Cell Marker Analysis in Acute Monocytic Leukemias

By Benjamin Koziner, Susan McKenzie, David Straus, Bayard Clarkson, Robert A. Good, and Frederick P. Siegal

Leukemic cells from nine cases of acute monocytic leukemia (AMoL) were characterized by multiple differentiation markers. Cells in most cases were phagocytic, carried an Fc receptor, and stained positively for alpha-naphthyl acetate esterase but negatively for naphthol AS-D chloroacetate esterase. However, subtle differences in marker expression were observed which suggested different degrees of leukemic cellular maturation or activation. Cell marker analysis proved to be a useful adjunct to conventional morphology in confirming the diagnosis and the recognition of the neoplastic cells in AMoL, and may ultimately provide insight into the functional state of these cells.

Diagnosis of acute monocytic leukemia (AMoL) remains largely based on clinical and morphologic features. Markedly elevated serum and urine muramidase levels and certain patterns of cytochemical staining of leukemic cells are also consistent with this diagnosis. Monocytes and their precursor cells show strong staining of cytoplasmic granules for alpha-naphthyl acetate esterase (α-NAE), while the granules of myeloid cells stain for naphthol AS-D chloroacetate esterase (CAE).

Recently, the use of techniques enabling detection of differentiation markers on the surface of mononuclear cells has contributed to a better delineation of the cellular types involved. Most human monocytes and tissue macrophages have surface receptors for complement and the Fc fragment of IgG, which are of developmental and functional significance, facilitating the attachment and further ingestion of opsonized particulate material. Although these markers may also be present on B lymphocytes and “third population” cells, the absence of intrinsic surface immunoglobulin and the ability to phagocytize help to differentiate monocytes from these other populations of mononuclear cells.

This study was an investigation of the neoplastic cells of a group of nine patients with AMoL for the presence of surface markers and phagocytic ability, an approach found to be useful in their further characterization.

MATERIALS AND METHODS

Patients

During a 1-yr period, nine consecutive patients with a diagnosis of acute monocytic or myelomonocytic leukemia (since a variable number of myeloid cells was found in all cases) were referred to our attention (Table I). The median age was 52 yr; there were five males and four females. All patients were either previously untreated or in relapse from their leukemic process at the

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Table 1. Clinical Characterization of Nine Patients With Acute Monocytic Leukemia

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Bone Marrow Differential (%)</th>
<th>Peripheral Blood Differential (%)</th>
<th>Cytomorphologic Differential</th>
<th>Serum Muramidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>V.K.</td>
<td>58</td>
<td>F</td>
<td>21.5 33.5 3.5 12 19 10.5</td>
<td>16 — 16 57 11</td>
<td>+ + +</td>
<td>58 + + 23 ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ 14 + 21 neg 28 neg 56</td>
<td></td>
</tr>
<tr>
<td>S.G.</td>
<td>47</td>
<td>M</td>
<td>68 12 — 2 14 4 61 — — — 27 12</td>
<td>+ + +</td>
<td>+ 33 + 20 neg 31 neg 27 + 9</td>
<td>ND</td>
</tr>
<tr>
<td>J.P.</td>
<td>60</td>
<td>M</td>
<td>33 8 3 7 38.5 10.5</td>
<td>1 — — 7 74 18</td>
<td>+ + +</td>
<td>58 + + 18</td>
</tr>
<tr>
<td>L.V.</td>
<td>77</td>
<td>F</td>
<td>78.5 13 0.5 0.5 1 6.5</td>
<td>8 6 1 29 24 32</td>
<td>+ + +</td>
<td>43 + + 6</td>
</tr>
<tr>
<td>J.M.</td>
<td>37</td>
<td>M</td>
<td>70 16.5 — 1.5 10 2 49 — 1 11 26 13</td>
<td>+ + +</td>
<td>+ 18 + 7 neg 50 neg 25 + 6</td>
<td>18</td>
</tr>
<tr>
<td>J.K.</td>
<td>48</td>
<td>F</td>
<td>28 4.5 2.5 5.5 5 54.5 5 15 2 3 5 63 12</td>
<td>+ + +</td>
<td>+ 30 + 9 neg 11 11 11</td>
<td>25</td>
</tr>
<tr>
<td>M.F.</td>
<td>57</td>
<td>F</td>
<td>43 5 4.5 6 33 8.5 8 — — 6 70 16</td>
<td>+ + +</td>
<td>+ 49 + 22 neg 30 neg 14 neg 15</td>
<td>ND</td>
</tr>
<tr>
<td>J.B.</td>
<td>45</td>
<td>M</td>
<td>37.5 1.5 11 8 35 7 32 1 3 10 35 19</td>
<td>+ + +</td>
<td>+ 53 + 9 neg 15 + 15 neg 16</td>
<td>ND</td>
</tr>
<tr>
<td>N.H.</td>
<td>40</td>
<td>M</td>
<td>70.5 3 1 0.5 21 4 97 — — 3 3</td>
<td>+ + +</td>
<td>+ 37 + 10 neg 29 + 17 neg 17</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ 10 + 9 260 neg 81 neg 17</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Bl, blasts; Pro, promyelocytes or promonocytes; My, myeloid; Gr, granulocytes; Mo, monocytes; Others, includes erythroid, lymphoid, and plasma cells; α-NAE, alpha-naphthyl acetate esterase; CAE, naphthol AS-D chloroacetate esterase; ND, not done. Degree of cytochemical staining; +, faint; + +, intermediate; + + +, strong; neg, negative.

