Leukemias With Megakaryoblastic Involvement: Clinical, Hematologic, and Immunologic Characteristics

By J.F. San Miguel, M. Gonzalez, M.C. Cañizo, E. Ojeda, A. Orfao, M.D. Caballero, M.J. Moro, P. Fisac, and A. Lopez Borrasca

The clinical, hematologic, and phenotypic features of 28 patients with acute leukemia with megakaryocytic involvement (AMKL) were analyzed. The prevalence of this type of leukemia in the entire series was 11.6%, with a higher incidence among patients with acute transformation of a previous myeloproliferative disorder (MPD) (24%) than among the transformed myelodysplastic syndrome (13%) patients. The incidence in the "de novo" ANLL was 8% and 16% among secondary leukemias. The presence of bone marrow fibrosis together with low WBC and normal or increased platelet counts despite a severe anemia are the most relevant features in these patients who otherwise displayed an apparently poor prognosis. Megakaryoblasts were morphologically recognized more frequently in the acute transformations of MPD than in de novo ANLL. Only two cases were considered pure AMKL, and in the remaining 26 patients, megakaryoblasts coexisted with other granulomonocytic and/or erythroid populations. Antiglycoprotein IIIa (anti-GP IIla) (C17) and anti-GP IIb/IIIa (CDw41, J5-I) antibodies are probably the best markers for AMKL, although the monoclonal antibody against GPIX (FMC25) was also positive in a majority of cases but in a lower percentage of cells. On the other hand, megakaryoblasts were generally negative for granulocytic or monocytic markers (CD13, CD14, CD15): the expression of HL-A-DR antigens in these cells was variable. Our present results indicate that megakaryoblastic involvement is more common than previously recognized. This is true not only in acute transformed leukemias but also in de novo ANLL. Although the diagnosis of these cases should be based on megakaryocytic markers, it is often possible to suspect a diagnosis according to certain clinical and hematologic features.

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VIM2; (b) granulocytic lineage: VINDs and FMC10 (both CD15); (c) monocytic lineage: FMC17, FMC33, Mo2, and UCHM1 (all CD14); (d) platelets: J15 and FMC24, both against glycoprotein IIb/IIIa (GPIIb/IIIa) (CD41), FMC25-anti-GPIX, protein complexed with GP Ib (CD42), and C17 (anti-GPIIIa); (e) erythroid: LICR LON R10 (anti-glycophorin A), OKT9 (transferrin-receptor); (f) precursor cells: GRB1 (anti-HLA-DR), 3C5 (CD34), OKT10 (CD38), and FMC8, or FMC56 (CD9); and (g) lymphoid: J5 (CD5), 3A1 (CD7), and Bi (CD20).

The reactivity and specificity of these MoAbs in AML has been reported previously. To minimize nonspecific Fc receptor binding, the cells were previously incubated at room temperature for 15 minutes with AB serum to block the Fc receptor and washed in a buffer containing phosphate-buffered saline-azide-bovine serum albumin–AB serum. Furthermore, in all experiments as a negative control we omitted the first antibody and used irrelevant isotype-matched MoAbs. A cell was considered positive when it presented evenly distributed fine staining on the cell surface.

For assessment of the involvement of a megakaryocytic lineage, a minimum of 10% blast cells, positive for one or more of the platelet-specific MoAbs, was required. The reason for using the 10% cutoff was that although 14% of the AML cases other than the megakaryoblastic leukemias had a maximum of 0% to 5% positive cells for the platelet-specific antibodies, none of these patients had 5% to 9% positivity. By contrast, in all the megakaryoblastic leukemias included in this paper, some of the MoAbs used always detected over 10% megakaryoblasts.

