Classifying Acute Leukemia by Immunophenotyping: A Combined FAB-Immunologic Classification of AML


A panel of commercially available monoclonal antibodies and five heteroantisera were used to distinguish and subtype 138 cases of acute leukemia (AL). The immunophenotype was compared with the French-American-British (FAB) classification obtained on the cases. The immunophenotype discriminated acute myelogenous leukemia (AML) from acute lymphoblastic leukemia (ALL) and recognized cases not distinguished by cytochemistry (22% of cases), and cases with separate populations of lymphoblasts and myeloblasts (one case). Using the immunologic panel and derived criteria to subtype AML, correspondence of the immunophenotype to the FAB subtypes M1, M2, M4, and M5 was possible in greater than 80% of cases. A combined classification of the immunophenotype and FAB morphology/cytochemistry was devised for AML subtyping. It is recommended that immunophenotyping should be done at least in all cases with negative or inconclusive cytochemistry. At present we suggest that until a “gold standard” for identifying leukemic subtypes is developed, the best method for typing acute leukemia is by using a combination of morphology, cytochemistry and immunophenotyping.

THE FRENCH-AMERICAN-BRITISH (FAB) classification of acute leukemia was proposed in 1976 to standardize the morphologic and cytochemical classification of acute leukemia so that comparisons could be made between institutions and between various trials. Romanowsky morphology and cytochemical stains were used, which included myeloperoxidase (MPO) and Sudan Black B (SBB) for granulocytic differentiation and nonspecific esterase (NSE) for monocytic differentiation. Initially nine subtypes were identified: six myeloid (M1 through M6) and three lymphoid (L1 through L3) but a further megakaryoblastic subtype M7 (as distinct from M7 [M1 + 2 + 4]) was added in 1985, though methods in addition to light microscopy were used for identification. In the interim various heteroantisera and monoclonal antibodies had been used to classify acute leukemia. Difficulties with FAB diagnostic criteria had also become apparent. The FAB investigators endeavored to improve their classification by introducing revisions in 1981 and in 1985; however, some difficulties remain in interpretation.17 Instituting an immunologic classification for comparative purposes had potential problems as different laboratories used various monoclonal antibodies and heteroantisera. Also, a myeloid subclassification corresponding to the FAB classification has not been well-defined using immunological reagents.

In this paper we describe how a panel of commercially available monoclonal antibodies and five heteroantisera were evaluated for distinguishing and subtyping acute leukemia cells. The purpose of our study was first to select from readily available immunologic reagents a useful panel of monoclonal antibodies and heteroantisera to classify acute leukemia so that comparisons could be made between various laboratories using similar reagents. Secondly, we wished to compare the immunophenotype obtained on each case with its corresponding FAB diagnosis. In particular we were interested in determining whether an immunophenotypic pattern corresponding with the FAB myeloid subtypes could be selected from the panel of monoclonal antibodies used. Our third aim was to derive, if possible, a combined FAB-immunological classification to subtype acute leukemia. In another paper we present a formal evaluation of the contribution of the immunophenotypic classification to the diagnostic concordance of leukemic subtypes.

MATERIAL AND METHODS

Morphologic, Cytochemical and Immunological Studies

The peripheral blood and bone marrows of 138 patients (122 adults, 16 children) with acute leukemia (AL) diagnosed over 2½ years, between December 1983 and June 1986, were tested for surface membrane, cytoplasmic, and nuclear antigens and were classified by the FAB Cooperative Group classification using Wright-stained smears and cytochemical stains. Cases of chronic myelogenous leukemia (CML) in blast crisis were not included in this study. The cytochemical stains used included periodic acid Schiff (PAS), SBB, MPO, nonspecific esterase-butyrate (NSE), and in some cases chloracetate esterase (CAE) and acid phosphatase (AP). According to the number of cells available, testing was done with the monoclonal antibodies and heteroantisera shown in Table 1 by using immunofluorescence microscopy. The monoclonal antibodies were selected for testing on the basis of previously reported type specificity. Some antibodies (AML2.23, PMN6, PMN29, FMC10, Leu M3, P1M1, and PLT1) were added as they became available.

FAB Classification

The FAB Cooperative Group classification with minor modification was used on the peripheral blood and bone marrow smears. The myeloid leukemias were subclassified according to the following criteria:

- **M1**: Predominance of myeloblasts with <10% granulocytic differentiation.

