients to be in groups II and IV. The preliminary finding of a larger prospective analysis involving over 200 patients demonstrates significant differences among these phenotypic groups with respect to complete response rate and disease-free survival (J.D. Griffin, personal communication). Moreover, expression of certain markers appears to be of independent prognostic significance: AML patients with MY7-positive leukemia exhibit a worse prognosis than do MY7-negative patients; the expression of monocyte antigen MY4 is also predictive of a poor response. Several studies of the biological implications of surface marker phenotype in AML are in progress.

A scheme for myeloid differentiation is shown in Fig 1. In an attempt to account for the FAB M4 leukemic cell (bearing features of both granulocytic and monocytic differentiation), Ball and Fanger have proposed that the normal M4 counterpart is an intermediate bipotential precursor cell capable of differentiating along either the monocytic or granulocytic path of differentiation. They further suggest that the myeloblast (M1 and M2), the progenitor of the M4 cell, is likewise bipotential. Given the ability of the promyelocytic leukemia cell line HL-60 to undergo subsequent differentiation toward mature monocytes or neutrophils depending on the nature of the inducing stimulus, it appears that the normal promyelocyte is not irreversibly committed to granulocytic maturation. Although these hypotheses are consistent with some experimental observations, considerable additional data are required.

Heterogeneity of surface marker expression by malignant AML cells. Most studies of AML indicate considerable heterogeneity in leukemic cell surface marker expression between patients as well as within a given individual. Typically, a patient is classified as positive for the expression of a marker if >10% to 20% of the patient’s leukemia cells display the determinant. Although certain antigens tend to be expressed by >50% of the leukemic cells of given individuals, variability is considerable. If surface marker expression correlates with the level of myeloid differentiation, these data suggest that the leukemic population is heterogeneous.

AML likely arises from leukemic myeloid progenitor cells, which in some cases can be grown in vitro in semisolid medium. The clonogenic leukemia cells (L-CFC) are, by definition, capable of limited proliferation (with a subset capable of self-renewal), a feature that distinguishes them from most leukemia cells that are terminally differentiated. Several groups have recently investigated the surface marker characteristics of L-CFC and compared them with the total leukemia population. The surface-marker phenotype of the total leukemia population, as determined by immunofluorescence analysis, may not predict the phenotype of the L-CFC as measured by inhibition of L-CFC growth after antibody-dependent, complement-mediated lysis. In general, the L-CFC has a pattern of...
antigenic expression that is more "immature" than that of the predominant phenotype of the total population. L-CFC have been subclassified using multiple markers whose expression on normal CFU-GEMM (Ia, MY9, S3-13, S8-6), early (day 14) CFU-GM (Ia, MY9, PM-81, S3-13, S8-6, S4-7), and late (day 7) CFU-GM (all of the preceding markers plus AML-2-23 and R1B19) are known. Three phenotypically distinguishable levels of differentiation have been identified.114,394 The degree of maturity, as based on morphology (FAB classification) and expression of "later stage" antigens (beyond the CFU-GM: Mo1, MY3, Mo2) of the total leukemia population, tends to correlate with the L-CFC maturation level (eg, CFU-GEMM level L-CFC are associated with M1 morphology and lack expression of late antigens), suggesting a limited potential for terminal differentiation.114,395 These data suggest that L-CFC are a distinct subset of clonogenic cells among the total leukemia population; these cells may arise at multiple points along the pathway of early myeloid differentiation.