time of this study. The number of peripheral blood leukemic cells, including atypical monocytic forms, promonocytes, and blasts, was in every instance above 10,000 cells/cu mm. The term promonocyte was used to describe large monocytic cells with folded nuclei, dispersed chromatin, and grayish blue cytoplasm with occasional azurophilic granulation (Fig. 1). Larger cells with oval or round nuclei, open chromatin structure containing one or more nucleoli, and abundant blue cytoplasm were categorized as blasts (Fig. 2).

Procedure

Nonspecific esterase activity using alpha-naphthyl acetate as substrate and esterase activity using naphthol AS-D chloroacetate as substrate were determined in unstained smears of peripheral blood and bone marrow as described by Yam et al.6
Fig. 1. Large promonocytes with folded nuclei and dispersed chromatin showing phagocytosis of latex particles.

Fig. 2. Blast cells with oval or round nuclei and open chromatin of histiocytic appearance.
The cells of all patients on which cytochemical staining was performed showed activity for α-NAE. Staining of variable intensity with CAE was seen in a minority of cells in all cases. This finding may have been due to a variable admixture of myeloid cells.

Serum muramidase determinations were performed as described by Parry et al. High levels were found in all patients studied, further confirming the monocytic nature of the leukemic process (normal range, 0-8 μg/ml).

**Mononuclear Cell Preparation**

Mononuclear cells were separated from heparinized whole blood or bone marrow by a Ficoll-Hypaque flotation method as previously described. Cell suspensions were incubated in 20% autologous plasma-Hanks' balanced salt solution (HBSS) for 1 hr at 37°C with 0.8-μm polystyrene particles to identify phagocytic cells. Viability of cells was determined by trypan blue dye exclusion. In all preparations, cells excluding dye exceeded 90% of the total.

The preparation of fluorescent antisera against heavy-chain classes of immunoglobulin and determination of surface immunoglobulin were performed as previously described. Indirect binding of aggregated IgG was carried out as described by Dickler and Kunkel.

High-affinity Fc receptors on mononuclear cells were determined with a human EA (Ripley) rosette technique modified from Fröland and Natvig. First, 100 μl of lymphocyte suspension (5 x 10⁶ cells/ml) was mixed with human erythrocytes (ORh) coated with 7S IgG of Ripley type, kindly provided by Dr. Marion Waller. The mixture was centrifuged at 50 g for 5 min, followed by incubation at 4°C for 30 min. A mononuclear cell surrounded by three or more red cells was considered a rosette. Criteria for positivity regarding the presence of receptors for the Fc fragment of IgG required that more than 50% of the leukemic cells form Ripley rosettes or bind aggregated IgG.

The morphological features of the leukemic cells were assessed on standard tetrachrome-stained smears of peripheral blood and bone marrow. In addition, permanent preparations of rosetting cells were made in a cytocentrifuge or after separation of the rosetting cells by slight modifications of the rosette density gradient method. Cytocentrifuge smears of only the rosetting cells were then prepared and evaluated. The proportion of leukemic cells bearing each characteristic was assessed, rather than the proportion of all mononuclear cells. However, in the majority of cases, most cells in the mononuclear cell layer were leukemic.

**RESULTS**

In ten normal controls, 45.5% ± 24% (mean ± 1 SD) of normal monocytes formed rosettes with IgG EA (Ripley), and 100% bound aggregated IgG and had surface IgG but did not have other heavy-chain classes of immunoglobulin on the surface. After short-term culture at 37°C in the absence of human serum, most cells did not stain for surface IgG, arguing in favor of the cytophilic nature of the immunoglobulin.