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Table 1. Antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Cluster Designation (CD Number)</th>
<th>Molecular Weight (kilodaltons, kd)</th>
<th>Specificity</th>
<th>Source</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leu 1</td>
<td>5</td>
<td>67</td>
<td>T lineage</td>
<td>Becton Dickinson</td>
<td>27</td>
</tr>
<tr>
<td>T3, T4, T6, T8, T11</td>
<td>3, 4, 1, 8, 2</td>
<td>19-29, 55, 45/12, 32, 50</td>
<td>T lineage</td>
<td>Coulter Immunology</td>
<td>28</td>
</tr>
<tr>
<td>OKT3</td>
<td>NA</td>
<td>90</td>
<td>T lineage</td>
<td>Orthodiagnostic</td>
<td>28</td>
</tr>
<tr>
<td>B1, B2, B4</td>
<td>20, 21, 19</td>
<td>35, 140, 95</td>
<td>B lineage</td>
<td>Coulter Immunology</td>
<td>12</td>
</tr>
<tr>
<td>BA1, BA2</td>
<td>24, 9</td>
<td>45/65/65, 24</td>
<td>B lineage</td>
<td>Hybritech</td>
<td>29</td>
</tr>
<tr>
<td>J5</td>
<td>10</td>
<td>100</td>
<td>Cell</td>
<td>Coulter Immunology</td>
<td>7, 12</td>
</tr>
<tr>
<td>HLA-DR (Ia)</td>
<td>NA</td>
<td>29-34</td>
<td>B lineage, monocytic, myeloblast</td>
<td>Becton Dickinson</td>
<td>30</td>
</tr>
<tr>
<td>Leu M1</td>
<td>NA</td>
<td>NA (x-hapten)</td>
<td>Granulocytic, monocytic</td>
<td>Becton Dickinson</td>
<td>31</td>
</tr>
<tr>
<td>My7, My9, Mol</td>
<td>w13, NA, 11</td>
<td>160, 68-74, 155/94</td>
<td>Granulocytic, monocytic</td>
<td>Coulter Immunology</td>
<td>10, 32, 37</td>
</tr>
<tr>
<td>My4, Mo2</td>
<td>w14, w14</td>
<td>55, 55</td>
<td>Monocytic</td>
<td>Coulter Immunology</td>
<td>10, 33</td>
</tr>
<tr>
<td>AML 2.23</td>
<td>NA</td>
<td>NA</td>
<td>Granulocytic, monocytic</td>
<td>Hybritech</td>
<td>9</td>
</tr>
<tr>
<td>Leu M3</td>
<td>NA</td>
<td>NA</td>
<td>Monocytic</td>
<td>Becton Dickinson</td>
<td>11</td>
</tr>
<tr>
<td>D5D6(CAML 1)</td>
<td>NA</td>
<td>NA</td>
<td>Granulocytic, monocytic</td>
<td>UCLA Tissue Typing Lab</td>
<td>18</td>
</tr>
<tr>
<td>PMN 6, PMN 29</td>
<td>NA</td>
<td>NA, NA, NA</td>
<td>Granulocytic</td>
<td>Hybritech</td>
<td>9</td>
</tr>
<tr>
<td>FMC10</td>
<td>15</td>
<td>NA</td>
<td>Granulocytic</td>
<td>AMD-Cedarlane</td>
<td>34</td>
</tr>
<tr>
<td>PLT1</td>
<td>NA</td>
<td>NA</td>
<td>pl. GP l/b/ll/a mega-karyocytic</td>
<td>AMD-Cedarlane</td>
<td>35, 36</td>
</tr>
<tr>
<td>FVIII R</td>
<td>NA</td>
<td>NA</td>
<td>Megakaryocytic</td>
<td>Coulter Immunology</td>
<td>37</td>
</tr>
<tr>
<td>EMcAb*</td>
<td>NA</td>
<td>NA</td>
<td>Megakaryocytic</td>
<td>Cappel</td>
<td>36, 38</td>
</tr>
<tr>
<td>TdT†</td>
<td>NA</td>
<td>NA</td>
<td>Erythroid/Lymphoid</td>
<td>B.S. Clarke</td>
<td>39</td>
</tr>
<tr>
<td>Immunoglobulin†, μ chain, kappa and lambda light chain</td>
<td>NA</td>
<td>NA</td>
<td>Lymphoid</td>
<td>Pharmacia</td>
<td>39</td>
</tr>
</tbody>
</table>

*Erythroid monoclonal antibody—not commercially available.
†Polyclonal.
NA, Not available.

