Fucose Binding Lectin for Characterizing Acute Myeloid Leukemia Progenitor Cells

By Ruud Delwel, Ivo Touw, Freek Bot, and Bob Löwenberg

The reactivity of acute myeloid leukemia cells (AML) was determined in 29 patients using the fucose binding lectin *Ulex europaeus* agglutinin (UEA) as surface marker. We show a marked heterogeneity in the UEA-binding abilities of the cells in these patients as determined by fluorescence analysis of the blasts labeled with the UEA coupled to the fluorescent molecule FITC. The results suggest a correlation between the capability of AML blast cells to bind UEA and cytologic maturation, because in 1 of 10 M1, 3 of 8 M2, 6 of 8 M4, and 1 of 3 M5 cytology types UEA binding to the leukemic cells was apparent. In 13 cases, the cells gave rise to colonies in vitro. The amount of UEA binding to AML colony-forming cells (AML-CFU) was determined by cell sorting and subsequent colony culture of UEA-negative, intermediate-positive, and highly fluorescent cells. AML-CFU from none of the four patients with M1 cytology were UEA positive, whereas they showed intense reactivity with the lectin in 1 of 4 cases with M2 cytology and in all 4 cases of M4. In these five cases with strongly UEA positive AML-CFU, the fluorescence distribution of the colony formers differed from that of the total leukemic population, indicating that AML-CFU represent a subpopulation of AML cells with specific UEA-binding properties. Normal bone marrow myeloid and multipotential colony-forming cells (CFU-GM, CFU-GEMM) showed low or no binding of UEA. UEA-FITC appears a useful reagent for membrane analysis of AML-CFU. In certain cases, UEA-FITC labeling may be applied to discriminate AML-CFU from normal hematopoietic progenitors.

SeveraI STUDIES have dealt with the detailed phenotyping of acute myeloid leukemia colony-forming cells (AML-CFU) in relation to normal bone marrow colony formers (CFU-GM, CFU-GEMM). The results of those studies indicate that AML-CFU can be recognized as the leukemic counterparts of normal hematopoietic progenitor cells. These investigations were performed with monoclonal antibodies, which have become the most common tools to identify surface phenotypes.

Lectins may also be of value to detect surface antigens. For example, the fucose-binding lectin from *Lotus tetragonolobus* (FBL-L) has been shown to react with human myeloid cells. The amount of cellular binding of the lectin increases progressively from myeloblasts toward mature granulocytes; monocytes show intermediate reactivity. CFU-GM mainly belong to the low FBL-L-binding bone marrow fraction. No information exists on the usefulness of the lectin for analysis of AML-CFU.

In the present study, we labeled AML blasts with the fucose binding lectin (FBL) *Ulex europaeus* agglutinin coupled to fluorescein-isothiocyanate (UEA-FITC) to determine whether UEA-binding to AML-CFU is different from that of normal CFU-GM and CFU-GEMM. We also compared UEA binding patterns of AML-CFU with that of the total AML blast population and with morphological classification of AML according to the criteria of the French-American-British (FAB) group.

It is shown that: (a) in certain cases, UEA binding to AML-CFU is markedly discrepant from that of normal CFU-GM and CFU-GEMM; (b) binding patterns of AML-CFU can be distinguished from those of total blast populations; and (c) UEA binding capacity of AML-CFU is positively correlated to cytologic (FAB) maturation.

**MATERIALS AND METHODS**

**Cell separation.** Cells from 29 patients with newly diagnosed AML and from three subjects without hematological disease were studied. AML cytology was classified according to the FAB criteria. All individuals had consented to donate bone marrow. AML blasts from bone marrow or peripheral blood were separated to >97% purity following discontinuous bovine serum albumin (BSA) density fractionation and E rosette sedimentation. The cells were then cryopreserved in aliquots of 30 x 10^6 cells in 7.5% dimethyl sulfoxide (DMSO) and 20% fetal calf serum (FCS) using a controlled freezing apparatus and storage in liquid nitrogen as described. Normal bone marrow cells were recovered from the interface after Ficoll-Isopaque centrifugation.