**CML.** CML is a myeloproliferative disorder characterized by a consistent chromosomal abnormality, the Philadelphia (Ph') chromosome. The Ph' chromosome results from a reciprocal translocation between chromosomes 9 and 22 designated t(9;22).396 This translocation results in the transfer of the c-abl oncogene from chromosome 9 to the Ph' chromosome and the variable reciprocal translocation of c-sti from chromosome 22 to 9.397 The target for leukemic transformation (Fig 3) appears to be at the level of the pluripotent stem cell, since the Ph' chromosome is present in all hematopoietic elements of patients with CML, including B and T cells.398-400 The clonal origin of CML is further indicated by analysis of patterns of expression of glucose-6-phosphate dehydrogenase (G6PD), and adenylate kinase isoenzyme.398,402 In the chronic phase of the disease, CML is characterized by an overproduction of relatively mature granulocytes. After a variable period of time, with a median of 3 years, most patients enter an acute phase (blast crisis) in which maturation no longer occurs. The acute phase resembles acute leukemia. Approximately one-third of patients with acute-phase CML demonstrate cells with lymphoid phenotype, including B and T cells.403-407 The cells of acute-phase CML involving myeloid cells is heterogeneous; typically the cells resemble myeloblasts, but erythroblasts, megakaryoblasts, and monoblasts can also be observed. Distinction between lymphoid and myeloid acute phase is important because patients with lymphoid blast crisis may respond to chemotherapy with vincristine (V) and prednisone (P).408,409,410 In making this diagnostic distinction, the characteristic expression of several myeloid markers (MY7, MY9, VIM-2, and Mo1/OKM1) on myeloid blast crisis cells and their lack of expression by lymphoid blast crisis cells (Table 9) provide information complementary to assays for the detection of CALLA, B1, TdT, and CD10.38,66,70,105,106,118,119,124,409,418,419

Surface marker analysis may allow further discrimination among the heterogeneous presentation of CML acute phase.

Four phenotypes were identified in 30 patients with this disorder based on antigen expression of normal myeloid, erythroid, megakaryocytic, and lymphoid cells.419 The cells of ten patients exhibited a phenotype corresponding to an immature myeloid cell (Ia, MY7, and Mo1-positive); all ten were negative for CALLA, B1, and TdT. These cases were felt to represent "myeloid" blast crisis; none of these patients responded to treatment with V and P. Cells from 11 patients expressed a phenotype corresponding to acute (early B) lymphoblastic leukemia cells (TdT, Ia, CALLA, and B1-positive; MY7 and Mo1-negative); six of nine evaluable patients had a complete response to V and P. Cells from one patient had the phenotype of erythroleukemia (glycoporphin A-positive); another patient’s cells expressed the phenotype of megakaryocytic leukemia (Plt-1-positive); one patient’s cells had features of both myeloid and lymphoid blasts on different cells. Cells from six patients did not express surface markers characteristic of any lineage; these were termed "undiifferentiated"; these cases were heterogeneous in expression of TdT; no complete responses to V and P were observed. Thus, in terms of response to V and P therapy, surface marker analysis provided useful prognostic information.

In another series of 45 patients with CML in blast crisis, 28 patients were classified as having "myeloid" blast crisis on the basis of reactivity with at least one of six antmyeloid monoclonal reagents (including VIM-D5 and VIM-2) and no surface expression of T or B cell immune markers or
TdT. Among these myeloid cases, however, only 11 patients expressed granulomonocytic antigens exclusively; the blast cells of 17 patients were additionally positive for platelet/megakaryocyte markers (16 cases) and/or erythroid determinants (three cases). Whether the same blast cell co-expressed myeloid, megakaryocytic, or erythroid antigens was not determined. Fourteen patients were classified as having “lymphoid” blast crisis with the phenotypic pattern of CALLA-positive ALL (10 cases), pre-B cell ALL (three cases), or “Null” ALL (one case). Two patients demonstrated a mixed myeloid and lymphoid blast cell phenotype, and a single patient was unclassifiable. Sixteen of these 45 patients were tested serially during the course of their illness and three demonstrated phenotypic changes. The immunological diagnosis of lymphoid blast crisis was associated with a higher rate of remission than was myeloid blast crisis (57% vs 4%, respectively) and a longer median survival.

Certain patients with the Ph1-chromosome are first diagnosed in the acute phase without a preceding history of a chronic phase. Either a chronic phase never existed or it was never detected. Surface-marker analysis may be as useful in the subclassification of these patients' cells as it is in the more typical acute phase that is preceded by a chronic phase.

