Restoration of Nonclonal Hematopoiesis in Chronic Myelogenous Leukemia (CML) Following a Chemotherapy-Induced Loss of the Ph' Chromosome

By Jack W. Singer, Zalmen A. Arlin, Vesna Najfeld, John W. Adamson, Sanford J. Kempin, Bayard D. Clarkson, and Philip J. Fialkow

After intensive chemotherapy, marrow cells of some patients with Philadelphia chromosome (Ph') positive chronic myelogenous leukemia (CML) become partially or completely Ph'-negative. However, without a second marker for the neoplastic clone, it could not be determined if these Ph'-negative cells arose from normal progenitors or were still members of an abnormal clone. In the present study, a patient with Ph'-positive CML, also heterozygous for glucose-6-phosphate dehydrogenase (G6PD), was studied before and after intensive chemotherapy. Prior to treatment only G6PD type B was detected in the patient's red cells, platelets, and granulocytes, and all unstimulated marrow metaphases had Ph'. After four cycles of chemotherapy, 76% of marrow cells were Ph'-negative, and approximately 80% of the granulocytes were nonclonal by G6PD analysis. Thus, the frequency of nonclonal cells by G6PD analysis correlated closely with that of the Ph'-negative cells. The data indicate that intensive chemotherapy can restore nonclonal and presumably non-neoplastic hematopoiesis in CML.

STUDIES OF FEMALES with chronic myelogenous leukemia (CML) and heterozygous at the glucose-6-phosphate dehydrogenase (G6PD) locus have shown that CML is a clonal neoplasm that arises in a stem cell common to platelets, granulocytes, red cells, and lymphocytes. The G6PD system can be used as a maker for these studies because the structural gene for this enzyme is on the X chromosome. Early in embryogenesis, one of the two X chromosomes in each somatic cell is inactivated, and the progeny of such cells show only the enzyme type of the remaining active X chromosome. Therefore, the tissues of females heterozygous for the common B gene (GdA) and a variant such as Gd'A, are mixtures of cells, some synthesizing type B, and others type A G6PD. Thus, in a G6PD heterozygote, if a neoplastic disease arises from a single cell, all malignant cells will have a single enzyme type.

The purpose of the present study was to determine whether residual normal stem cells exist in CML. Prior studies from this laboratory of G6PD heterozygotes with CML failed to demonstrate the presence of significant numbers of residual normal committed granulocytic stem cells (CFU-C). However, there is evidence that Philadelphia chromosome (Ph') negative cells are present in some cases of CML. Approximately 20%-40% of patients with CML aggressively treated with combination chemotherapy become at least transiently Ph'-negative.

To determine whether these Ph'-negative cells are normal, i.e., nonclonal, we studied a patient with Ph'-positive CML who was heterozygous for G6PD and who obtained a partial Ph'-negative remission after combination chemotherapy. The degree of hematopoietic normalcy in peripheral blood cells determined by G6PD closely correlated with the frequency of Ph'-negative cells.

CASE REPORT

A.J., a 33-yr-old black female, was well until January 1978 when she developed pneumonia and leukocytosis. After resolution of the pneumonia, the white blood count (WBC) remained elevated at 30,000/cu mm. Over the next 15 mo, no treatment was given. She presented herself to Memorial Hospital in March 1979 with increasing fatigue. Physical examination revealed only an enlarged uterus. The hemoglobin was 11.7g/dl, the platelet count was 487,000/cu mm, and the WBC was 30,100/cu mm with 45% neutrophils, 22% band neutrophils, 1% eosinophils, 4% basophils, 2% monocytes, 6% lymphocytes, 6% metamyelocytes, 12% myelocytes, and 2% nucleated red blood cells. A pregnancy test was positive. A marrow smear showed hypercellularity with myeloid hyperplasia.

On 3/28/79, after informed consent was obtained, the patient underwent a therapeutic abortion. Splenectomy and appendectomy were done on 4/4/79 following a single intravenous injection of Thiotope. On 5/8/79, she began the first cycle of chemotherapy on the L-15 protocol, which included daunorubicin (45 mg/sq m i.v. daily for 2 days), cytosine arabinoside (12.5 mg/sq m i.v. followed by continuous infusion of the same drug at 100 mg/sq m daily for 4 days), and 6-thioguanine (100 mg/sq m every 12 hr for 4 days). Three subsequent courses of chemotherapy with the same medications and doses was administered starting on 6/15/79, on 7/23/79, and on 8/29/79.
NONCLONAL HEMATOPOIESIS IN CML

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Fig. 1. Giemsa-banded karyotype from marrow prior to therapy. Arrows point to Ph' (22q-) and the 9q-. Also note the extra chromosome 19.

