Fetal Erythropoiesis in Juvenile Chronic Myelocytic Leukemia

By Susan F. Travis

Red cell enzymes, 2,3-diphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP), were evaluated in a 23-mo-old boy with juvenile chronic myelocytic leukemia (JCML) at the onset of his illness and 6 mo later during the accelerated phase. The activities of the age-dependent red cell enzymes, hexokinase, aldolase, pyruvate kinase, and glucose-6-phosphate dehydrogenase, were elevated, as were the concentrations of red cell 2,3-DPG and ATP, consistent with a young red cell population metabolizing at an increased glycolytic rate. The activities of the non-age-dependent red cell enzymes, phosphoglucone isomerase (PGI), phosphofructokinase (PFK), glyceraldehyde-3-phosphate dehydrogenase (G3PD), phosphoglycerate kinase (PGK), and enolase (ENO), which have previously been reported to demonstrate differences in enzyme activity unique to the neonatal period, were investigated in a 2-yr-old boy with JCML in the beginning of his illness and 6 mo later at the onset of the accelerated ("blastic") phase, in order to evaluate possible changes in enzyme activity with progression of the disease.

Acquired Enzymopathies have been described in congenital hypoplastic anemia and in various dyserythropoietic states, such as congenital dyserythropoietic anemia, chronic refractory anemia with and without ringed sideroblasts in the bone marrow, preleukemia, and overt leukemia. These hematologic disorders are also often associated with a mild to moderate increase in fetal hemoglobin (HbF), and some of the acquired red cell enzyme abnormalities are similar to those normally seen in fetal red cells, although no consistent or specific pattern has been described in any of these disorders. The one neoplastic condition in which there is a marked increase in HbF production is juvenile chronic myelocytic leukemia (JCML). The gamma chain of HbF in JCML has a glycine to alanine ratio (\( \gamma/\alpha \)) at \( \gamma \) similar to that observed in newborn infants. Other striking similarities to cord blood red cells, such as a decreased percentage of HbA2, decreased activity of red cell carbonic anhydrase isozymes B and C, decreased tilters of red cell I antigen, and increased activity of glucose-6-phosphate dehydrogenase (G6PD), have led to the suggestion that JCML may represent a reversion to fetal erythropoiesis or a congenital disorder associated with failure of normal red cell differentiation. Thus, it was anticipated, and has been demonstrated, that the activities of several red cell enzymes in this disorder would be similar to those observed in cord red cells.

In this study, the activities of the age-dependent red cell enzymes, pyruvate kinase (PK), G6PD, hexokinase (HK), and aldolase (ALD), and the non-age-dependent red cell enzymes, phosphoglucone isomerase (PGI), phosphofructokinase (PFK), glyceraldehyde-3-phosphate dehydrogenase (G3PD), phosphoglycerate kinase (PGK), and enolase (ENO), which have previously been reported to demonstrate differences in enzyme activity unique to the neonatal period, were investigated in a 2-yr-old boy with JCML in the beginning of his illness and 6 mo later at the onset of the accelerated ("blastic") phase, in order to evaluate possible changes in enzyme activity with progression of the disease.

Materials and Methods

Blood from the subject was collected in tubes containing dried sodium heparin (Vacutainer; Becton-Dickinson and Co.; Rutherford, NJ). Extracts for determination of red cell 2,3-diphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP) were prepared at the bedside; 2 ml of heparinized blood was immediately pipetted into 4 ml chilled 2N perchloric acid, reextracted, and neutralized by methods previously described. For the assay of glycolytic enzymes, heparinized blood was centrifuged at 400 rpm in a PR-J (IEC) centrifuge at 4°C. The white cell and platelet-rich plasma were removed. The red cells were then diluted in at least 5 parts cold buffered saline with glucose (buffered to pH 7.4, containing 200 mg/dl glucose) and filtered 3 times at 4°C through double layers of Whatman No. 2 filter paper. After each filtration, the resuspended cells were recentrifuged with removal of residual buffy coat and the process was then repeated. The red cells were then washed in cold buffered saline and reconstituted to a hematocrit of 70%–80%. Red cell 2,3-DPG and ATP and glycolytic enzymes were determined as previously described and compared to results previously reported in term infants and subjects with reticulocytosis who were evaluated in this laboratory.

Red cell carbonic anhydrase was measured by the method of Itada and Forster (kindly performed by Dr. Esther Chow, University of Pennsylvania). Leukocyte alkaline phosphatase was determined by alkali denaturation, fetal hemoglobin determinations by alkali denaturation, the Kleihauer-Betke slide elution test, and muramidase were performed using standard techniques.

Bone marrow chromosomes were evaluated using the technique of marrow culture with Colcemid (GIBCO, Grand Island, NY) for 3–5 hr, harvesting, and staining with Giemsa.