Table 2 shows our findings in the group of nine patients with AMoL. The leukemic cells were phagocytic (latex positive) in seven patients. In five of these seven cases (V.K., S.G., J.P., L.V., and J.M.), the phagocytic cells carried a receptor for the Fc fragment of IgG recognized by both formation of IgG EA rosettes (Fig. 3) and binding of aggregated IgG. In two other cases (J.K., M.F.), the leukemic cells bound IgG aggregates but failed to form Ripley rosettes. In another case (J.B.), the leukemic cells in peripheral blood exhibited phagocytosis and displayed an Fc receptor recognized by the binding of aggregates, but the bone marrow cells, predominantly of blastic morphology, failed to express these characteristics. The cells of patient N.H. bound aggregated IgG, but did not phagocytize latex particles.
Table 2. Characterization of Leukemic Monocytes by Phagocytic Ability and Surface Markers

<table>
<thead>
<tr>
<th>Patient</th>
<th>Tissue Studied</th>
<th>Latex-ingesting Cells (%)</th>
<th>Ripley Rosette</th>
<th>Ind. Agg. Binding</th>
<th>SlgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>V.K.</td>
<td>PB</td>
<td>95</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S.G.</td>
<td>PB</td>
<td>42</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>J.P.</td>
<td>PB</td>
<td>74</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>L.V.</td>
<td>PB</td>
<td>65</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>BM</td>
<td>94</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>J.M.</td>
<td>PB</td>
<td>24</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>BM</td>
<td>20</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>J.K.</td>
<td>PB</td>
<td>57</td>
<td>—</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M.F.</td>
<td>PB</td>
<td>50</td>
<td>—</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>J.B.</td>
<td>PB</td>
<td>40</td>
<td>—</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N.H.</td>
<td>PB</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>BM</td>
<td>7</td>
<td>—</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Abbreviations: Ind. Agg. Binding, indirect aggregate binding; SlgG, surface IgG; PB, peripheral blood; BM, bone marrow; ND, not done.

DISCUSSION

Acute monocytic leukemia is recognized as the neoplastic proliferation of predominant monocytic forms involving bone marrow, peripheral blood, and, less frequently, lymphoid and connective tissue. Although the morphology of the cells involved may vary widely from primitive forms to well-differentiated monocytes, monoblasts and promonocytes usually predominate. A variable ad-
mixture of myeloid elements as well as abnormalities of the erythrocytic and megakaryocytic sectors are frequently observed.\textsuperscript{15}

Mouse promonocytes, monocytes, and macrophages express surface receptors for the Fc fragment of IgG and phagocytic ability which become increasingly prominent with progressive maturation.\textsuperscript{16,17} Normal human monocytes are also phagocytic and carry Fc receptors on their surface, which can be demonstrated readily by the binding of aggregated IgG. Cells with more net binding ability for IgG (approximately half of these cells using present techniques) also form IgGEA (Ripley) rosettes as described above.

Several examples of leukemic monocytes exhibiting phagocytosis and surface receptors for Fc have been reported, suggesting the usefulness of this approach in better defining the cellular type involved.\textsuperscript{18-21} In the present study, there was a predominance of phagocytic cells recognized by the ingestion of latex particles, which also in most cases carried an “avid” receptor for the Fc fragment of IgG as detected by both the formation of IgGEA rosettes and binding of aggregated IgG. Some cells failed to form Ripley rosettes but still bound aggregates and had surface IgG.

In one patient large numbers of latex-ingesting monocytic and undifferentiated forms were present in the peripheral blood, but the bone marrow cells, which had more “blastic” morphology, failed to show either phagocytic ability or a surface receptor for Fc. This would suggest differences in stages of differentiation of the leukemic cells in the two compartments. In another patient the leukemic cells carried the receptor for Fc but failed to phagocytize. The subtle variations in marker patterns from case to case may reflect varying degrees of cellular differentiation, with neoplastic proliferation at different stages of development, or different levels of transitory surface activation. The combined investigation of other functional and morphological features of monocytes, such as growth in soft agar culture,\textsuperscript{22} and electron microscopic characteristics,\textsuperscript{23} may further dissect differentiative levels among these cells. It might be clinically useful to delineate these differences in cellular maturation of leukemic cells and correlate them with clinical behavior.

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