**Colony cultures for AML-CFU, CFU-GM, and CFU-GEMM.** Cultures from AML cells were performed in the PHA-lymophocyte feeder (PHA-LF) system. Colonies in this system from purified blasts are always of leukemic nature as demonstrated by morphology, immunophenotyping, and cytogenetic markers. Myeloid colonies (CFU-GM) from normal marrow were grown in a semi-solid culture containing human placenta conditioned medium (HPCM; 20% vol/vol) as a source of colony-stimulating factor (CSF). Mixed colonies (CFU-GEMM) were described by Fauser and Mennel with slight modifications. Normal bone marrow cells were cultured in a 1:1 mixture of Iscove's modified Dulbecco's medium (IMDM, GIBCO, Gent, Belgium), 1.1% methylcellulose, 30% autologous heparinized plasma, 7.5% of a medium conditioned by leukocytes in the presence of 1% PHA (PHA-LCM), 1 U sheep erythropoietin per milliliter (step III, Connaught, Willodale, Ontario, Canada), BSA, transferrin, lectin, sodium selenite, and 2-mercapto-ethanol. Dishes were incubated at 37°C and 100% humidity in an environment of 5% CO2 in air. Cultures were scored at day 15. Mixed colonies were defined as containing red hemoglobinized cells plus at least 5% nonhemoglobinized translucent cells of various sizes. Disputable mixed colonies were always verified cytologically after they were lifted off the plate with a fine-drawn Pasteur pipette.

**Labeling of cells with UEA-FITC and FACS.** AML blast cells or bone marrow nucleated cells were suspended in phosphate-buffered saline (PBS) containing 5% FCS at a cell concentration of

**From the Dr Daniel den Hoed Cancer Center, Rotterdam, The Netherlands.**

Supported by The Netherlands Cancer Foundation "Koningin Wilhelmina Fonds."

Submitted July 23, 1985; accepted Feb 12, 1986.

Address reprint requests to Dr B. Löwenberg, The Dr Daniel den Hoed Cancer Center, PO Box 5201, 3008 AE Rotterdam, The Netherlands.

© 1986 by Grune & Stratton, Inc.

0006-4971/86/6801-0004$03.00/0
The cells were then washed three times with PBS and resuspended in 4 °C with 20 μL of UEA-FITC (Polysciences Inc, Warrington, Pa). The cells were then washed three times with PBS and resuspended in PBS with 5% FCS at a cell concentration of 2 x 10^5 cells per milliliter. Cell sorting was performed on a FACS 440 (Becton Dickinson, Sunnyvale, Calif). Excitation was performed with the laser at 488 nm (0.4 W). For the fluorescence measurements, a 530/30 band pass filter was used. The instrument was calibrated with fluorescent standard beads (1.0 μm and 2.83 μm in diameter) (Polysciences Inc).

Dead cells among the leukemic population were excluded from analysis by elevating the threshold level of the forward light scatter. Fractions of different fluorescence intensity were sorted following a sterile procedure and plated separately in colony culture for determining the number of colony-forming cells as a function of the amount of UEA binding. Usually, between 1.5 to 3.0 x 10^5 cells per fraction were collected. Sorting rate was between 1,500 and 2,000 cells/second. Data analysis was performed using a Hewlett Packard 86B system.

May-Gr"unwald-Giemsa staining. After FACS, normal bone marrow cells or peripheral blood cells were suspended in 50% FCS in PBS (4 x 10^5 cells in 0.5 mL) and centrifuged onto clean glass slides (500 rpm for 5 minutes). The slides were air-dried and, after fixation in methanol (15 minutes), they were stained with May-Gr"unwald’s solution for 3 minutes and then counterstained with 4% (vol/vol) Giemsa in PBS. Slides were then washed and air-dried. Differential counts were performed at x1,000 magnification under oil, counting 250 cells.

RESULTS

UEA-binding to AML blasts and AML progenitors. The reactivity of purified AML cells to the fluorescent FBL (UEA-FITC) was studied. In 11 of 29 cases, the AML blasts reacted with the lectin (Table 1). The data are indicative of a correlation between the binding of UEA and morphologic maturation of AML cells according to the FAB classification. The blasts of only 1 of 10 patients with AML-M1 cytology were UEA reactive, whereas in 3 of 8 cases of AML-M2, 6 of 8 AML-M4, and 1 of 3 AML M5, the cells showed significant UEA binding.

