Acute Leukemia After a Primary Myelodysplastic Syndrome: Immunophenotypic, Genotypic, and Clinical Characteristics


We studied the nature of blast cells in 41 patients with acute leukemia following a previous primary myelodysplastic syndrome (MDS) by a combined multiparameter analysis including morphologic, immunophenotypic, and molecular genetic (Igs, T-cell receptor (TCR)-β, γ, and δ and the major breakpoint cluster region [M-bcr]) investigations. In addition, the clinical and hematologic characteristics according to the immunophenotype of blast cells were analyzed. Our results show that, although the granulocytic and/or monocytic lineages are those most commonly involved in these acute leukemias, other cell components, including the megakaryocytic and lymphoid, may be present (12% and 15% of the cases, respectively). Moreover, both morphologic and phenotypic studies show the frequent coexistence of two or three cell populations. Interestingly, in all cases the lymphoblastic component constantly displayed an early B phenotype (CD19+, CD10+, TdT+). Upon analyzing whether the type of MDS conditioned any differences in the immunophenotype of blast cells, we observed that, although the lymphoid lineage may be involved in all MDS subgroups, some differences emerge within the myeloid leukemic transformations.

Thus, the refractory anemias with excess of blasts (RAEB) and RAEB in transformation displayed a significantly higher incidence of myeloblastic and megakaryoblastic transformations, while in the RA, RA with ring sideroblasts and chronic myelomonocytic leukemia, the granulo-monocytic phenotype predominated. In addition, our results show that the clinical and hematologic characteristics of these patients may be partially related to the immunophenotype of the blast cells. Ig heavy chain gene rearrangements were found in two of 19 patients analyzed (11%), one with a hybrid leukemia (lymphoid-myeloid) and the other with a granulomonocytic phenotype. Two other hybrid transformations analyzed were in germ line configuration, γ and δ gene rearrangements were found in 21% and 37% of these acute transformations, respectively. The TCR-β and M-bcr were in germ line configuration in all 19 cases studied. In summary, immunophenotype and molecular studies point to a pluripotent stem cell with preferential myeloid commitment as the target cell of leukemias following a primary MDS.

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Materials and Methods

Patients

Forty-one patients with an overt AL (>30% blast cells) following a previous primary MDS were included in the study. All investigations were performed at the time of transformation into AL. The median number of blast cells in the specimens analyzed was 65%, with 87% of the cases having more of 50% of blast cells. The initial diagnosis of the MDS according to the French-British (FAB) criteria was: refractory anemia (RA), 10 cases; RA with ring sideroblasts (RAS), six cases; RAEB, 14 cases; RAEB in transformation (RAEBT), six cases; and chronic myelomonocytic leukemia (CMML), 5 cases. The median age at presentation of the AL was 72 years, 86% of the cases being over 60
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years. All patients were managed conservatively until the development of overt AL. At the time of diagnosis of AL, 18 patients were treated with intensive chemotherapy including daunorubicin/cytarabine (ARA-C)/thioguanine; nine received low dose ARA-C and thioguanine and 14 (all over 75 years of age and with a poor performance status) were treated with supportive therapy. No significant differences in overall survival were observed according to the treatment used.

Cell Morphology

BM and peripheral blood (PB) smears were stained with May-Grünwald-Giemsa (MGG) and cytochemical methods for peroxidase and nonspecific esterase both with and without sodium fluoride inhibition.

Immunologic Markers

Mononuclear cells obtained at the moment of diagnosis of acute transformation were isolated by Ficoll-Hypaque density gradient centrifugation from PB (76%) and/or BM (24%) and analyzed by direct and indirect immunofluorescence with a terminal deoxynucleotidyl transferase (TdT) heteroantiserum (Supertechs, Bethesda, MD) and a panel of monoclonal antibodies (MoAbs) whose reactivity and specificity has been previously described. For early myeloid cells we used My9 (CD33) and My7 (CD13); for granulocytic lineage, VIMDS and FMC10 (CD15); for monocytic lineage, FMC17 and leuM3 (CD14); for megakaryoblasts, J15 (CD41, anti-glycoprotein [GP] IIb/IIIa), C17 (CD61, anti-GPIIb/IIIa), FMC25 (CD42a, anti-GPIX), and AN51 (CD10, anti-GPib); for erythroid lineage, LICR LON R10 (anti-glycophrin A); for precursor cells, GRB1 (anti-IL-3ADR) and FMC56 (CD9); and for lymphoid lineage, B4 and leu12 (CD19), B1 (CD20), 3A1 (CD7), and J5 (CD10).

