Clonal Origin of Cells Restricted to Monocytic Differentiation in Acute Nonlymphocytic Leukemia

By Anna Maria Ferraris, Giorgio Broccia, Tullio Meloni, Letizia Canepa, Mario Sessarego, and Gian Franco Gaetani

Two patients with acute monocytic leukemia and heterozygous for the Mediterranean variant of the X-linked enzyme, glucose-6-phosphate dehydrogenase (G6PD), were investigated to determine the number and type of progenitor cells involved. Mosaicism for Mediterranean G6PD was assessed by the different rate of utilization of 2-deoxyglucose-6-phosphate (2dG6P) by normal and Mediterranean variants of G6PD. The monocytic blasts were found to express one type of G6PD only, indicating their clonal origin from a common progenitor cell, whereas all other hematopoietic cell populations tested expressed the heterozygous phenotype. The finding of a unique involvement of the monocytic line in two cases of acute nonlymphocytic leukemia (ANLL) represents further evidence of heterogeneity of stem cell involvement in ANLL.

CASE REPORTS

Case 1

A 63-year-old woman was diagnosed as having acute nonlymphocytic leukemia in February 1983. There was no previous history of exposure to radiation or leukemogens. Physical examination on admission was unremarkable, except for diffuse purpura and a liver edge palpable 4 cm below the right costal margin. The blood count showed a hemoglobin of 11 g/dL, 0.1% reticulocytes, platelets 20,000/μL, and white cells 48,000/μL with a differential count of 94% blasts, 4% granulocytes, and 2% lymphocytes. A marrow aspirate was hypercellular; the erythroid and megakaryocytic components were virtually absent and replaced by a homogeneous population of large immature cells with abundant basophilic cytoplasm, rare azurophilic granules, and occasional pseudopodia; the nucleus was often cleaved, with one to three prominent nucleoli (Fig 1). More than 95% of these cells stained positively for peroxidase and α-naphthyl acetate esterase; the latter reaction was completely inhibited by sodium fluoride. Immunologic studies showed that the blast cells uniformly lacked receptor for sheep erythrocytes, surface and cytoplasmic immunoglobulins, and were DR positive and common acute lymphocytic leukemia antigen (CALLA) negative. These features were considered to be consistent with a FAB M5 type of ANLL.

Of seven marrow metaphases studied after 12 hours in culture without mitogen, all had the following karyotype: 46,XX,del(11)(q13). Poor specimen conservation did not allow more detailed cytogenetic analysis.

Remission induction chemotherapy with high-dose cytosine arabinoside was begun; after two courses of chemotherapy, a marrow aspirate showed a slightly reduced cellularity with less than 4% blasts. At that time, no chromosomal abnormalities were detected in 11 marrow metaphases. After two months on maintenance treatment, the patient’s disease relapsed, and a second remission was not achieved despite aggressive chemotherapy. She died of gastrointestinal hemorrhage five months after diagnosis.

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Case 1

In October 1983, this 48-year-old woman was found to have acute monocytic leukemia. She presented with fever, easy bruising, and marked gum hypertrophy. The white cell count was 71,000/µL, with 95% blasts, platelets 18,000/µL, and Hb 6.7 g/dL. The marrow aspirate contained almost exclusively monocytic blasts. Morphological, cytochemical, and immunologic characteristics of circulating and marrow blast cells were compatible with a FAB M5 ANLL.7 No chromosome abnormalities were detected in ten marrow metaphases studied without culture or mitogen stimulation.

The patient received induction combination chemotherapy with daunorubicin and cytosine arabinoside and achieved a complete clinical and hematologic remission five weeks after diagnosis. She is currently receiving maintenance therapy and remains in remission to date.

MATERIALS AND METHODS

Samples of skin, hair follicles, and peripheral blood were examined for G6PD mosaicism. Techniques used for skin fibroblast culture, hemopoietic cell separation, and characterization of cell fractions, and preparation of cell extracts for determination of relative 2dG6P utilization have been described previously.24 Platelet contamination was checked in all white cell preparations with direct count and was found to be negligible. Erythrocyte contamination was eliminated with osmotic shock. The degree of contamination of each cell fraction was less than 3% by examination of cytocentrifuge slides stained with May-Grünewald-Giemsa (MGG), alpha-naphtyl acetate esterase (ANAE), and peroxidase. In order to obtain sufficient number of cells with homogeneous characteristics, monocytic cultures were established by the method of Packard et al.9 Briefly, heparinized peripheral blood was centrifuged over a Ficoll-Hypaque gradient; interface cells were collected, washed, and resuspended at a concentration of 5 × 10⁶ cells per mL in Fischer’s medium with 25% horse serum. One milliliter of cell suspension was added to each 35-mm Petri dish containing a 24 × 22 mm glass coverslip. After a two-hour adherence phase at 37 °C in 5% CO₂, plates were washed three times, and the adherent cells overlaid with 2 mL of culture medium, which was subsequently changed twice per week. After two to three weeks, the coverslips were removed and adherent cells harvested with gentle hypotonic treatment. Part of the cell suspension was used for 2dG6P assay; part was cytocentrifuged and the slides stained with MGG and ANAE.