**M2:** More than 30% myeloblasts with >10% differentiating granulocytes, NSE <20%.

**M3:** (a) Hypergranular promyelocytes with numerous Auer rods on Wright-stain or CAE. (b) A variant form showing cells with bilobed, multilobed or reniform nuclei (NSE-negative) and relative scarcity of hypergranular promyelocytes or of primitive cells with multiple Auer rods.

**M4:** Monocytic cells with >20% but <80% NSE-butyrate positivity.

**M5:** Monocytic cells with >80% NSE positivity. (a) Monoblastic, poorly differentiated. (b) Monocytic, differentiated

**M6:** More than 50% erythroblasts with >30% myeloblasts excluding the erythroid cells.

The criteria used for the myeloid subclassification were similar to the revised FAB classification recently published and were introduced in our laboratory in 1983 as a result of the FAB reports in 1981 and 1982 and from examination of our data.

**Cell Preparation and Immunofluorescence**

**Monoclonal antibodies.** This has been described in detail in a previous paper. In brief: after Ficoll-Hypaque gradient separation, cells were incubated for 30 minutes with the monoclonal antibody (McAb). After three washes the cells were then incubated for 30 minutes with fluorescein-conjugated antismouse immunoglobulin. To increase the reactivity of some monoclonal antibodies (My9, My7, My4, Leu M1, PMN 6/29, and B4) biotin-conjugated antismouse immunoglobulin was used as the second antibody and for the third stage fluorescein conjugated avidin was added. After three further washes, cytospin preparations were made and examined under the fluorescent microscope.

**Heteroantisera**

**Surface immunoglobulin.** Cells were incubated with fluorescein-conjugated antihuman immunoglobulin for 30 minutes. After three washes, cytospin preparations were made and examined using a fluorescent microscope.

**Cytoplasmic immunoglobulin.** After fixing for five minutes in acetone, cytospin preparations of cells were incubated for 30 minutes with fluorescein-conjugated antihuman immunoglobulin. After three washes, the preparations were examined using a fluorescent microscope.

**Terminal deoxynucleotidyl transferase.** An indirect immunofluorescent assay using rabbit anti-terminal deoxynucleotidyl transferase (anti-TdT) was performed as previously described.

**Criterion for all methods.** Cases where more than 25% of mononuclear cells were reactive with antibody were considered positive.

**Comparison of the Derived Immunophenotype with the FAB Classification**

Two experienced observers independently classified (using the FAB classification as described above), randomly ordered, and
IMMUNOPHENOTYPING ACUTE LEUKEMIA

Results and Discussion

Comparison of the FAB Classification by the Two Observers

Of the 138 cases, there was agreement in the FAB diagnosis of 83% of the cases (113 cases). In the remaining 17% (23 cases), 15 cases showed FAB subtype differences (M1/M2 [4], M1/M5 [1], M2/M4 [2], M4/M5 [2] or L1/L2 [6]) while eight cases were unclassifiable (AUL). In 30 cases (22%), there was negative cytochemical staining. The two observers were concordant in their FAB diagnosis in 20 of these cases. Of the remaining 10 cases, eight were undifferentiated and two were minor subtype differences.

Initial Selection of Antibodies

The reagents used to type acute leukemia are shown in Table 1. Some reagents showed a lack of specificity, poor reactivity, an irregular response to differentiation, or maturation or insufficient positivity in AML and were dropped from our panel. The reagents ultimately used to immunophenotype are shown in Tables 2, 3 and 4.

Relationship Between Immunophenotype and FAB Classification in AML

The reactivity pattern of 75 cases of AML with monoclonal antibodies against myeloid differentiation antigens is shown in Table 2. It will be noted that 87% of the cases were identified by My9 and 75% by My7. Of these 75 cases, 12 were My7 and My9; five My7 and My9; and three My7 and My9. The remaining 55 cases (73%) were positive for both antibodies (My7, My9). Ninety-six percent of the cases of AML expressed either My9, My7, or both. Three of the 75 cases that failed to be identified by My7 and My9 were later recognized by other criteria to be myeloid in origin. In two of the patients, the bone marrows were considered to be M0 by morphology and NSE reaction (>80%) and were both My4-positive. A further case, SBB- and MPO-positive, was negative with all myeloid antibodies used (My7, My9, My4, and Leu M1).