Cells were analyzed either by fluorescence microscopy (43% of the cases) (Leitz Ortholux, Wetzlar, Germany) or flow cytometry (53% of the cases) (FACScan; Becton Dickinson, Mountain View, CA). To avoid the false-positivity due to contaminating normal cells, the results were referred to blast cells identified either by phase contrast or multiparameter analysis of the list mode data using the color-based "Paint-A-Gate" software (FACScan; Becton Dickinson). Moreover, the results were correlated with the morphology assessed on cytocentrifuge slides prepared with mononuclear cells and the percentages corrected according to the number of residual normal cells. For the assessment of lineages, a minimum of 15% blast cells positive for one or more of the specific MoAbs was required. The presence of mixed-lineage immunophenotypes was assessed by appropriate individual double stainings, using the following marker combinations: TdT/CD13 labeled with tetramethylrhodamine isothiocyanate (TRITC) and fluorescein isothiocyanate (FITC), respectively, as previously described. CD19/CD13 and CD61/CD13 analyzed by direct immunofluorescence (IF) technique in flow cytometry (CD13 being phycoerythrin [PE]-labeled and the other antibodies FITC-labeled). To minimize nonspecific Fc receptor binding, the cells were incubated at room temperature for 15 minutes with AB serum to block the Fc receptors and washed in a buffer containing phosphate-buffered saline–bovine serum albumin–AB (PBS-BSA-AB) serum. Additionally, as a negative control, we have used irrelevant isotype-matched MoAbs in all steps of the experiments.

DNA Analysis

High molecular weight DNA was obtained by lysis of leukemic cell samples with sodium dodecyl sulfate (SDS) and proteinase K, followed by extraction with phenol-chloroform and chloroform and further precipitation with ethanol. The DNA samples were digested with restriction endonucleases BamHI, EcoRI, HindIII, or Bgl II (Boehringer, Mannheim, Germany), electrophoresed in 0.8% agarose gel, blotted, and hybridized as described elsewhere. The probes were labeled by a random priming method to a specific activity of 1 to 2 x 10^7 cpm/μg of DNA. After hybridization, the filters were washed stringently at 65°C, dried, and autoradiographed.

Ig gene analysis was performed using genomic probes: an Ig heavy-chain joining (JH) region probe (6 kb, BamHI-HindIII fragment). TCR-β chain gene rearrangement was detected using a 770-bp cDNA constant region probe (M131B10BB1). The configuration of the TCR-γ genes was analyzed by use of the H60 probe, a 0.77 EcoRI-HindIII fragment containing Jγ1.3 segments and hybridizes to both Jγ1.3 and Jγ2.3 gene segments. TCR-γ gene rearrangements were detected with J616 (a 1.5-kb Sac I fragment), which recognizes J61 sequences, and the J62 probe R21XH (a 2.3-kb HindIII-EcoRI fragment).

Analysis of M-bcr breakpoint. Two probes were used to identify M-bcr breakpoint locations: a 0.6-kb fragment, corresponding to a Pot-1-Bgl II fragment hybridizing intronic sequences located between M-bcr exons 3 and 4, designated as the 3' M-bcr probe; and a 2-kb probe corresponding to a Bgl II-HindIII fragment containing exon 1 and all sequences comprised in subregion 0, designated the 3' M-bcr probe.

Statistical Analysis

Clinical disease characteristics were correlated with the immunophenotype of blast cells using both the χ² test and the Fisher's test for categorical variables.

RESULTS

Morphology

Because the FAB criteria frequently cannot be applied for AL following a previous MDS, a descriptive morphologic classification was used; the blast cells being assigned to one of the following possible cell lineages: granulocytic, monocytic, erythroid, megakaryocytic, or lymphoid. The existence of a pure single blast cell population (always granulocytic or monocytic) was evidenced in 14 cases (34%), while the coexistence of two or three cell populations was recognized in 17 (41%) and seven (17%) cases, respectively. Among these mixed leukemias, megakaryoblasts were morphologically suspected in six cases (15%) and erythroid blast cells in three (7%). Additionally, in eight patients, (19%) lymphoblasts, together with other myeloid cell components, were recognized. In three patients the blast cells were considered morphologically to be undifferentiated. The distribution of these patients, according to the FAB proposals, was as follows: one MO, five M1, two M2, 14 M4, eight M5, two M6, three M7, and six mixed (myeloid-lymphoid) leukemias. Nevertheless, as mentioned above, it should be noted that the FAB criteria cannot be easily applied to these types of leukemias.