**MONOCLONAL ANTIBODY THERAPY**

Several investigators have attempted to treat lymphoid or myeloid leukemias with monoclonal antibodies. In some studies, patients with advanced B cell-derived CLL received TIO1 monoclonal antibody. TIO1 could be safely infused and led to transient reductions in circulating leukemia cells; there was, however, no sustained effect on the bone marrow, involved lymph nodes, or other organs. This therapy resulted in some intravascular cell injury, but destruction in the spleen, liver, and lungs was probably more important. Similar results have been reported in patients with adult T cell leukemia/lymphoma, ALL, and AML treated with other monoclonal antibodies. Patients with cutaneous T cell lymphoma who received TIO1 or anti-Leu-1 have had only transient improvement in skin lesions and lymphadenopathy. Side effects of monoclonal antibody therapy are usually minor. Respiratory distress following the rapid infusion of monoclonal antibody has been described, and some patients have demonstrated transient elevation of serum creatinine and hepatic enzymes.

Monoclonal antibody therapy has several shortcomings that must be addressed. First, treatment with antibodies such as TIO1 results in modulation of the antigen from the cell surface, which prevents antibody binding to the tumor cells. The TIO1 antigen-antibody complex is pinocytosed into the cytoplasm, a phenomenon that might be advantageous when drugs or toxins are linked to the antibody to enhance its cytotoxicity. Antigen in the circulation poses another potential problem because it might prevent the antibody from reaching the tumor cells. Furthermore, murine antibodies can stimulate production of human anti-mouse antibodies which lead to antibody neutralization. This situation may be correctable by treatment with high initial doses of antibody (> 500 mg) or by simultaneous treatment with immunosuppressive drugs to induce tolerance. In addition, the heterogeneity of antigen expression of tumor cells may necessitate therapy with more than one antibody. Clearly, monoclonal antibody therapy for leukemia and lymphoma is in its earliest stages.

An interesting therapeutic approach with monoclonal antibodies involves the use of anti-idiotype monoclonal antibody reactive with the idiotype of the immunoglobulin on malignant B cells. Such an antibody is by definition specific for a patient's tumor cells. A patient with B cell lymphoma in an accelerated phase who was unresponsive to conventional therapies was treated with an IgG2 anti-idiotype monoclonal antibody. Following eight intravenous (i.v.) infusions, the patient entered a complete remission that has been sustained for > 3 years. Results were less impressive in other lymphoma patients treated with this approach with ~50% achieving short-lived partial remissions. We developed several monoclonal anti-idiotype antibodies to cells from patients with leukemia and lymphoma. The first patient to undergo treatment had advanced CLL. Sequential anti-idiotype monoclonal antibody therapy with IgG2a and IgG1 antibody provided no benefit. His therapy was limited because of circulating idiotype immunoglobulin that blocked the binding of the anti-idiotype antibody to the leukemia cells. We were able to reduce the circulating idiotype sufficiently with extensive plasmapheresis.

Although anti-idiotype antibody therapy remains an interesting area of investigation, its applicability is limited by patient specificity (ie, antibodies are “tailor-made” for a single patient) and the presence of antibody in the serum of many patients. Recent data indicate that some tumors are biclonal; this would require the use of more than one antibody. In addition, the tumor cell idiotype may be unstable due to somatic mutation within the immunoglobulin variable region genes.

A number of centers are studying toxin and drug conjugates with murine antibodies directed toward human tumors; clinical trials have just begun. Antisera and monoclonal antibodies conjugated to radionuclides for tumor imaging have been extensively studied; this subject was recently reviewed. We have used the TIO1 antibody conjugated to indium for imaging in 12 patients with cutaneous T cell lymphoma. Tumors as small as 0.5 cm have been localized; however, nonspecific uptake of the immunoconjugate in the liver and spleen has prevented critical evaluation of these organs. This difficulty has been partially circumvented by the administration of intracutaneous injections of the immunoconjugate which cause it to be carried via the lymphatics directly to lymph node sites of disease. This procedure does not, of course, facilitate visualization of extralymphatic disease.