MATERIALS AND METHODS

Marrow and peripheral blood samples obtained in New York were drawn into preservative-free heparin (10 U/mL) and shipped air-express to Seattle. A full-thickness skin biopsy obtained from the patient on 4/4/79 was tested for G6PD directly by previously published methods. The 10/4/79 sample was obtained in Seattle at the time marrow was harvested for cryopreservation and later autologous transplantation.

Preparations of platelets, red blood cells, and granulocytes were obtained from each sample and tested for G6PD isoenzyme content by previously published methods. Marrow cells were studied for chromosomes directly, after 24 hr in culture, or both. Marrow or peripheral blood cells were also cultured for 72 hr in the presence of phytohemagglutinin (PHA) (Burroughs Wellcome). Metaphases from direct preparations and 24 hr cultures were stained with Giemsa and analyzed for numerical abnormalities and for Ph'. Banding patterns of chromosomes in marrow and blood cells were obtained using a modified Giemsa banding technique. The chromosomes were classified according to the Paris Conference and its supplement.

Peripheral blood or marrow cells were cultured for CFU-c and erythroid burst (BFU-c) growth on six occasions. PHA-conditioned medium was used as a source of colony-stimulating factor. Granulocyte colonies of over 40 cells were counted on an inverted microscope after 14 days of incubation in a 5% CO₂ - 95% air humidified incubator, and individual colonies were then electrophoresed to determine G6PD type. Erythroid bursts that formed in the presence of 2.5 U of sheep plasma erythropoietin (Step III; Connaught Laboratories, Willowdale, Ontario, Canada) were counted, and their G6PD type was determined after 10–12 days of culture.

RESULTS

Studies Prior to Splenectomy

Cytogenetics. Each of 31 banded marrow cells had the Ph¹ with the common translocation—t(9;22) (q34.0;q11.1). In addition, 28 of 31 cells had 47 chromosomes with an extra no.19 (Fig. 1). This trisomy 19 in Ph¹-positive cells was also detected in 10/50 marrow cells grown in the presence of PHA. The other 40 cells were normal.

G6PD. Approximately equal amounts of A and B enzyme activities were found on analysis of skin. In

Table 1. Cytogenetic Studies of Marrow Cells and Direct G6PD Analyses of Circulating Blood Cells

<table>
<thead>
<tr>
<th>Date</th>
<th>Therapy</th>
<th>No. Cells With Ph¹/No. Cells Studied</th>
<th>G6PD (%)A</th>
<th>Red Cells</th>
<th>Granulocytes</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>4/4/79</td>
<td>None</td>
<td>31/31*</td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>6/5/79</td>
<td>Cycle 1</td>
<td>92/100†</td>
<td></td>
<td>0</td>
<td>5–10</td>
<td></td>
</tr>
<tr>
<td>7/24/79</td>
<td>Cycle 2</td>
<td>29/67</td>
<td></td>
<td>10</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>8/21/79</td>
<td>Cycle 3</td>
<td>6/18</td>
<td></td>
<td>20</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>9/27/79</td>
<td>Cycle 4</td>
<td>1/6</td>
<td></td>
<td>45</td>
<td>40</td>
<td>—</td>
</tr>
<tr>
<td>10/4/79</td>
<td></td>
<td>12/50</td>
<td></td>
<td>35</td>
<td>40</td>
<td>30</td>
</tr>
</tbody>
</table>

*Twenty-eight of 31 had trisomy 19 in addition to Ph¹.
†Three of 92 cells had trisomy 19 in addition to Ph¹.
Studies After Chemotherapy

Cytogenetics. The results of cytogenetic analyses following each cycle of chemotherapy are shown in Table 1. There was a progressive loss of Ph'. After the end of the fourth cycle of chemotherapy, only 12 of 50 cells were Ph'-positive. Cells with trisomy 19 were no longer seen after the first cycle of chemotherapy.

G6PD. With chemotherapy, an increase in type-A enzyme activity was detected in red cells, granulocytes, and platelets (Table 1; Fig. 2). At the end of the fourth cycle of chemotherapy, 30%–40% of the G6PD activity found in these cells was type A. Since the ratio of type-A to type-B activity in the patient's skin biopsy was 1:1, it is assumed that there is an amount of type-B G6PD activity from normal cells equal to the amount G6PD type-A activity detected. Therefore, the percent of normal activity is approximately twice the measured percent A activity. Thus, 80%–90% of the activity in red blood cells and granulocytes following cycle 4 was from presumably normal cells.

