Reexpression of Normal Stem Cells in Erythroleukemia During Remission

By A. M. Ferraris, L. Canepa, C. Marenzi, G. Baule, T. Meloni, E. Salvidio, G. Forteleoni, and G. F. Gaetani

A patient with erythroleukemia and heterozygous for the Mediterranean variant of the X-linked enzyme glucose-6-phosphate dehydrogenase (G6PD) was studied to determine the number and type of progenitor cells in which the disease arose. G6PD mosaicism was assessed by the different rate of utilization of 2-deoxyglucose-6-phosphate (2dG6P) by normal and Mediterranean variants of G6PD. Erythroleukemia is established as a clonal disease involving a precursor cell common to the erythroid and myeloid lines. After intensive chemotherapy, restoration of nonclonal hemopoiesis is achieved, as indicated by the reappearance of the mosaic phenotype in hemopoietic cell populations.

ERYTHROLEUKEMIA is a disorder of controversial definition characterized by uncontrolled proliferation of erythroblasts and myeloblasts in the bone marrow.1 A central, and as yet unresolved, issue is whether the abnormal erythroblasts in this disease are neoplastic cells or whether the morphological abnormalities reflect the microenvironment of myeloblastic leukemia.2,3

We report evidence that erythroleukemia is a clonal disease involving a precursor cell common to the erythroid and myeloid lines. The achievement of a complete clinical and hematologic remission coincided with the restoration of nonclonal hemopoiesis, supporting the hypothesis that in erythroleukemia during remission, the marrow is repopulated by normal stem cells.

We used as a cellular marker the X-linked alloenzyme system of glucose-6-phosphate dehydrogenase (G6PD). Since in female cells only one X chromosome is genetically active, cell populations from heterozygotes will express a double-enzyme phenotype if derived from many cells, while a clonal origin will be indicated by a single-enzyme phenotype. To investigate cellular mosaicism for the Mediterranean variant of G6PD we determined the 2-deoxyglucose-6-phosphate (2dG6P) utilization of different tissues. This method is based on the different utilization of 2dG6P by the normal B enzyme and the Mediterranean variant and allows detection of a population of normal or G6PD-deficient cells as low as 5%.4

CASE REPORT

A 67-yr-old woman of Sardinian origin was admitted in January 1982 for anemia. There was no history of preceding illnesses nor of consumption of drugs. Physical examination revealed sternal tenderness, splenomegaly, and several lymph nodes palpable in the laterocervical and supraclavicular regions. The blood count showed Hb 6.4 g/dl, platelets 20,000/cu mm, and WBC 1,600/cu mm with more than 95% peroxidase-positive blasts. Reticulocyte count was 250,000/cu mm, and nucleated red cells were detected in the peripheral blood. All other hematologic and serologic tests were normal, including hemoglobin electrophoresis. A bone marrow aspirate revealed an increased cellularity, with 40% abnormal erythroid precursors (multiple nuclei, nuclear fragments, giant forms); periodic acid-Schiff (PAS) reaction was positive in 10% of these cells. With the Prussian blue stain, siderotic granules were found in the cytoplasm of most erythroblasts, but no ringed sideroblasts were present. The residual marrow cellularity was represented by a homogeneous population of peroxidase-positive blasts, with occasional promyelocytes; Auer rods were seen in a few blast cells. Chromosomal analysis of eight marrow metaphases studied without culture or mitogen stimulation showed a normal karyotype. A diagnosis of erythroleukemia was made,5 and the patient was started on remission induction combination chemotherapy.6 After three courses she went into clinical and hematologic remission.

At the time of diagnosis, samples of skin and hair follicles were tested for 2dG6P utilization and, once the patient’s heterozygous state for G6PD Mediterranean was established, further studies were undertaken to ascertain the G6PD mosaicism expressed by the different cell populations in this erythroleukemic patient.

MATERIALS AND METHODS

Samples of skin, hair follicles, and peripheral blood were examined at diagnosis and at the time of remission. Cell purification procedures were carried out as previously described.7

Part of the skin biopsy was directly tested. Fibroblasts were cultured in Eagle’s supplemented minimal essential medium. Clones were obtained by plating 10-20 cells in 80-mm Petri dishes; the resulting colonies were isolated with cloning cylinders and propagated. Cells were collected by trypsinization and lysed by freezing and thawing.

Purified cell preparations were tested for 2dG6P utilization according to Ferraris et al.; experiments were carried out in duplicate. The 2dG6P method allows detection of a 5% mosaicism for G6PD Mediterranean. Eighteen normal G6PD heterozygotes acted as controls.

RESULTS

Relative 2dG6P utilization of the different cell populations isolated from the patient at diagnosis and...
at the time of remission are reported in Table I. The 2dG6P utilization of hair follicles, skin biopsy, and uncloned fibroblasts is consistent with a heterozygous condition for G6PD Mediterranean.\textsuperscript{4} At diagnosis, circulating blast cells and erythrocytes exhibited a 2dG6P utilization compatible with a fully G6PD-deficient phenotype.\textsuperscript{4} The assay done during remission showed that granulocytes, platelets, erythrocytes, monocytes, T and B lymphocytes expressed the same G6PD mosaicism as tissues of nonhemopoietic origin.