Case Report

J.L. was a healthy boy until age 23 mo, when he presented with fever, otitis media, pericarditis, bilateral pleural effusions, diffuse
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adenoaptathy, and an enlarged liver and spleen. Laboratory investigation revealed mild anemia, leukocytosis, and thrombocytopenia (Table 1). The hemoglobin A4 was decreased (1.1%) and the fetal hemoglobin was markedly increased by alkali denaturation (39.5%).

Both parents had a normal hemoglobin electrophoresis. The leukocyte alkaline phosphatase (44 and 31) and an increase in the hemoglobin. The bone marrow showed marked myeloid and erythroid heterogeneous distribution. Both parents had a normal hemoglobin

tachypnea, and progressive hepatosplenomegaly, thrombocytopenia, and anemia. Chemotherapy with cytosine arabinoside resulted in marked improvement in his state of well being, but little change in

the next 4 mo was punctuated by numerous respiratory infections, adenopathy, and an enlarged liver and spleen. Laboratory investigation revealed mild anemia, leukocytosis, and thrombocytopenia (Table 1). The hemoglobin A4 was decreased (1.1%) and the fetal hemoglobin was markedly increased by alkali denaturation (39.5%).

Table 1. Laboratory Data at Presentation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Adults (n = 20)</th>
<th>Subjects With Young RBC Population (n = 10)</th>
<th>Term Infants (n = 10)</th>
<th>JCMRL</th>
<th>A*</th>
<th>B*</th>
<th>% ∆</th>
</tr>
</thead>
<tbody>
<tr>
<td>G6PD</td>
<td>206.4 ± 24.2</td>
<td>277.6 ± 43.6</td>
<td>334.5 ± 38.7</td>
<td>395.5</td>
<td>384.1</td>
<td>-2.9</td>
<td></td>
</tr>
<tr>
<td>HK</td>
<td>11.5 ± 1.9</td>
<td>17.0 ± 3.1</td>
<td>24.2 ± 3.8</td>
<td>18.7</td>
<td>15.6</td>
<td>-16.6</td>
<td></td>
</tr>
<tr>
<td>PGI</td>
<td>319.6 ± 36.8</td>
<td>405.1 ± 48.2</td>
<td>525.9 ± 46.7</td>
<td>354.5</td>
<td>504.7</td>
<td>+42.4</td>
<td></td>
</tr>
<tr>
<td>PFK</td>
<td>196.2 ± 24.7</td>
<td>211.6 ± 31.0</td>
<td>139.5 ± 13.9</td>
<td>205.0</td>
<td>191.5</td>
<td>-6.6</td>
<td></td>
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<tr>
<td>ALD</td>
<td>39.5 ± 4.7</td>
<td>60.0 ± 8.9</td>
<td>62.4 ± 7.9</td>
<td>63.5</td>
<td>57.2</td>
<td>-9.9</td>
<td></td>
</tr>
<tr>
<td>G3PD</td>
<td>4,088 ± 487.8</td>
<td>5,907.7 ± 750.6</td>
<td>5,056 ± 655.2</td>
<td>7,266.9</td>
<td>8,889.5</td>
<td>+18.2</td>
<td></td>
</tr>
<tr>
<td>PK</td>
<td>3,181 ± 255.5</td>
<td>3,587.9 ± 316.2</td>
<td>4,412.9 ± 321.9</td>
<td>5,135.2</td>
<td>5,066.6</td>
<td>-1.3</td>
<td></td>
</tr>
<tr>
<td>ENO</td>
<td>156.3 ± 35.3</td>
<td>212.4 ± 39.8</td>
<td>346.0 ± 33.5</td>
<td>401.9</td>
<td>413.6</td>
<td>+2.9</td>
<td></td>
</tr>
<tr>
<td>PK</td>
<td>240.8 ± 42.3</td>
<td>392.7 ± 51.2</td>
<td>385.6 ± 57.6</td>
<td>481.2</td>
<td>504.2</td>
<td>+4.8</td>
<td></td>
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<tr>
<td>2,3-DPG</td>
<td>4,423 ± 1,907</td>
<td>4,586 ± 529</td>
<td>4,691 ± 383</td>
<td>5,443</td>
<td>3,538</td>
<td>-35.4</td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>1,024 ± 100</td>
<td>1,114 ± 222</td>
<td>1,056 ± 144</td>
<td>1,950</td>
<td>1,490</td>
<td>-23.6</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Red Cell Enzyme Activity (U/100 ml RBC) and Concentration of 2,3-DPG and ATP (nmol/ml RBC)

Fetal Hb

Kleihauer-Betke <1% positively stained cells — — 72.5 74.4

Alkali denaturation <2% — 66.0 ± 15.0 39.5 36.4

RESULTS

Red cell glycolytic enzymes, G6PD and ATP, were evaluated at presentation (A) and 6 mo later (B) (Table 2). The activities of the age-dependent red cell enzymes, G6PD, HK, ALD, and PK, were increased when initially evaluated (A), as were the concentrations of red cell 2,3-DPG and ATP. The hemoglobin (Hb) was 8.7 g/dl and the reticulocyte count was 5% at that time. The non-age-dependent enzymes, ENO, PGK, and G3PD, were also elevated when compared to red cells from both subjects with a young red cell population and term infants. Red cell PGI and PFK activities were compatible with the age of the red cell population.