Survival for patients with ALL following relapse has not improved over the past several years with chemotherapy drugs. Allogeneic bone marrow transplantation clearly leads to improved survival, but only 30% to 40% of patients have matched donors. An alternative method to allogeneic bone marrow transplantation would make use of monoclonal antibodies to cleanse autologous bone marrow prior to bone marrow transplantation. Patients who are in clinical remis-
sion are likely to have morphologically undetectable tumor cells in their bone marrow; these cells may be identified and destroyed in vitro by specific antibodies and complement or antibodies conjugated to toxins. In one recently reported study, patients with ALL in second or subsequent remission had their bone marrow treated with a mixture of the BA-1, BA-2, and BA-3 monoclonal antibodies and rabbit complement. All the patients were prepared for transplantation with cyclophosphamide and fractionated total body irradiation. Engraftment occurred in all the patients and 7 of the 23 patients were relapse-free from 6 to 32 months (median 21 months) posttransplantation. All but one of the deaths was caused by recurrent leukemia. The researchers concluded that autologous bone marrow transplantation using in vitro-treated marrow was safe, allowed engraftment, and resulted in prolonged survival in some patients with ALL in second or subsequent remission. Similar results have been reported for ALL patients treated with the J5 monoclonal antibody and complement. Relapse of leukemia in these patients may result from the inadequacy of the preparative regimen used to treat the patients prior to transplantation, inadequate removal by the in vitro treatment with monoclonal antibody and complement, or possibly to the lack of the putative graft vs leukemia effect described in allogeneic bone marrow transplantation. Even in allogeneic transplantation, in which the preparative regimens are identical to those of autologous transplantation, >50% of the ALL patients relapse, suggesting that an insufficient preparative regimen may be the factor leading to relapse in autologous transplantation as well.

In another study, patients with advanced B cell non-Hodgkin's lymphoma underwent in vitro bone marrow treatment with the anti-B1 antibody and complement. Ten of 17 patients are disease-free at a median follow-up of 22 months (L.M. Nadler, personal communication). Despite the presence of the B1 antigen on mature B cells, B cells recovered within the first few months after transplantation, suggesting that the normal B cell progenitor does not express the B1 antigen.

Another approach to cleansing bone marrow in vitro is the use of monoclonal antibodies conjugated to toxins. In one study, whole ricin was conjugated to the T101 and 3A1 antibodies. It was demonstrated that 95% of the tumor colonies were killed whereas 96% of bone marrow progenitor cells survived. Similar results were reported for a panel of anti-T cell monoclonal antibodies conjugated to intact ricin. Other investigators reported results of an immunotoxin synthesized with pokeweed antiviral protein and the B43 antibody directed against Burkitt lymphoma cells. Immunotoxins may prove to have advantages over antibody and complement; not all antibodies fix complement, and immunotoxins may have greater cytotoxic capability.

The use of monoclonal antibodies and antibody immunoconjugates in the treatment and radioimaging of cancer is in its infancy. Although much work must still be done to address the problems of monoclonal antibody therapies, studies in animal tumor models and humans have clearly demonstrated that antibodies alone or antibody conjugates can be safely administered with minimal adverse effects; in selected cases, these may have diagnostic and therapeutic value. Nonspecific localization of antibody in the reticuloendothelial system, host antibody response, and antigenic heterogeneity are major obstacles to safe and effective treatment with monoclonal antibodies. These issues are under investigation in animal models and humans. Although anti-idiotype antibodies are highly specific and have produced excellent responses in a small number of patients, problems such as biconality of some lymphomas, instability of the idiotype, and the difficulty of tailoring antibodies to individual patients clearly limit the role of anti-idiotype therapy. The utility of purging bone marrow in vitro with antibodies and complement (or antibodies coupled to toxins) is limited to only a few diseases. However, studies have demonstrated that tumor cells can be removed from the bone marrow following in vitro treatment with antibody and complement; treated bone marrow can successfully engraft, and a number of patients have remained disease-free for >2 years. Whether this is related to the in vitro treatment is unknown. This treatment may prove to be an important application of monoclonal antibody therapy, and it bypasses most of the problems associated with in vivo monoclonal antibody serotherapy. Perhaps the most important future role for monoclonal antibody therapy will be in patients with minimal disease in the "adjuvant" setting, in whom antibody conjugates may eliminate micrometastatic deposits of tumor cells. This remains to be addressed in controlled trials.