From surveying our initial results we recognized that both My7 and My9 antibodies would be useful as an initial screen for acute myeloid leukemia. Pooling My7 and My9 antibodies was first undertaken when insufficient cells had been obtained by marrow aspirate or on peripheral blood. In 20 further cases pooled My7/My9 antibodies were compared with the results when the reagents were used individually. Using the pooled reagents, an increase in the intensity of the reaction, and sometimes in the number of positive cells, was observed in the majority of cases. In a similar manner, we now pool Leu 1 and Tll (see Table 4).

Table 2. Reactivity Pattern of Acute Myeloid Leukemia with Monoclonal Antibodies

<table>
<thead>
<tr>
<th>FAB (No.)</th>
<th>My9</th>
<th>My7</th>
<th>Leu M1</th>
<th>My4</th>
<th>PMN</th>
<th>AML</th>
<th>la</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1(18)</td>
<td>14/17 (82)</td>
<td>11/17 (65)</td>
<td>0/18 (0)</td>
<td>1/18 (5)</td>
<td>0/10 (0)</td>
<td>1/6 (17)</td>
<td>9/15 (60)</td>
</tr>
<tr>
<td>M2(17)</td>
<td>12/15 (80)</td>
<td>13/15 (87)</td>
<td>15/17 (88)</td>
<td>1/16 (6)</td>
<td>2/10 (20)</td>
<td>1/5 (20)</td>
<td>7/11 (64)</td>
</tr>
<tr>
<td>M3(8)</td>
<td>6/6 (100)</td>
<td>6/6 (100)</td>
<td>3/6 (50)</td>
<td>0/7 (0)</td>
<td>4/6 (67)</td>
<td>4/4 (100)</td>
<td>1* (8) (13)</td>
</tr>
<tr>
<td>M4(15)</td>
<td>15/15 (100)</td>
<td>13/15 (87)</td>
<td>13/15 (87)</td>
<td>12/15 (80)</td>
<td>7/13 (54)</td>
<td>6/7 (86)</td>
<td>14/15 (93)</td>
</tr>
<tr>
<td>M5(14)</td>
<td>11/13 (85)</td>
<td>6/13 (46)</td>
<td>13/14 (93)</td>
<td>14/14 (100)</td>
<td>1/9 (11)</td>
<td>3/4 (75)</td>
<td>13/13 (100)</td>
</tr>
<tr>
<td>M6(2)</td>
<td>1/2</td>
<td>1/1</td>
<td>1/1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>M7(1)</td>
<td>1/1</td>
<td>1/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Total Non-mono</td>
<td>34/41 (83)</td>
<td>32/40 (80)</td>
<td>19/43 (44)</td>
<td>2/42 (5)</td>
<td>6/27 (22)</td>
<td>6/16 (38)</td>
<td>17/35 (49)</td>
</tr>
<tr>
<td>Total mono (M4, 5)</td>
<td>26/28 (93)</td>
<td>19/28 (68)</td>
<td>26/29 (90)</td>
<td>26/29 (90)</td>
<td>8/22 (36)</td>
<td>9/11 (82)</td>
<td>27/28 (96)</td>
</tr>
<tr>
<td>Total AML (75)</td>
<td>60/69 (87)</td>
<td>51/68 (75)</td>
<td>45/72 (63)</td>
<td>28/71 (39)</td>
<td>14/49 (29)</td>
<td>15/27 (56)</td>
<td>44/63 (70)</td>
</tr>
<tr>
<td>ALL</td>
<td>6/50 (12)</td>
<td>7/50 (14)</td>
<td>5/47 (11)</td>
<td>1/49 (2)</td>
<td>0/19 (0)</td>
<td>2/8 (25)</td>
<td>34/44 (77)</td>
</tr>
</tbody>
</table>

*pPromyelocytic variant.
†EMcAb > 25%
‡PLT1 and PLM1 > 25%
We found that all four cases of FAB M3 including a variant form\(^9\) gave a positive reaction with AML 2.23. All of these cases were My\(^4\) but the variant form was Ia\(^+\) in contrast to the hypergranular form which was Ia\(^-\). The combination of Ia\(^-\), AML 2.23\(^+\), and My\(^4\) was not observed in FAB M1, M2, M4, or M5.