Of the complete series of patients, in 13 cases the cells were capable of giving rise to colonies in vitro. To determine the quantitative level of surface binding of UEA-FITC to AML-CFU, cells were sorted into separate fractions of varying fluorescence intensity and seeded into PHA-LF. AML-CFU from subjects with a relatively undifferentiated AML morphology, ie, all four M1 cases, showed no or minimal capacity for UEA-binding (Table 2), whereas AML precursor cells from one of four patients with M2 and four of four patients with M4 cytology exhibited high UEA binding. The colony formers of the single patient with acute monoblast leukemia (M5) did not bind detectable UEA to their surface. In Fig 1, four typical examples are given of the fluorescence distribution profiles of AML blast cells and AML-CFU. The cells from three patients also formed colonies in the other culture system, ie, with HPCM as stimulator. The distributions of AML-CFU as assessed in these cultures were found to be identical to those of AML-CFU grown in the PHA-LF method (data not shown).

UEA binding to normal bone marrow cells, CFU-GM, and CFU-GEMM. For comparison, the UEA-binding properties of normal marrow cells, CFU-GM and CFU-GEMM were determined. Figure 1 gives an example of the UEA-FITC fluorescence distribution of Ficoll-separated normal marrow cells and CFU-GM. CFU-GEMM, not plotted, showed essentially identical UEA binding properties as CFU-GM. It is evident from this and the data from the separate experiments shown in Table 2 that low amounts of UEA-FITC bind to the cell surface of normal CFU-GM and CFU-GEMM.

Table 1. Binding of UEA-FITC in Relation to FAB Classification of AML

<table>
<thead>
<tr>
<th>Classification*</th>
<th>UEA Negative</th>
<th>UEA Positive†</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1 (n = 10)</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>M2 (n = 8)</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>M4 (n = 8)</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>M5 (n = 3)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total N = 28</td>
<td>18</td>
<td>11</td>
</tr>
</tbody>
</table>

UEA, Ulex europaeus agglutinin; AML, acute myelogenous leukemia.
*Morphologic subtypes based on the classification of the French-American-British (FAB) Cooperative Group.†
†Of the 11 UEA-positive cases, 100% of the cells were UEA reactive in 7 patients (1 M1, 2 M2, 4 M4), and 15% to 20% of the cells reacted with the lectin in the other 4 cases (1 M2, 2 M4, 1 M5).

Table 2. Distribution of Nucleated Cells, AML-CFU, CFU-GM, and CFU-GEMM Relative to the Amount of UEA Binding

<table>
<thead>
<tr>
<th>Patient</th>
<th>FAB*</th>
<th>UEA Negative Cells (%)</th>
<th>CFU (%)</th>
<th>UEA Weakly Positive Cells (%)</th>
<th>UEA Strongly Positive Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M1</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>M1</td>
<td>95</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>M1</td>
<td>97</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>M1</td>
<td>97</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>M2</td>
<td>91</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>M2</td>
<td>91</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>M2</td>
<td>94</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>M2</td>
<td>39</td>
<td>32</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>9</td>
<td>M4</td>
<td>22</td>
<td>10</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>M4</td>
<td>24</td>
<td>18</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>M4</td>
<td>13</td>
<td>33</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>M4</td>
<td>49</td>
<td>33</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>M5</td>
<td>98</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1. BM, bone marrow.
Percentage of total recovered (%).
*Classification according to the FAB nomenclature.† Negative fluorescence (log scale); channel 0-90.
Weakly positive fluorescence; channel 91-130.
Strongly positive fluorescence; channel 131-255.
cytes and most of the eosinophils showed high UEA reactivity whereas neutrophilic granulocytes were intermediately reactive with this sugar (data not shown). The reactivity of the two lectins, however, differs somewhat. FBL-L has a higher binding capacity for granulocytes than for monocytes.16–18 Our results show the opposite for UEA, ie, intense binding to neutrophils. Studies in larger numbers of patients appear of interest in order to evaluate the usefulness of UEA as a reagent in the classification of AML.

The fluorescence histograms shown in Table 1 reveal three discrete types of reactivity among the leukemias: a group of cases of fully UEA-negative AML (N = 18), a second group of AML containing ~20% UEA-positive cells (N = 4) and a positive for UEA. Basophilic granulocytes were equally distributed; lymphocytes were UEA negative.