RESULTS

Clinical and hematologic features. In 28 of the 245 patients studied with adult acute leukemia other than lymphoblastic, the involvement of the megakaryocytic lineage was assessed. The frequency was higher in the group of acute transformation of a previous MPD (24.3%, ten of 41 cases) than among those with transformed MDS (13.6%, three of 22 cases). The incidence in de novo ANLL was 8% (14 of 176 cases). ANLL staining was negative in the pure megakaryoblastic forms between the undifferentiated blasts and micromegakaryocytes, the majority of these de novo leukemias being classified as undifferentiated, monocytic, or erythroid due to the predominant cell component (Table 3).

The ANAE reaction was generally positive, including the two cases of pure AMKL. Nevertheless, five cases with an important megakaryoblastic population were completely negative. This staining was NaF resistant in 30% of the cases. ANBE staining was negative in the pure megakaryoblastic case assayed, and six additional patients tested simultaneously for ANAE and ANBE displayed the same pattern (ANAE+, ANBE–), which has been claimed to be of use for the diagnosis of AMKL. The PAS reaction stained over 10% megakaryoblasts and some of these cases (ANAE+, ANBE–) were also observed (Fig 1). These latter forms were more frequently seen in the acute transformations of previous MPDs, which led us to suspect, in morphological terms, the presence of a megakaryoblastic component in all but one of these patients (Table 2). On the other hand, in only one ANLL case was it possible to recognize transitional forms between the undifferentiated blasts and micromegakaryocytes, the majority of these de novo leukemias being classified as undifferentiated, monocytic, or erythroid due to the predominant cell component (Table 3).

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Table 1. Clinical and Hematologic Data in Patients With Megakaryoblastic Leukemia

<table>
<thead>
<tr>
<th>Leukemia</th>
<th>Age</th>
<th>Sex</th>
<th>Hematopoeisis (%)</th>
<th>Splenomegaly (%)</th>
<th>Lymphadenopathy (%)</th>
<th>BM Fibrosis (%)</th>
<th>Hb (g/dL)</th>
<th>WBC (x 10^9/L)</th>
<th>Blast Cells (%)</th>
<th>Plaiklets (%)</th>
<th>Complete Remission</th>
<th>Survival (mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>De novo ANLL (14)</td>
<td>64 ± 16</td>
<td>1</td>
<td>1</td>
<td>24</td>
<td>28</td>
<td>7</td>
<td>65</td>
<td>8.3 ± 1.8</td>
<td>28 ± 40</td>
<td>86 ± 23</td>
<td>171 ± 248</td>
<td>21 ± 7.6</td>
</tr>
<tr>
<td>Acute transformation</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MGD (10)</td>
<td>54 ± 11</td>
<td>1.5</td>
<td>1</td>
<td>100</td>
<td>100</td>
<td>10</td>
<td>90</td>
<td>7.6 ± 1.9</td>
<td>62 ± 88</td>
<td>48 ± 14</td>
<td>183 ± 202</td>
<td>10 ± 2.4</td>
</tr>
<tr>
<td>MDS (3)</td>
<td>64 ± 4</td>
<td>1</td>
<td>2</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>7.3 ± 0.4</td>
<td>9 ± 4</td>
<td>57 ± 5</td>
<td>44 ± 22</td>
<td>0 ± 2.0</td>
</tr>
<tr>
<td>Secondary ANLL (11)</td>
<td>68</td>
<td>F</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>10</td>
<td>90</td>
<td>12</td>
<td>0 ± 1</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SD.
*One in still in complete remission.

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In the group of acute transformations, all patients died within the first 2 months except one with chronic myelogenous leukemia (CML) in blast crisis who achieved complete remission after two daunorubicin, Ara-C, thioguanine (DAT) courses. However, 3 months later this patient relapsed and is currently receiving reinduction therapy.

Morphology and cytochemistry. The morphological appearance of the blast cells varied from case to case; they were generally undifferentiated, either with a small lymphoid-looking appearance or larger in size with more abundant basophilic cytoplasm and cytoplasmic blebs. Blast cells surrounded by shed platelets and recognizable micromegakaryocytes were also observed (Fig 1). These latter forms were more frequently seen in the acute transformations of previous MPDs, which led us to suspect, in morphological terms, the presence of a megakaryoblastic component in all but one of these patients (Table 2). On the other hand, in only one ANLL case was it possible to recognize transitional forms between the undifferentiated blasts and micromegakaryocytes, the majority of these de novo leukemias being classified as undifferentiated, monocytic, or erythroid due to the predominant cell component (Table 3).

