Unbalanced Globin Chain Synthesis in Congenital Dyserythropoietic Anemia

By Marilyn A. Hruby, R. George Mason, and George R. Honig

Hematologic evaluation of a 5-yr-old girl with lifelong anemia demonstrated the characteristic findings of congenital dyserythropoietic anemia (CDA) type II. Globin chain synthesis was studied in vitro by measuring the incorporation of L-leucine-14C into globin by peripheral blood and bone marrow erythroid cells. In cells from the child and from both of her parents an abnormal balance between the synthesis of the α and non-α globin components of hemoglobin was observed, the α chains being synthesized in excess. Neither parent demonstrated microcytosis, hypochromia, or other findings suggestive of β-thalassemia trait.

T THE CONGENITAL DYSERYTHROPOIETIC anemias (CDA) are a group of rare disorders characterized by ineffective erythropoiesis and a moderate to severe degree of refractory anemia. Characteristic morphologic changes present in the bone marrow of affected patients are confined to the erythroid precursors, and include multinuclearity, karyorrhexis, and, in some cases, megaloblastosis. Associated findings include elevated levels of unconjugated bilirubin,1 serologic abnormalities,2 abnormal changes in the activity of some erythrocyte enzymes,3 and evidence of iron overloading. Although cytochemical and electron microscopic studies have indicated that a disturbance of DNA synthesis may exist in the developing normoblasts in these conditions,4,5 the pathogenic mechanism remains to be defined.

In this report we present an additional example of CDA occurring in a 5-yr-old girl in whom severe anemia was present since infancy. Globin chain synthesis studies performed in this patient demonstrated an imbalance of α and non-α chain synthesis which represents a previously unreported finding in this disorder.

METHODS AND MATERIALS

Hematologic studies were performed by standard methods. For determination of the rate of plasma iron clearance, 3.0 μCi of 59Fe were injected intravenously, and serial blood samples...
were taken between 10 and 90 min for determination of plasma radioactivity. Red cell utilization
was determined from blood samples taken on days 4, 6, 8, and 10 following injection of the radio-
iron. Red cell survival studies were done by a standard $^{51}$Cr procedure. Hemoglobin electrophoresis was done in starch gel according to the method of Smithies. Quantitation of hemo-
globin A$_2$ was performed by acrylamide disc electrophoresis. Fetal hemoglobin was determined by an alkali denaturation procedure and by the slide elution test. The procedure for detection of heat precipitable hemoglobin was as described by Dacie et al.

Globin synthesis in vitro was studied by methods previously described. Heparinized blood
and aspirated bone marrow samples were centrifuged at 4°C to remove the plasma, and the cells
were washed three times in a cold saline solution (sodium chloride 0.140 M, potassium chloride
0.005 M, magnesium chloride 0.0015 M). The cells were suspended in 9 volumes of the saline
solution supplemented with Tris-hydrochloride, pH 7.4, glucose, hemin, and a mixture of L-amino
acids. Uniformly labeled L-leucine-$^{14}$C (270 mCi/mmole, New England Nuclear, Boston, Mass.) $\mu$Ci/ml was added, and the cell suspension was incubated in a metabolic shaker at
37°C for 4 hr. The cells were recovered by centrifugation and lysed with 5 volumes of water.
The cell stroma were removed and globin was prepared from the total soluble fraction by pre-
cipitation in an acetone-HCl mixture at -20°C. Chromatography of the globin was performed
on a column of carboxymethyl-cellulose by the procedure of Clegg et al. For determination
of incorporated radioactivity in the effluent fractions, 0.5 mg of bovine serum albumin was added
to each fraction, and the protein was precipitated by addition of 3 volumes of 10% trichloracetic
acid. The precipitates were collected on Millipore filter discs which were dried and fastened to
aluminum planchets. The samples were counted in a low-background gas-flow counter with a thin
window. Incorporated radioactivity into each globin chain was determined by adding together the
total recovered radioactivity of effluent fractions comprising the globin chain peak.

CASE REPORT AND RESULTS

W. H., a 5$\frac{1}{2}$-yr-old girl, was referred to the University of Illinois Hospital in February 1972 for evaluation of anemia. The child was born to a 37-yr-old mother after 7$\frac{1}{2}$ mo gestation and had a birth weight of 4 lb. No problems were noted in the neonatal period. Severe anemia with a hemoglobin concentration of 3 g/100 ml was first detected at 7 mo of age. A transfusion of packed red cells was given, and oral iron therapy was begun. At 13 mo of age the child was again noted to be severely anemic. Her reticulocyte count was reported to be normal at that time, and a bone marrow preparation demonstrated erythroid hyperplasia with megaloblastic changes. Her serum iron level and iron binding
capacity were normal. Folic acid orally and later intramuscularly was given without apparent benefit, and the child continued to require periodic transfusions.