The second sample (B) was obtained 6 mo later at the onset of the accelerated phase of the illness. The Hb at the time was 11.2 g/dl, and the reticulocyte count had decreased to 1.0% (2 mo after the spleen was removed). There was a mild to moderate decrease in the activities of the age-dependent red cell enzymes, G6PD, HK, and ALD, and the non-age-dependent enzyme, PK. The decrease in HK activity (16.6%) was greater than that observed for other red cell enzymes (2.9%–9.9%). The concentrations of red cell 2,3-DPG and ATP also decreased (35.4% and 23.6%, respectively). There was a marked increase in PGI activity (42.4%) and an 18.2% increase in G3PD activity. The activities of PK and ENO increased slightly.

DISCUSSION

The pattern of red cell glycolytic enzymes and G6PD in term infants differs from that observed in

resulted in no definitive response. His condition continued to deteriorate and he expired 15 mo after diagnosis. Autopsy was not obtained.

* A, data obtained at onset of illness; B, data obtained 6 mo later, at onset of accelerated phase.
At the onset of the illness, the activities of the red cell enzymes, G6PD, HK, ALD, become adult values by 1-2 yr erythropoiesis. In prior studies, the activities of values seem to represent passage from fetal to adult changes toward normal adult cell, and developmental characteristics out of proportion to the age of the red cell population. However, the activities of certain red cell enzymes are elevated consistent with the age of the red cell population. Red cell PFK and PGK, and ENO were comparable to adults. Enzyme activity tends to be increased, consistent with a young red cell population. However, the activities of certain red cell enzymes are elevated out of proportion to the age of the red cell population (PGI, G3PD, PGK, ENO), whereas others, such as PFK and carbonic anhydrase, are decreased. These characteristics appear to be unique to the “fetal” red cell, and developmental changes toward normal adult values seem to represent passage from fetal to adult erythropoiesis. In prior studies, the activities of PFK, G6PD, PGK, and ENO were comparable to adult values by 1–2 yr of age.

The present study has demonstrated that the activity of red cell enzymes in JCML is similar to values obtained in newborn infants and has provided the opportunity to demonstrate that these “fetal” characteristics become more marked with progression of the disease. At the onset of the illness, the activities of the age-dependent red cell enzymes, G6PD, HK, ALD, and PK, were elevated, as were the concentrations of red cell ATP and 2,3-DPG, which were consistent with a young red cell population and an increased glycolytic rate. However, the activities of the non-age-dependent red cell enzymes, G3PD, PGK, and ENO, were also elevated, out of proportion to the age of the red cell population, to levels that were similar to or greater than those obtained in term infants. Red cell PFK and PG1 activities were normal. Carbonic anhydrase activity was decreased. Six months later, when the illness entered an accelerated phase, the activities of the age-dependent red cell enzymes and the concentrations of red cell ATP and 2,3-DPG had decreased, which was consistent with the decrease in reticulocyte count and increase in hemoglobin that occurred after the spleen was removed. However, the “fetal” characteristics persisted or increased; the activities of red cell PGK and ENO remained markedly elevated and G3PD activity increased further. Red cell PGI activity, which had been normal, increased 42.4% to 504.7 U/100 ml RBC, a value similar to the mean obtained in term infants.

Prior studies of red cell enzyme activity in JCML have not provided sequential or consistent data. In all cases, however, red cell ENO, G6PD, and 6-phosphogluconic dehydrogenase activities were increased and PGK and phosphoglycerate mutase activities were normal or increased. PK activity was normal or increased in all patients, except the child reported by Gahr et al., who demonstrated red cell enzyme activity with “fetal” characteristics similar to the present case, but who also manifested signs of dyserythropoiesis: very high activity of HK and low PK activity.

The activity of G3PD in term infants was not inappropriately elevated for red cell age in the present study or in the premature infants reported by Gahr et al., although Konrad et al. have reported significantly increased red cell G3PD activity in term infants when compared to subjects with reticulocytosis. The activity of red cell G3PD in our patient with JCML, however, was markedly elevated when compared to term infants and increased with progression of the illness to a level 70% higher than the mean activity obtained in term infants. Red cell G3PD activity in the child reported by Gahr et al. was also 70% greater than the mean activity obtained in premature infants. Significantly increased activity of G3PD has also been reported in congenital dyserythropoietic anemia. Thus, it is conceivable that the marked increase in red cell G3PD activity in these two children with JCML may be more indicative of dyserythropoiesis than “fetal” erythropoiesis.

The present study has provided further evidence that red cell enzyme activity in JCML is similar to values obtained in newborn infants and that, as the illness progresses, these abnormalities become more marked, which is consistent with the suggestion that JCML probably represents a reversion to “fetal” erythropoiesis.

REFERENCES

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