As previously reported,\(^10\) My\(^4\) reacted positively in the majority (90%) of cases of FAB M4 and M5. It gave a positive reaction in only two of 42 cases (5%) classified as nonmonocytic by the FAB classification and a negative NSE reaction. In seven cases NSE-butyrate and My\(^4\) reactivity were not concordant.

Eighty-six cases which typed as AML were tested with anti-TdT and two cases were positive. One of the cases was also Tll- and sheep red cell receptor-positive and was considered to be a case of acute mixed lineage leukemia (AML-L).\(^26\)\(^,\)\(^27\) Two further cases diagnosed as AML by the FAB classification showed myeloid markers plus a single lymphoid marker (Tll) on immunophenotyping\(^28\) (See Table 3).

By observing certain patterns using monoclonal antibodies against myeloid differentiation antigens, a myeloid immunophenotype corresponding to the FAB myeloid subclassification was recognized for M1, M2, M4, and M5 (Table 5).\(^46\)

Figure 1 shows that by the immunophenotypic criteria, 85% of cases diagnosed as M1, 93% as M2, 81% as M4 and 100% as M5 corresponded to their appropriate FAB morphologic diagnosis. The hypergranular FAB M3 appeared to be Ia\(^-\), AML 2.23\(^+\), and My\(^4\), but our numbers are too small to draw a firm conclusion. Criteria for the diagnosis of M6\(^49\) and M7\(^50\) have been published in the literature.

**Table 4. Monoclonal Antibodies for Classifying Acute Leukemia**

<table>
<thead>
<tr>
<th>First Line</th>
<th>Acute Myeloid Leukemia</th>
<th>2nd Line‡</th>
<th>Acute Lymphoid Leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>My 7/9</td>
<td>Leu M1, My4, EMcAb(^5), la, PMN 6/29, AML 2.23</td>
<td>VFIIR, PLT1, Gp IIb/IIIa</td>
<td>Ta, J5, cu(^+), Slg(^+), T3, T6, T9</td>
</tr>
</tbody>
</table>

*Heteroantiserum.
†An addition would be Leu 9, (CD7, 40 kd).\(^23\)
‡Second and third line subsequently performed according to lineage established by the first line.
§EMcAb, erythroid monoclonal antibody; not commercially available.

Relationship Between Immunophenotype and FAB Classification in ALL

As previously demonstrated, there was a lack of correlation between the FAB L1, L2 classification and the ALL immunophenotype.\(^2,\)\(^13\)\(^,\)\(^15\) Furthermore, one case classified as FAB L3 showed no SIg positivity and typed as cALL (B4\(^+\), J5\(^+\), Cu\(^-\), Slg\(^-\)).\(^30\)\(^,\)\(^31\)

Our immunophenotypic results with acute lymphoid leukemia correspond to those reported in the literature.\(^8\)\(^,\)\(^12\)\(^,\)\(^29\)\(^,\)\(^32\)\(^,\)\(^52\)\(^,\)\(^53\) The reactivity pattern with monoclonal antibodies of 51 patients classified as ALL are shown in Table 3. B4 was specific for non-T lineage ALL being positive in all patients tested, and negative in 13 cases of T-ALL. Anti-TdT was positive in all except six cases of B-ALL showing positive SIg. Because BA2 was positive in four out of nine cases of AML and two out of three cases of T-ALL, and because BA1 was considered less reliable than B4 for subtyping Null ALL, both antibodies were dropped from our panel.\(^34\) Twelve cases (24%) diagnosed as ALL by the FAB classification showed lymphoid markers and a myeloid marker on immunophenotyping.\(^46\)

Cases With Disagreement Between the Morphological Diagnosis and the Immunophenotype

Table 6 shows the results of 14 cases where there was disagreement between the morphologic diagnosis and the immunophenotype. Thirteen of the cases were undifferentiated by cytochemical staining. Eight of the cases were originally unclassifiable (AUL, M1, or L2) by morphology.

**Table 5. Myeloid Immunophenotypic Subclassification**

<table>
<thead>
<tr>
<th>M1</th>
<th>17</th>
<th>0</th>
<th>1</th>
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<tbody>
<tr>
<td>M2</td>
<td>2</td>
<td>14</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>M4</td>
<td>1</td>
<td>13</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>M5</td>
<td>0</td>
<td>1</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

**Table 6. Myeloid Immunophenotypic classification and the FAB-cytochemical classification.**

Fig 1. Comparison of results of the immunophenotypic myeloid subclassification and the FAB-cytochemical classification.