Immunophenotype

The blast cells in the majority of acute transformations of MDS (85%) displayed a myeloid phenotype (Table 1), while in six cases (15%) a hybrid (myeloid-lymphoid) phenotype was detected (Tables 1 and 2). Double staining showed that all six of these hybrid cases corresponded to bilineal leukemias in which, together with the lymphoid...
component, different myeloid lineages were involved (Table 2). Interestingly, the lymphoid cells consistently displayed an early B phenotype (TdT⁺, CD19⁺, CD10⁻). Furthermore, these lymphoid components were present in all MDS subgroups, except in CMML, and were specially frequent in RAS. Within the myeloid leukemic transformations, five phenotypical subgroups could be recognized (Table 3), with relevant differences among the MDS subgroups. Thus, while most RA (67%), RAS (100%), and CMML (80%) expressed monocytic Ags (CD14) either alone (monocytic phenotype) or in combination with granulocytic Ags (CD15) (granulomonocytic phenotype), this finding was exceptional in the RAEB (8%) and RAEBt (20%) cases ($P < .001$). By contrast, the myeloblastic (positivity for only CD13/CD33) and megakaryoblastic (CD14/61⁺) phenotypes were more frequently detected in the RAEB and RAEBt cases (Table 3). In all megakaryoblastic transformations, megakaryoblasts coexisted with other myeloid components. No cases of erythroid transformation were detected by immunologic means in the present series. One patient expressing CD13 Ag and a morphologic myeloblastic transformation exhibited TdT activity in 80% of cells but lacked other lymphoid markers (CD19⁻, CD10⁻, CD7⁻), thus considered to be a TdT⁺ myeloblastic transformation.

**Clinical and Hematologic Characteristics According to the Immunophenotype**

The most relevant clinical characteristics, according to the immunophenotypic of blast cells, are summarized in Table 4. The myeloblastic transformations were preceded by a short chronic phase that was clinically characterized by the absence of visceromegalies. By contrast, patients with granulomonocytic transformations displayed frequent organ involvement and a short survival. The megakaryoblastic transformations predominated in females, showed a high incidence of hepato-splenomegaly (60% of cases), and were

<table>
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<tr>
<th>Table 1. Immunophenotype of AL Following MDS</th>
<th>Table 3. Immunophenotypical Findings in AML Following MDS</th>
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<tbody>
<tr>
<td><strong>Myeloid</strong></td>
<td><strong>Hybrid</strong></td>
</tr>
<tr>
<td>RA (n = 10)</td>
<td>9* (90%)</td>
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<tr>
<td>RAS (n = 6)</td>
<td>4 (67)</td>
</tr>
<tr>
<td>RAEB (n = 14)</td>
<td>12 (86)</td>
</tr>
<tr>
<td>RAEBt (n = 6)</td>
<td>5 (83)</td>
</tr>
<tr>
<td>CMML (n = 5)</td>
<td>5 (100)</td>
</tr>
<tr>
<td>Total (n = 41)</td>
<td>35 (85)</td>
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</tbody>
</table>

*One case TdT⁺; CD19⁺.

| RA (n = 9)  | 2* (0) | 0 (0) | 6 (1) | 1 (15) |
| RAS (n = 4) | 0 (0) | 2 (33) | 0 (0) | 1 (17) |
| RAEB (n = 12) | 5 (50) | 1 (17) | 5 (50) | 1 (17) |
| RAEBt (n = 6) | 2 (17) | 1 (17) | 5 (83) | 1 (17) |
| CMML (n = 5)  | 1 (20) | 1 (20) | 1 (20) | 1 (20) |

MO + GR-MO phenotypes in RA/RAS v RAEB/RAEBt $P < .001$; MB + MK phenotypes in RA/RAS v RAEB/RAEBt $P < .044$.

Abbreviations: MB, myeloblastic; GR, granulocytic; MO, monocytic; MK, megakaryocytic.

*In all megakaryocytic transformations megakaryoblasts coexisted with another myeloid component.

| RA (n = 1) | 0 (0) | 1 (1) | 0 (0) |
| RAS (n = 2) | 1 (1) | 1 (1) | 0 (0) |
| RAEB (n = 2) | 1 (1) | 1 (1) | 0 (0) |
| RAEBt (n = 1) | 1 (1) | 1 (1) | 0 (0) |

Abbreviations: MB, myeloblastic; LY, lymphoid; GR, granulocytic; MO, monocytic.

The most relevant clinical characteristics, according to the immunophenotypic of blast cells, are summarized in Table 4. The myeloblastic transformations were preceded by a short chronic phase that was clinically characterized by the absence of visceromegalies. By contrast, patients with granulomonocytic transformations displayed frequent organ involvement and a short survival. The megakaryoblastic transformations predominated in females, showed a high incidence of hepato-splenomegaly (60% of cases), and were the group with the highest proportion of response to therapy, leading to a relatively longer survival (4.6 months).