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Bone marrow fibrosis was present in most patients with a previous MPD (90%) but was absent in all of the three megakaryoblastic transformations of MDS. The patient with secondary leukemia had severe BM fibrosis, as did 65% of the de novo leukemias.

The mean survival rates of the 14 patients with de novo AMKL was 7.6 months. Three patients achieved complete remission with conventional therapy for AML, and one is still in continuous complete remission.

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pattern preferentially in the blebs. Patients with an erythroid component were not computed.

**Immunophenotype.** A notorious megakaryoblastic population (>30% positive blast cells) was detected in 15 patients (seven de novo AMKL, seven acute transformations of MPD, and the secondary leukemia), whereas in the remaining 13 cases the percentage of positive blast cells for platelet markers ranged between 10% and 30% (Tables 2 and 3).

Only two cases, both CGL in blast crisis (CGL-BC) (cases 18 and 19), were considered pure megakaryoblastic leukemias since in the remaining patients the megakaryocytic component coexisted with another cell population (mixed cases): granulomonocytic (19 cases), erythroid (three cases), or both granulomonocytic and erythroid (two cases). In addition, a lymphoid (TdT+, J5+) component coexisting with a granulomonocytic and megakaryoblastic cell population was detected in two patients (one de novo leukemia and

<table>
<thead>
<tr>
<th>Table 2. Immunologic Markers in Patients With AMKL Following a Previous MPD (Cases 15 to 24), MDS (Cases 25 to 27), and Multiple Myeloma (Case 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lymphoid</strong></td>
</tr>
<tr>
<td><strong>Case/Reference</strong></td>
</tr>
<tr>
<td>15/506</td>
</tr>
<tr>
<td>16/783</td>
</tr>
<tr>
<td>17/224</td>
</tr>
<tr>
<td>18/1892</td>
</tr>
<tr>
<td>19/1418</td>
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<td>20/2363</td>
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<tr>
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<td>22/491</td>
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</tr>
<tr>
<td>24/2345</td>
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</tr>
<tr>
<td>24/2345</td>
</tr>
<tr>
<td>25/630</td>
</tr>
<tr>
<td>26/885</td>
</tr>
<tr>
<td>27/2038</td>
</tr>
<tr>
<td>28/2854</td>
</tr>
</tbody>
</table>

*Abbreviations: MY, myelocytic; ER, erythroid; CMF, chronic myelofibrosis; MO, monocytic; MK, megakaryoblastic; MM, multiple myeloma; PV, polycythemia vera.*
CHARACTERIZATION OF MEGAKARYOBLASTIC LEUKEMIAS

One transformation of chronic myelofibrosis). The single expression of either common ALL antigen (five cases) or TdT activity (one case) was not considered specific for the assessment of a lymphoid lineage.

Some discrepancies in the percentage of cells detected by the two anti-GP Ib/IIa antibodies (CDw41)—J15 and FMC24—were observed: FMC24 was negative in two J15-positive cases, whereas in two other cases it reacted with twice the number of cells as compared with J15 (Tables 2 and 3). There were no major differences between J15 and C17 (GP IIIa), although in a pure megakaryoblastic case (no. 19) the latter antibody reacted with 70% blast cells compared with only 15% J15-positive cells. Six cases (no. 4, 8, 10, 11, 16, and 24) displayed a significantly lower reactivity with anti-GP IX (FMC25) than with anti-GP Ib/IIa (J15); furthermore, two cases, negative for FMC25, expressed J15.