At the onset of the disease, the 2dG6P utilization ratios between hemopoietic and nonhemopoietic cells indicated a clonal proliferation of a precursor common to the erythroid and myeloid lines. During remission, this ratio was around 1, consistent with the finding that the degree of G6PD mosaicism is the same in all tissues of normal heterozygote individuals.\textsuperscript{7} In the 18 heterozygous controls tested, the ratio between hemopoietic and nonhemopoietic cells was 0.95 ± 0.12 (mean ± SD).

Moreover, 28 clones derived from the patient's cultured skin fibroblasts were tested, and 12 of them found to express a relative 2dG6P utilization around 30%, while the other 16 were within the range of normals, thus confirming the presence of both G6PD alloenzymes in nonhemopoietic tissues (Fig. 1).

At the time of diagnosis, the patient's erythrocytes were assayed for G6PD, and the activity was 20 U/100 ml RBC (normal value 140 ± 20 U). The apparent discrepancy between a 2dG6P utilization within the G6PD-deficient range and an enzymatic activity in the lower limits of heterozygote values is explained by the patient's considerable reticulocytosis at that time. G6PD Mediterranean reticulocytes express a higher activity than mature red cells;\textsuperscript{8} the 2dG6P relative utilization, being a peculiar characteristic of the enzyme, is not affected by cell aging. During remission, when the reticulocyte count was normal, the activity was 85 U/100 ml RBC, a value within the heterozygote range.

**DISCUSSION**

The alloenzyme system of G6PD has often been proved a very useful tool to investigate the clonal origin of several hematopoietic diseases.\textsuperscript{3} Of particular interest are the results obtained studying subjects with myeloproliferative disorders, who are heterozygous for G6PD. Chronic myeloid leukemia, for example, has been demonstrated to be a clonal disease involving a multipotent stem cell, with no residual normal progenitors detectable even in the clinical remission phase.\textsuperscript{10} On the other hand, reports on acute nonlymphocytic leukemias studied with the G6PD marker seem to indicate that, in some cases, the neoplastic event hits a cell already committed to myeloid differentiation.\textsuperscript{11} However, the data obtained from studies on acute nonlymphocytic leukemias with G6PD as a marker did not help to clarify the difficulties still persisting concerning the prognostic significance of the morphological classifications. Erythroleukemia has been classified in the past both as a chronic or acute myeloproliferative disorder and has also been regarded as a diserythropoietic syndrome.\textsuperscript{2}

**Table 1. 2dG6P Utilization Values of Different Cell Populations**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>2dG6P (%)</th>
<th>2dG6P Ratio (Hemopoietic/Nonhemopoietic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonhemopoietic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair follicles</td>
<td>9.2</td>
<td>—</td>
</tr>
<tr>
<td>Skin</td>
<td>9.8</td>
<td>—</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>9.3</td>
<td>—</td>
</tr>
<tr>
<td>Hemopoietic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>31.9</td>
<td>3.38</td>
</tr>
<tr>
<td>Blasts</td>
<td>31.4</td>
<td>3.33</td>
</tr>
<tr>
<td>Remission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>9.1</td>
<td>0.96</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>9.7</td>
<td>1.03</td>
</tr>
<tr>
<td>Platelets</td>
<td>9.7</td>
<td>1.03</td>
</tr>
<tr>
<td>Monocytes</td>
<td>10.4</td>
<td>1.10</td>
</tr>
<tr>
<td>B lymphocytes</td>
<td>9.8</td>
<td>1.04</td>
</tr>
<tr>
<td>T lymphocytes</td>
<td>10.4</td>
<td>1.10</td>
</tr>
</tbody>
</table>

*G6PD B: 3.79 ± 0.73; G6PD Mediterranean: 35.15 ± 6.05.*\textsuperscript{4}
We have been able to study a case of erythroleukemia in a subject heterozygous for the Mediterranean variant of G6PD. Erythroleukemia is demonstrated as a clonal disease involving the erythroid and myeloid lines: the heterozygous phenotype present in the patient’s nonhemopoietic tissues was not detectable in the cells of hemopoietic origin, which expressed a single-enzyme phenotype.

A very interesting finding comes from the evidence that, following adequate chemotherapy, a true remission state is achieved, as demonstrated by the reappearance of the heterozygous phenotype in hemopoietic cells. The malignant clone has been completely eradicated and the potential for normal hematopoiesis restored. These results provide useful information to determine the frequency of origin and expression in progenitors with restricted or multipotent differentiation in acute nonlymphocytic leukemias, compared to chronic myeloproliferative disorders. The controversial evidence derived from cytogenetic studies of clonal involvement of erythropoiesis in the malignant process is resolved by the establishment of erythroleukemia as a clonal disorder of a multipotent stem cell. These data might prove of relevance in the understanding of the mechanisms of hemopoietic cell differentiation in leukemic disorders.

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REFERENCES

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