The hybrid transformations were outstanding owing to the frequent tendency to infiltrate skin and/or the central nervous system (CNS) (3 of 5 patients; Table 4).

All transformations from a previous MDS displayed severe anemia and thrombocytopenia despite having low white blood cell (WBC) counts, except the hybrid transformations that exhibited a more intense leukocytosis together with a higher BM infiltration and a more pronounced thrombocytopenia (Table 5). No relevant differences were observed among the remaining phenotypic subgroups. Thus, the myeloblastic transformations only stood out by having more preserved hemoglobin (Hb) levels, while the granulomonocytic and megakaryocytic subgroups had a similar hematologic profile (Table 5).

**Molecular Genetic Studies**

Rearrangement of the Ig heavy chain region was found in two of the 19 patients analyzed (11%). One of these patients corresponded to a hybrid (B-lymphoid/myeloid) leukemia following a previous RAEB and the other to a blast crisis (BC) of CMML with a granulo-monocytic phenotype (Tables 6 and 7). Two other hybrid transformations analyzed were in germline configuration. The TCR-β gene was detected in germline configuration in all the patients studied (Table 6). TCR-γ gene rearrangements were observed in four of the 19 cases, all of them with a previous diagnosis of either RAEB or RAEBt (Table 7) with a random phenotypic distribution. In two patients, the TCR-γ gene was the only locus being rearranged, while in the remaining two cases, either IgH or TCR-δ gene rearrangements coexisted. Rearrangements of the TCR-δ locus were detected in seven of 19 patients studied with the J61 and J62 probes. No correlation with the FAB classification was observed (Table 7). Interestingly, regarding the immunophenotypic distribution, all three hybrid leukemias showed the TCR-δ gene in germline configuration, while in two of the three megakaryoblastic leukemias it was rearranged (Table 6). Two TCR-δ gene recombinations coexisted with either IgH or TCR-γ gene rearrangements. In addition, in these 19 patients the organization of the BCR
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Additionally, so far the immunologic and molecular features of these leukemias have not been studied with a systematic approach in a uniform series of patients. The nature of blast cells in leukemias following a previous MDS has been classically assumed to be of myeloid (granulocytic and/or monocytic) lineage. However, our recent results show that, although this is the commonest form of transformation, other cell lineages (including the lymphoid) may also be involved, supporting the notion that the target cell for these leukemias could be a pluripotential stem cell. The involvement of the megakaryocytic lineage has been previously suspected on morphologic grounds, which was also the case in the present series. However, for a definitive diagnosis, ultrastructural (platelet peroxidase) or immunologic (GPIIIa or GPIIb/IIIa) markers are required. Although indirect data support evidence of this lineage involvement in MDS, until now the presence of megakaryoblasts in acute transformation of MDS has not been established. The incidence of megakaryocytic transformations in the present series (12%) was slightly higher than that reported in de novo acute myeloid leukemia (AML) (8%) but lower than that found in BC of myeloproliferative disorders (31% to 35%). Moreover, in MDS (similar to the picture found in AML and BC of CML) megakaryoblasts generally coexist with other myeloid blast cells. Although erythroleukemia following a previous history of MDS has been reported, the low incidence of erythroid transformation is outstanding in the present series (three cases morphologically suspected and none by immunologic means), particularly if it is considered that abnormalities of the erythroid precursors are one of the commonest features of MDS. Because glycophorin A is a relatively mature erythroid marker, it is conceivable that the incidence of this lineage involvement has been underestimated; nevertheless, using the same MoAbs, erythroleukemias were diagnosed in 4% of our de novo AML.

Acute lymphoid leukemias (ALL) following a primary MDS have been previously reported either as a pure population of lymphoblasts or coexisting with other myeloid components. In the present series the incidence was 14%, being more frequent among RAS patients; this is in agreement with some of the individual case reports. Interestingly, in all our cases, lymphoblasts displayed an early B phenotype (TdT+; CD19+; CD10-) that differed significantly from that found in the lymphoblastic transformations of CML that are generally CD10+. This finding raises intriguing questions about the molecular mechanisms responsible for such diversity in antigenic expression.