Cell Culture Results

The numbers of granulocytic colonies and erythroid bursts grown on each occasion and the numbers of colonies that showed G6PD type A or B are given in Table 2. Figure 3 shows the serial frequencies of normal granulocytic colonies and erythroid bursts. Overall, a higher frequency of normal erythroid colonies compared to granulocytic colonies was observed ($p < 0.05; \chi^2$). However, possibly due to the small number of observations, no statistically significant differences between the frequencies of type-A BFU-e and CFU-c were found in individual samples.

DISCUSSION

The availability of a patient with Ph'-positive CML and heterozygous for G6PD who was treated with intensive therapy provided an unusual opportunity to examine the relationship of Ph' to the neoplastic clone. Results of a prior study of chromosomes in granulocytic colonies suggested that in at least some patients, Ph'-negative committed stem cells persist. However, several other studies failed to confirm this observation. Additional evidence for the presence of Ph'-negative stem cells comes from studies demonstrating complete or partial loss of the Ph' following intensive chemotherapy.

In a previous study of G6PD markers in granulocytic colonies, we found little evidence for appreciable numbers of residual normal committed stem cells in CML. Only 2/1308 tested colonies had the nonclonal enzyme type. The discrepancy between these results and the cytogenetic analysis of colonies suggested the possibility that the Ph'-negative cells found in patients

Table 2. Serial Studies of CFU-c and BFU-e Numbers and G6PD Types of Individual Colonies

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample</th>
<th>Colonies $10^5$ Cells Plated</th>
<th>G6PD: Number of Colonies</th>
<th>Bursts $10^5$ Cells Plated</th>
<th>CFU-c</th>
<th>BFU-e</th>
</tr>
</thead>
<tbody>
<tr>
<td>4/4/79</td>
<td>Marrow</td>
<td>21</td>
<td>0 15</td>
<td>2.5</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>5/8/79</td>
<td>Blood</td>
<td>52</td>
<td>0 15</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6/5/79</td>
<td>Blood</td>
<td>4</td>
<td>0 15</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7/13/79</td>
<td>Marrow</td>
<td>80</td>
<td>0 19</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7/24/79</td>
<td>Marrow</td>
<td>97</td>
<td>4 39</td>
<td>13</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>8/27/79</td>
<td>Marrow</td>
<td>121</td>
<td>9 20</td>
<td>30</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>10/4/79</td>
<td>Blood</td>
<td>14</td>
<td>4 11</td>
<td>5</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Marrow</td>
<td>90</td>
<td>8 17</td>
<td>18</td>
<td>14</td>
<td>13</td>
</tr>
</tbody>
</table>
after intensive therapy might still be part of the CML clone.

To test this hypothesis, a patient with Ph'-positive CML and heterozygous for G6PD who was receiving therapy on an established protocol was studied. After 4 cycles of chemotherapy, 76% of directly prepared metaphases were Ph'-negative. If these Ph'-negative cells were part of the CML clone, the single enzyme (type B) phenotype should have persisted. Instead, we found a strong correlation between the frequency of Ph'-negative cells and the percent normal granulocytes determined by G6PD \( r = 0.96; p < 0.01 \). As seen in Table I, the percentage of normal red blood cells and platelets also increased progressively. The frequency of normal red cells lagged behind, however, due to the slower turnover of this cell type.

The frequency of detected normal erythroid bursts and granulocytic colonies increased substantially following chemotherapy. By the end of the fourth cycle, 65% of marrow BFU-e and 100% of marrow BFU-e derived colonies tested were normal. As shown in Fig. 3, the estimated frequency of normal BFU-e was consistently greater than that of CFU-c \( p < 0.05 \). This apparent discrepancy suggests the possibility of differences in vivo or in vitro regulation of early committed progenitors in CML, as has been previously demonstrated in polycythemia vera.

The present data strongly suggest that the Ph'-negative marrow cells that appeared in this patient did not arise from the dominant CML clone. The results, however, do not exclude the possibility that Ph'-negative cells from the CML clone may be present in small numbers. The existence of such cells is suggested by the report that cells in some lymphocyte populations derived from the CML clone are Ph'-negative. We conclude that Ph'-negative stem cells that are not members of the CML clone persist in some patients with CML and that conversion to the Ph'-negative state by intensive chemotherapy is accompanied by the reexpression of previously undetected normal stem cells.

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REFERENCES


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