To draw a firm conclusion.
In the remaining six cases there had been FAB concordance but the result was in disagreement with the immunophenotype. One of these cases showed negative reactivity with all monoclonal antibodies used despite FAB M1 morphology and strong positivity with MPO and SBB. The immunophenotype included three cases with a mixed lineage phenotype and a single case with a dual population of lymphoid and myeloid cells. The final diagnosis for therapeutic purposes was considered as either ALL or AML, according to the predominant lineage observed on combined morphologic, cytochemical, and immunologic assessment.

**DISCUSSION**

The FAB classification provides a common language for comparing and treating acute leukemia; however, difficulties with some of the diagnostic criteria are evident. The major difficulties include distinguishing M1 from L2 in cases where cytochemical staining is negative and M1 from M2 through their distinction has been improved in the recent revised FAB classification.

From our initial investigation, a panel of commercially available antibodies to discriminate and subtype acute leukemia was devised and used in the Hamilton region. Since its initiation, the panel has been tested prospectively on 58 ALL cases. It was developed to obtain the maximum of information using the smallest number of tests. In addition, the panel is performed in sequence so that the most useful information is likely to be derived from specimens which contain an insufficient number of cells for complete analysis. The first line screen is used to distinguish acute myeloid from acute lymphoid leukemia and to separate B and T lineage ALL. It will also identify some cases of AMLL. The second panel discriminates a rare case not recognized by the first panel and further subtypes acute myeloid and lymphoid leukemia. Cases not defined by the first two panels are investigated by a third panel which includes antibodies that recognize megakaryocyte/platelet differentiation or further discriminate subtypes of ALL.

The immunophenotype will discriminate cases of acute lymphoid from myeloid leukemia which remain undifferentiated by cytochemistry (22% of cases) and thereby clarifies the majority of the disagreements (M1 v L2) noted between observers using the FAB classification. In addition, cases with a mixed lineage phenotype (13% of cases) and, less commonly, dual populations of lymphoid and myeloid cells (<1% of cases) could be identified. It seems the immunophenotype should be regarded as a reliable method of classifying acute leukemia. Monoclonal antibodies with the same cluster differentiation (CD) number as defined by the First and Second Leukocyte Antigen Workshop could possibly be used to replace some of the monoclonal antibodies utilized in our panel; however, despite their having the same CD number, we found differences in the reactivity of My4 and MO.

The subclassification of acute lymphoblastic leukemia by immunophenotyping is well-documented. To date all our cases of non-T-ALL have shown B4 reactivity. Null ALL was identified in the panel by reactivity with anti-TdT and B4 only, but Anderson et al have observed presumed cases of non-T ALL that were TdT and B4. As previously reported the ALL immunophenotype did not correspond with the FAB classification.

In most previous reports using different antibodies there has been little correlation between the FAB myeloid subtypes and antigenic phenotype in AML; however, the expression of certain antigens have correlated with the FAB classification. Using our immunologic panel and derived criteria to subtype AML, correspondence of the immunophenotype to the FAB subtypes M1, M2, M4, and M5 was...
Table 7. Criteria for FAB Myeloid Subclassification Using Morphology, Cytochemistry and Immunophenotype