CONCLUSION

The application of hybridoma technology and the exciting discoveries in molecular biology over the past 10 years have led to major advances in our understanding of the cellular origin of leukemia and lymphoma and will likely lead to a better understanding of the etiology of these diseases. Utilizing these techniques, it is now possible to more accurately diagnose and classify these disorders, sometimes guiding therapeutic decisions. It is also possible to use molecular probes to detect minimal residual disease. In the future, monoclonal antibodies conjugated to isotopes, drugs, and/or toxins will likely have a role in the therapy of certain leukemias and lymphomas. We look forward to this exciting new era in cancer therapy and diagnosis.

ACKNOWLEDGMENT

The authors are grateful to Dr Robert Peter Gale for his critical review of this manuscript, and to Jerrie CeBulky for her skills, patience, and tenacity in typing this manuscript.

REFERENCES

cytes during early phases of differentiation. Proc Natl Acad Sci USA 74:4012, 1977


18. Zipf TF, Lauzon GJ, Longnecker BM: A monoclonal antibody detecting a 39,000 kDa molecule that is present on B lymphocytes and chronic lymphocytic leukemia cells but is rare on acute lymphoblastic leukemia blasts. J Immunol 131:3064, 1983


32. Royston I, Majda JA, Baird SM, Meserve BL, Griffiths JC: Human T cell antigens defined by monoclonal antibodies: The 65,000-dalton antigen of T cells (T65) is also found on chronic lymphocytic leukemia cells bearing surface immunoglobulin. J Immunol 125:725, 1980


36. Burns GF, Boyd AW, Beverley PCL: Two monoclonal anti-human T lymphocyte antibodies have similar biologic effects and recognize the same cell surface antigen. J Immunol 129:1451, 1982


CLASSIFICATION OF LEUKEMIA AND LYMPHOMA


47. Morishima Y, Kobayashi M, Yang SY, Collins NH, Hoffman MK, Dupont B: Functionally different T lymphocytic subpopulations determined by their sensitivity to complement-dependent cell lysis with the monoclonal antibody 4A. J Immunol 120:1091, 1982


64. Todd RF, van Aghoven A, Schlossman SF, Tchoror C: Structural analysis of differentiation antigens Mo1 and Mo2 on human monocytes. Hybridoma 1:329, 1982


112. Perussia B, Acuto O, Terhorst C, Faust J, Lazarus R, Fanning V, Trinchieri G: Human killer cells analyzed by B73.1, a monoclonal antibody blocking Fc receptor functions. II. Studies of


139. Jones NH, Borowitz MJ, Metzgar RS: Characterization and distribution of a 24,000 molecular weight antigen defined by a monoclonal antibody (DU-ALL-1) elicited to common acute lymphoblastic leukemia (cALL) cells. Leuk Res 6:449, 1982


CLASSIFICATION OF LEUKEMIA AND LYMPHOMA 23
147. Leder P: The genetics of antibody diversity. Sci Am 246:102, 1982


224. Rosen PI, Feinstein DI, Pattengale PK, Tindle BH, Wil-


CLASSIFICATION OF LEUKEMIA AND LYMPHOMA


297. Catovsky D, Petitit JE, Galetto J, Okas A, Galton DAG: The


345. Stuart AE, Volsen SG, Zola H: The reactivity of Reed-Sternberg cells with monoclonal antisera at thin section and ultrastructural levels. Pathology 14:1, 1981


386. Griffin JD, Surface marker analysis of acute myeloblastic leukemia, in Bloomfield CD (ed): Chronic and Acute Leukemia in Adults. Boston, Martinus Nijhoff, 1985, p 113


419. Griffin JD, Todd RF III, Ritz J, Nadler LM, Canellos GP,


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