Upon analyzing the expression of myeloid markers on megakaryoblasts although specific double labeling was not systematically performed, the percentage results suggested that megakaryoblasts are generally negative for CD13/11 (granulomonocytic), CD15 (granulocytic), and CD14 (monocytic) antigens. Therefore, we were dealing with different populations of cells, as was suspected according to the morphological and cytochemical findings. Nevertheless, in three cases there was an overlap of My7- (CD13) and J15 (CDw41)-positive cells, thus suggesting that individual blast cells may simultaneously express platelet and granulomonocytic antigens. Double labeling performed in one of these cases showed the occasional presence of My7+ J15+ cells.

In a mixed erythroid-megakaryocytic secondary leukemia, (case 28) half of the cells exhibited platelet glycoproteins and the other half glycoporphin A, almost all cells being positive for OKT9.

The expression of precursor cell markers on megakaryoblasts is difficult to assess in the present series due to the frequent involvement of more than one lineage. Therefore, we shall only comment on the pure cases and those with a predominant megakaryocytic component. HLA-DR and 3C5 were clearly positive in several of these patients, including one pure megakaryoblastic case. However, both of them were negative in the other pure case (no. 19), which showed 70% C17+ cells, whereas FMC8 (CD9) was positive in both cases (Table 2).

DISCUSSION

Using a systematic approach, we analyzed the presence of megakaryoblasts in all acute leukemias other than ALL in patients who were referred to our Institution for immunophenotyping. The prevalence of leukemias with involvement of the megakaryoblastic lineage in the entire series was 11.4%.

The relatively common presence of megakaryoblasts as a part of the leukemic transformation in patients with MPD has been previously reported, although the percentages are highly variable: 51%,23 23%,22 and 10%.21 In the adult cases of de novo ANNL, this incidence is less documented and ranges between 11.8% in the series reported by Huang et al2 and 8.4%, 3.6%, and 1.6% in the series of Ruiz-Arguelles et al,16 Van der Reijden,24 and Neame et al,25 respectively. However, these series were either short or included secondary leukemias, or not all cases were systematically tested with platelet antibodies, so these incidences should be regarded with caution. In view of the number of individual cases reported, the frequency of AMKL in children seems to be higher.26-29 Nevertheless, the incidence in the series studied at the University of Minnesota (12%)28 is similar to that found in our adult series. No references exist concerning the prevalence of megakaryoblastic involvement in secondary leukemias, although several cases have been reported.4,11,12 Something similar occurs with the MDS evolving to acute leukemia, the incidence in our previous analysis41 being 22%, slightly higher than in the present study (12%).

Some of the clinical and hematologic characteristics of the adult patients with de novo AMKL (BM fibrosis, low WBC count, and normal or increased numbers of platelets despite severe anemia) may be helpful in suspecting such a diagnosis. BM fibrosis should be considered secondary to activating fibroblast factors synthesized by megakaryocytes11,12 rather than as an intrinsic part of the disease.3,13 Nevertheless, in agreement with previous reports,11 this BM fibrosis was not a constant feature in our patients. Adult patients with AMKL respond poorly to conventional chemothera-
In this series, only 21% of the de novo AMKL patients achieved complete remission. The high mean age of this group of patients (64 ± 16 years) could partially explain these disappointing results.

The highly polymorphic appearance of megakaryoblasts and the absence of a specific cytochemical staining pattern makes the use of other methods such as PPO with EM or immunologic markers essential for the diagnosis of these leukemias.

Only two cases were considered pure megakaryoblastic leukemias. In the remaining 26 patients, the megakaryoblastic component coexisted with the involvement of another cell population. The coexistence of several cell lineages has been observed in patients with megakaryoblastic transformation of CGL. However, this possibility is generally ignored in de novo AMKL although in certain individual cases it has been suspected. Such a coexistence of blast cells from different cell lineages might suggest that the target cells for the malignant transformation in leukemias with megakaryoblastic involvement correspond to a less differentiated stem cell (probably CFU-GEMM) than that of the granulocytic and/or monocytic leukemias (CFU-GM).