RESULTS

Both patients were heterozygous for Gdβ and Gdα, as indicated by the 2dG6P relative utilization of skin, hair follicles, and cultured skin fibroblasts (Table 1).2,8

Case 2

Peripheral blood cells were assayed for G6PD mosaicism prior to chemotherapy and to any transfusion. Although only B-type G6PD was found in circulating blast cells, platelets, erythrocytes, and granulocytes exhibited a 2dG6P utilization consistent with a heterozygous phenotype (Gdβ/Gdα).2 Studies on peripheral blood cells were repeated at the time of clinical remission: erythrocytes, platelets, and granulocytes all showed the heterozygous phenotype. Monocytes harvested from 14-day cultures expressed the same heterozygote value.

At the time of relapse, a clonal origin was again demonstrated for the blast cells, but a G6PD mosaicism was present in the erythrocyte preparation. Attempts to purify other hemopoietic cell populations were unsuccessful (Table 1).

Table 1. Glucose-6-Phosphate Dehydrogenase in Hemopoietic and Nonhemopoietic Cells From Two Patients With Acute Monocytic Leukemia*

<table>
<thead>
<tr>
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<th>Case 1</th>
<th>Case 2</th>
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<tbody>
<tr>
<td>Diagnosis</td>
<td>Remission</td>
<td>Relapse</td>
</tr>
<tr>
<td>Hair follicles</td>
<td>11.8</td>
<td>8.1</td>
</tr>
<tr>
<td>Skin</td>
<td>11.9</td>
<td>8.0</td>
</tr>
<tr>
<td>Fibroblasts</td>
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<td>2.7</td>
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<tr>
<td>Erythrocytes</td>
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<td>7.8</td>
</tr>
<tr>
<td>Platelets</td>
<td>11.4</td>
<td>7.4</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>10.5</td>
<td>7.7</td>
</tr>
<tr>
<td>Monocytes</td>
<td>10.0</td>
<td>7.5</td>
</tr>
<tr>
<td>Rosetting cells</td>
<td>7.2</td>
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</tbody>
</table>

*G6PD mosaicism is expressed as percentage of relative 2dG6P utilization (G6PD B: 3.79 ± 0.73; G6PD Mediterranean: 35.15 ± 6.05; heterozygotes will express a 2dG6P utilization between these values and related to their degree of G6PD mosaicism).2
type was detected in all hemopoietic cell populations tested (erythrocytes, platelets, granulocytes, and monocytes from 20-day culture).

DISCUSSION

The two patients considered in this article were heterozygous for G6PD B and the common Mediterranean variant in tissues of nonhemopoietic origin. The morphological and cytochemical characteristics of the blast cells were remarkably similar, allowing a diagnosis of acute monocytic leukemia (M5 ANLL) for both cases.7

At presentation, only one type of G6PD was expressed in the leukemic cells, suggesting that monocytic leukemia is a clonal disease in these patients. A rather unexpected finding was the persistence of the double enzyme phenotype in the other hemopoietic cell populations, indicating that the leukemic event had occurred at the level of a progenitor cell with differentiation limited to the monocyte pathway.

Several patients with ANLL who were heterozygous for G6PD have been investigated6,10 from the analysis of four cases, Fialkow and coworkers6 have suggested the heterogeneity of ANLL, with the involvement of a multipotent stem cell in elderly patients and the preferential expression in cells with differentiation restricted to the granulocyte-monocyte line in children. On the basis of the results reported above, we suggest that ANLL with limited differentiative ability may also occur in adults.

The clinical course of our patients has also confirmed the possibility of effectively suppressing the malignant clone with conventional chemotherapy, with consequential reexpression of the heterozygous G6PD phenotype in all hemopoietic cell populations.6,10 The finding of a unique involvement of the monocyte line, with persistence of the normal heterozygous phenotype in erythrocytes and platelets in both cases, and also in the mature granulocytes in case 1, gives further support to the hypothesis of the heterogeneity of stem cell involvement in ANLL.6

Studies of direct cell preparations and hemopoietic colonies in chronic myeloproliferative disorders and acute leukemias have convincingly demonstrated that, in these diseases, monocytes share a clonal origin from a progenitor cell common to the myeloid, erythroid, and megakaryocytic lineages.6,11 In the cases considered in this article, the progenitor cell seems to be hit at a more differentiative stage of development. An alternative explanation is that the pluripotent stem cell involved in the leukemia is not capable of differentiating into mature granulocytes, erythrocytes, and platelets. The heterozygous phenotype expressed by these cell populations during the acute phase of the disease would therefore represent the residual normal preleukemic cellularity, not yet overcome by the neoplastic proliferation.

It is possible to hypothesize the presence of at least two major subtypes of ANLL. Leukemias with a multiline involvement could represent the acute phase of preleukemic syndromes or the blastic expression of previously undiagnosed myeloproliferative disorders. A multistep pathogenesis is the most likely explanation in these cases, with involvement of at least two steps: one causing proliferation of a clone of genetically unstable pluripotent hemopoietic stem cells, and the other causing the transformation and uncontrolled growth of the preleukemic clone.1,12 The second category of ANLL could include the cases reported here and the two young patients studied by Fialkow6: the restricted differentiative ability of the progenitor cell involved in the leukemic process would suggest a one-step pathogenesis of these leukemias, a hypothesis supported by the complete disappearance of the abnormal clone and the absence of residual chromosomal abnormalities during remission.

Discrimination between ANLL with restricted or multipotent differentiative expression may be of critical significance for prognostic and therapeutic implications; investigation of other patients with ANLL is necessary to determine more accurate correlations with clinical and morphocytochemical parameters.

REFERENCES


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