**DISCUSSION**

In this study, we examined the reactivity of the UEA to AML blast cells and AML-CFU. The findings were related to the maturation stage of AML (FAB) and to UEA reactivity of normal bone marrow nucleated cells, CFU-GM and CFU-GEMM. Lectin binding to the surface of AML blast cells among the patients (N = 29) showed considerable variability. The cell samples from 18 patients were UEA negative; the blasts from 11 patients were UEA positive. Fluorescence histograms indicate that UEA reactivity is heterogeneously distributed among blasts, indicating subsets with relatively low and high UEA surface binding.

Although the number of cases was limited, a correlation is suggested as regards the capacity of the cells to bind UEA and their morphology. The M1 cases with one exception were negative, whereas cells from three of eight M2 cases and six of eight M4 cases reacted with the lectin. The cells from one of three cases of an acute monoblastic leukemia (M5), showed UEA reactivity. The data are compatible with the results from investigations with FBL-L.28 In the latter studies, it was demonstrated that 5 of 6 M4 cases and the 1 M2 case reacted with this FBL-L, whereas their 1 M5 case was FBL-L negative. No AML cells classified as M1 were examined in that particular study. FBL-L and UEA are both reactive with α-L fucose. UEA can be washed from the cells with this sugar (data not shown). The reactivity of the two lectins, however, differs somewhat. FBL-L has a higher binding capacity for granulocytes than for monocytes.16–18 Our results show the opposite for UEA, ie, intense binding to monocytes and an intermediate binding to neutrophilic granulocytes. Studies in larger numbers of patients appear of interest in order to evaluate the usefulness of UEA as a reagent in the classification of AML.

The fluorescence histograms shown in Table 1 reveal three discrete types of reactivity among the leukemias: a group of cases of fully UEA-negative AML (N = 18), a second group of AML containing ~20% UEA-positive cells (N = 4) and a
third series of seven cases, in which 100% of the cells reacted with the lectin. Because we had no sufficient cell material to study colony growth among cases of each of these three groups, we were not able to correlate these classes to colony-forming abilities or AML-CFU phenotypes. However, it appeared that of the fully UEA-positive AML cases with colony growth, the majority of the AML-CFU were recovered from the UEA weakly positive fraction in one case and, in the other 5 cases, from the UEA strongly positive fraction.

Sorting experiments using UEA-FITC fluorescence show that in several cases AML-CFU can be recognized as a subgroup of cells with specific lectin-binding properties. This is consistent with the results of immunophenotyping, which have indicated that AML-CFU are frequently different from the total AML population in their antigenic cell surface profile.9,12,13

The relation between UEA binding and FAB categories holds up when binding is expressed for the AML-CFU subpopulation. In 4 M1 cases, UEA binding to AML-CFU was low (Table 2), whereas in 1 of 4 M2 and 4 of 4 M4 patients, a high level of UEA binding sites was demonstrated on the cell membrane. Variations in the expression of membrane antigens of AML-CFU have been noticed by different groups.12,14 Correlations between immunophenotypes of AML-CFU and AML cytology have been established but have provided controversial results. On the basis of the antigenic cell surface structure, Lange and co-workers classified AML-CFU as counterparts of the in vitro multipotent stem cell (CFU-GEMM), "early" CFU-GM or "late" CFU-GM. These investigators did not find a correlation with FAB classification of AML cells.1 However, using another panel of monoclonal antibodies, Sabbath and colleagues concluded that the antigenic makeup of AML-CFU tended to correlate with the maturation stage of the cells.2 We demonstrate that CFU-GM and CFU-GEMM were generally UEA negative or weakly positive. We suggest that AML-CFU with little UEA reactivity are generally of a more immature phenotype, whereas AML-CFU with high binding capacities are more differentiated into monocytic directions.

Morstyn and colleagues have shown that early as well as late CFU-GM have low affinity for FBL-L.18 In our studies, AML-CFU in 5 patients (1 AML-M2, 4 AML-M4) showed high reactivity with the lectin UEA and, in 8 cases, AML-CFU were recovered from the UEA low-reactive or negative fractions. It is possible that the discrepant UEA-binding capacities of AML-CFU and normal colony formers can be exploited in certain cases for recognizing AML clonogenic cells in the bone marrow during complete remission.

REFERENCES

Fucose binding lectin for characterizing acute myeloid leukemia progenitor cells

R Delwel, I Touw, F Bot and B Lowenberg