A comparative analysis of the platelet markers used in this study is in keeping with other observations showing that MoAbs against the GP Ib complex (GP Ib plus GP IX) (CDw42) detect a lower proportion of blast cells than do anti--GP Ib/IIIa and anti--GP IIIa. This would be because the former marker (CDw42) appears later during megakaryocytic differentiation. Nevertheless, the anti--GP IX used in the present study (FMC25) proved to be a good megakaryocytic marker since it was positive in 92% of the megakaryocytic cases tested. This proportion is higher than that reported by Bethelheim et al (44%) and Erber et al (80%) with an anti--GP Ib MoAb (AN51). The difference can be related to the binding epitope for these MoAbs in the GP Ib complex (GP Ib for AN51 and GP IX for FMC25).

The discrepancies between the two anti--GP Ib/IIIa (J15 and FMC24) used in this study might be due to the lack of specificity of FMC24 since it has been seen to be positive in some AML cases, which suggests that although it was clustered by the Oxford workshop into CDw41 it could be directed against a different epitope. Only minimal differences between the reactivity of J15 and C17 were observed. However, C17 (anti--GP IIIa) allowed us to establish the diagnosis of a pure megakaryoblastic leukemia with an otherwise minimal expression of the other GP, which supports the notion that this GP is the first to be expressed during megakaryoblastic maturation. Conflicting results have been reported regarding HLA-DR expression by megakaryoblasts. In our work, it was observed that there were negative cases compared with other clearly positive ones. Koike et al and Vinci et al suggest that this antigen probably appears early in the differentiation of this lineage and is subsequently lost, which could account for the discrepant findings. Nevertheless, the HLA-DR antigen would appear, at least on some occasions, after GP IIIa. The cells in the pure megakaryoblastic leukemia mentioned earlier displayed a high reactivity for GP IIIa, whereas the other GPs were only expressed in a minority of cells, thereby indicating that we were dealing with very early megakaryoblasts. These cells were lacking in HLA-DR antigen, which would indicate that GP IIIa might be acquired earlier than the HLA-DR marker in the maturation of megakaryoblasts.

The expression of myeloid markers on megakaryoblasts has received very little attention. However, Bethelheim et al reported the occasional finding of a remarkable overlap in the percentage of blast cells with granulomonocytic and platelet markers, as was also observed in our study. These results together with the recent report by Koike et al point to the notion that megakaryoblasts may be able to express early myeloid markers such as M9 (CD33) and more rarely My7 (CD13), although they would be negative for specific granulocytic (CD15) and monocytic (CD14) markers.

The transferrin receptor, although absent in platelets, has been described in one case of AMKL. We have had the opportunity to study a mixed erythroid-megakaryocytic secondary leukemia in which almost all cells reacted with OKT9. However, since this marker was negative in all other cases explored, it seems that megakaryoblasts do not have transferrin receptors; the aforementioned findings should be interpreted as an aberrant expression of a marker due to the neoplastic nature itself of the blast cells.

In summary, involvement of the megakaryocytic lineage in acute leukemias occurs more frequently than previously suspected; antiplatelet GPs are the most useful tools for such a diagnosis in routine laboratory assays. However, some hematologic and clinical features as the presence of morphologically more differentiated cells of the megakaryocytic lineage, BM fibrosis, low WBC count, and normal or increased platelet count may also be of help in suspecting this type of leukemia in which another cell component is generally present together with the megakaryoblastic population.

Acknowledgment

We are grateful to the following colleagues: for the gift of the monoclonal antibodies used in this study, Drs H. Zola (FMC10, FMC56, FMC24, FMC25), W. Knapp (VIM2, VIMDS), P.A.W. Edwards (LICR, LON/R10), A. McMichael (J15), P. Tetteroo (C17), F. Garrido (GRB1), B. Haynes (3A1), R. Tindle (3C5), P. Beverley (UCHM1); to all physician-hematologists of Castilla-Leon who sent us blood samples for immunophenotyping; and to G. Ercilla, M.T. Gonzalez, and P. Fernandez for technical assistance.

REFERENCES

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