<table>
<thead>
<tr>
<th>FAB M1</th>
<th>+ / - 90% Blasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBB, MPO &gt; 3% and/or My 7/9 &gt; 25%</td>
</tr>
<tr>
<td></td>
<td>Very little maturation (&lt;10%), Leu M1 &lt; 25%</td>
</tr>
<tr>
<td>M2</td>
<td>&gt; 30% Blasts with maturation</td>
</tr>
<tr>
<td></td>
<td>&gt; 10% More mature myeloid cells, &gt; 25% Leu M1</td>
</tr>
<tr>
<td></td>
<td>&lt; 20% NSE and &lt; 25% My 4</td>
</tr>
<tr>
<td>M3a</td>
<td>Hypergranular Promyelocytes</td>
</tr>
<tr>
<td></td>
<td>Multiple Auer rods (Wright-stain, CAE, or phase contrast microscopy)</td>
</tr>
<tr>
<td></td>
<td>AML 2.23 &gt; 25%, ia &lt; 25%, My4 &lt; 25%</td>
</tr>
<tr>
<td>M4</td>
<td>&gt; 30% Blasts</td>
</tr>
<tr>
<td></td>
<td>Monocytic differentiation</td>
</tr>
<tr>
<td></td>
<td>NSE &gt; 20%, &lt; 80% and/or My 4 &gt; 25%, &lt; 55%</td>
</tr>
<tr>
<td></td>
<td>My 4 between 45-55%, PMN 6/29 &gt; 25%</td>
</tr>
<tr>
<td>M5</td>
<td>&gt; 30% Blasts</td>
</tr>
<tr>
<td></td>
<td>Monocytic differentiation</td>
</tr>
<tr>
<td></td>
<td>NSE &gt;80% and/or My 4 &gt; 55%</td>
</tr>
<tr>
<td></td>
<td>My 4 between 45-55%, PMN 6/29 &lt; 25%</td>
</tr>
<tr>
<td>M6</td>
<td>&gt; 30% Blasts (Nonerythroid population)</td>
</tr>
<tr>
<td></td>
<td>&gt; 50% Erythroid precursors (Total marrow cells) E McAb positive</td>
</tr>
<tr>
<td>M7</td>
<td>&gt; 30% Blasts</td>
</tr>
<tr>
<td></td>
<td>PLT1, or PLM1, or FVIIIIR &gt; 25% blasts positive</td>
</tr>
</tbody>
</table>

possible in over 80% of cases (Fig 1). Using the immunophenotype together with the FAB morphology and cytochemistry (Table 7) it is possible to obtain almost perfect concordance between observers. It should be emphasized that this scheme is based on the testing of a relatively small sample (75 AML cases). Combining FAB M1, M2, and M4 as so-called M7 is unnecessary when using the combined FAB/immunophenotypic classification as the various myeloid subtypes can be distinguished.

Should all cases of acute leukemia be immunophenotyped? A major difficulty of the FAB classification is encountered in cases with negative cytochemical staining. This was found in 30 of our 138 cases (22%). Though our two observers were concordant in 20 of these 30 cases, in five of the concordant observations there was disagreement between the concordant FAB classification and the immunophenotype. Of the remaining ten cases, eight were undifferentiated (M1 or L2). Immunophenotyping may therefore be useful in the cases with negative cytochemistry. Clarifying the diagnosis in this group of patients will allow therapy to be given according to the type of leukemia diagnosed. A controlled study will be required to ascertain whether this results in an improved therapeutic outcome. Acute mixed lineage leukemia is found in 13% of our cases and could rarely be recognized without immunophenotyping. If therapeutic outcome with standard therapy is found to be inferior to outcome in single lineage leukemia in future studies (in progress), an argument could be made for applying immunophenotyping to all cases of AML.

At this time, the immunophenotype cannot be regarded as the "gold standard" for classifying all cases of acute leukemia. Unfortunately, there is no "gold standard." To date, the most widely accepted classification system has been the FAB classification and for this reason the design of this study was to compare the immunophenotyping of acute leukemia with the FAB classification. The purpose of our paper is to provide data which may improve current systems. The process will hopefully help to establish a classification with biologic meaning or prognostic relevance; however, further controlled studies will have to be done to determine whether therapeutic decisions based upon immunophenotyping in adults improve the outcome as is possible in ALL in childhood. Such studies cannot be done until the classification is firmly established.

The possibility that the antigenic phenotyping of AML with monoclonal antibodies may identify groups which are of prognostic importance has been recently reported. A correlation with My1 reactivity and successful remission induction has been suggested. In addition, Griffin has noted a correlation of My7 reactivity with poor prognosis in AML, an observation noted previously with My4. The FAB classification has only been minimally successful in identifying groups of clinical relevance.

Following the recent introduction of probes to test for rearrangement of the immunoglobulin or T cell antigen receptor genes, it seemed that a test would be available to distinguish acute lymphoid from myeloid leukemia with certainty; however, recently immunoglobulin and T cell antigen receptor rearrangement have not been noted in cases with the AML phenotype. Thus in cases with a somatic rearrangement of the immunoglobulin or T cell antigen receptor gene, acute lymphoid leukemia may be suspected but is not proven. Cases with a germ-line configuration on DNA analysis would suggest nonlymphoid leukemia. The addition of karyotyping to the morphological and immunologic assessment should also contribute to the detailed cellular phenotype in acute leukemia.

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Classifying acute leukemia by immunophenotyping: a combined FAB-immunologic classification of AML

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