In the present study, we also analyzed the possible immunophenotypical differences according to the type of MDS. Except in CMML, which seems to bear a certain lineage fidelity in the transformation (monocytic or granulomonocytic), in the other MDS, all cell lines, including the lymphoid, may be involved. However, the type of MDS did condition, at least partially, a preferential model of transformation. Thus, in RA and RAS the granulo-monocytic immunophenotypes predominate, while the myeloblastic and megakaryoblastic transformations were significantly more frequent among the RAEB and RAEBt. Moreover, our recent results suggest that the disease characteristics may be at least partially related to the type of blast cells. Thus, patients with hybrid transformation displayed frequent skin and CNS involvement together with a higher degree of BM infiltration and leukocytosis, features that could be attributed to the lymphoid component present in these transformations. Additional studies including larger series of patients would shed further light on these correlations.

<table>
<thead>
<tr>
<th>Table 4. Clinical Characteristics of AL Following MDS According to the Immunophenotype</th>
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<tbody>
<tr>
<td>Age (y)</td>
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<tr>
<td>--------</td>
</tr>
<tr>
<td>Myeloblastic (n = 10)</td>
</tr>
<tr>
<td>Granulocytic/monocytic (n = 20)</td>
</tr>
<tr>
<td>Megakaryoblastic (n = 6)</td>
</tr>
<tr>
<td>Hybrid (n = 6)</td>
</tr>
</tbody>
</table>

Results expressed as median, except for survival (X ± SD).

Abbreviations: PR, partial response to therapy (decreased ≥ 50% of BM blast cells). No cases with complete remission were found.

*Skin infiltration (confirmed by biopsy).
†One case with both skin and CNS infiltration (confirmed by biopsy and lumbar puncture, respectively).

Table 5. Biologic Characteristics of AL Following MDS According to Immunophenotype

<table>
<thead>
<tr>
<th>Immunophenotype</th>
<th>Hb (g/dL)</th>
<th>WBC (x 10^9/L)</th>
<th>Platelets (x 10^9/L)</th>
<th>Blasts BM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloblastic (n = 10)</td>
<td>9.2</td>
<td>15</td>
<td>28</td>
<td>67</td>
</tr>
<tr>
<td>Granulocytic/monocytic (n = 20)</td>
<td>7.5</td>
<td>11</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>Megakaryoblastic (n = 5)</td>
<td>8.3</td>
<td>11</td>
<td>30</td>
<td>55</td>
</tr>
<tr>
<td>Hybrid (n = 6)</td>
<td>6.9</td>
<td>23</td>
<td>16</td>
<td>72</td>
</tr>
</tbody>
</table>

Results expressed as median.
while the TCR-P gene was always in germline, two patients had rearranged Ig heavy chain sequences. In one of them, the reorganization of the IgH region was expected because the rearranged case was a CMML with a bona fide myeloid origin for the target cell of leukemias following a primary MDS.43,44 Moreover, using molecular analysis, Smadja et al37 have recently reported rearrangement of M-bcr in a patient with RAEB that evolved into RAEBt. Here, we have investigated the origin for the target cell of leukemias following a primary MDS.

The presence of the Philadelphia (Ph) chromosome has been observed exceptionally in MDS.45,46 Moreover, using molecular analysis, Smadja et al37 have recently reported rearrangement of M-bcr in a patient with RAEB that evolved into RAEBt. Here, we have investigated the origin for the target cell of leukemias following a primary MDS.

In summary, we have found that all cell lineages, including the megakaryocytic and lymphoid, may be involved in these patients, pointing to a pluripotent stem cell origin. In addition, certain particular phenotypic characteristics, such as the early B-cell phenotype of the lymphoid transformation, suggest the existence of intriguing ontogenic differences with other related situations, such as the lymphoid BC of CML (pre-B phenotype). The present findings also show that the immunophenotype of blast cells is partially related to the disease characteristics. Finally, molecular genetic studies illustrate unexpected rearrangements of the IgH and the TCR-γ and δ genes. Further investigations should be performed to clarify the pathogenic role of these molecular abnormalities in the leukemias following a primary MDS.

ACKNOWLEDGMENT

We are grateful to the following colleagues: for the gift of monoclonal antibodies used in this study, Drs H. Zola (FMCl0, FMCS6, FMCS 25), W. Knapp (VIM2, VIMD3), P.A.W. Edwards (LICR, LON/R10), A. McMichael (J15), P. Tetteroo (C17), F. Garrido (GRBl), and B. Haynes (3A1); for providing us the probes used in this study, S.J. Korsmeyer (JH, CK), T.H. Rabbits (TP, TY, T8), and J.M. Goldman (3’M-bcr, 5’M-bcr); and to all physician-hematologists of Castilla-Leon who sent us blood samples for study.
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Acute leukemia after a primary myelodysplastic syndrome: immunophenotypic, genotypic, and clinical characteristics [see comments]

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