In February 1969 the patient was reevaluated. A Schilling test and serum vitamin B$_{12}$ level were normal. Assays of erythrocytic pyruvate kinase and

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1972, the child was noted to be pale but nonicteric with marked bossing of her frontal, parietal and maxillary bones. Her growth and development were normal for her age. Her liver was enlarged with the edge palpable 5 cm below the costal margin. No edema or clubbing was noted in her extremities.

Her hemoglobin concentration was 5.5 g/100 ml, hematocrit 16%, and white blood count 19,600 cell/cu mm with a differential count of 33% segmented neutrophiles, 63% lymphocytes, 3% monocytes, and 1%, eosinophiles. A reticulocyte count was 5.4%, and platelet count 540,000/cu mm. Nucleated red cells, 15/100 white cells counted, were present in the peripheral blood. The red cell morphology was abnormal and showed poikilocytosis, anisocytosis, and occasional target cells and spherocytes. Supravital staining did not demonstrate erythrocyte inclusions. The red cell indices are shown in Table 1. Approximately 5% of erythrocytes in the child’s peripheral blood gave a strong staining reaction for fetal hemoglobin by the slide elution test. A bone marrow smear again demonstrated erythroid hyperplasia. In addition the late stage normoblasts exhibited karyorrhexis, multinuclearity, and nuclear fragmentation (Fig. 1); no megaloblastoid changes or chromatin bridges were found.

Other laboratory studies are summarized in Table 2. Erythrokineic studies (Table 3) showed mild shortening of the red cell survival, a rapid iron clearance, an increased rate of iron turnover, and reduced iron incorporation into hemoglobin. These results reflect a marked degree of ineffective erythropoiesis characteristic of the dyserythropoietic group of anemias. The Ham test and the acid thrombin test were performed with red cells from the patient using serum

**Table 1. Hematologic Values of the Patient and Her Family**

<table>
<thead>
<tr>
<th>Normal</th>
<th>W, H.</th>
<th>Mother</th>
<th>Father</th>
<th>Sister</th>
</tr>
</thead>
<tbody>
<tr>
<td>M 14-18</td>
<td>F 12-16</td>
<td>5.5</td>
<td>13.2</td>
<td>13.4</td>
</tr>
<tr>
<td>F 42-52</td>
<td>M 37-47</td>
<td>16</td>
<td>37</td>
<td>40</td>
</tr>
<tr>
<td>0.5-1.5</td>
<td>5.4</td>
<td>0.8</td>
<td>1.6</td>
<td>0.7</td>
</tr>
<tr>
<td>84-99</td>
<td>88</td>
<td>88</td>
<td>101</td>
<td>84</td>
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<td>27-31</td>
<td>31.5</td>
<td>30.9</td>
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<td>29.1</td>
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<td>32-36</td>
<td>35.0</td>
<td>35.7</td>
<td>33.4</td>
<td>35.0</td>
</tr>
<tr>
<td>AA</td>
<td>AA</td>
<td>AA</td>
<td>AA</td>
<td>AA</td>
</tr>
<tr>
<td>1.5-1.7</td>
<td>5.8</td>
<td>1.0</td>
<td>0.8</td>
<td>1.1</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>&lt;3.0</td>
<td>&lt;1.0</td>
<td>2.5</td>
<td>2.5</td>
<td>2.8</td>
</tr>
</tbody>
</table>

![Fig. 1. Photomicrograph of the bone marrow preparation showing erythroid hyperplasia and karyorrhexis of normoblast nuclei.](image-url)
samples from 10 ABO-compatible normal donors, and all were negative. Red cell agglutination studies demonstrated a titer of 1:640 against anti-I; no agglutination occurred with anti-i. Chromosome studies performed with cells from peripheral blood revealed a normal female karyotype pattern.

The parents of the patient are unrelated, and the mother appeared hematologically normal. Results of hematologic studies of the family members are summarized in Table 1. The father's blood smear demonstrated macrocytosis which may have been related to alcohol intake and a poor diet. Both parents and the 3-yr-old sister were found to have normal hemoglobin electrophoresis patterns and normal levels of fetal hemoglobin and hemoglobin A2 in their blood. Neither of the parents demonstrated fetal hemoglobin-containing cells in their peripheral blood by the slide elution test.

The pattern of incorporation of L-leucine-14C into globin by red cells from the patient is shown in Fig. 2. Two anomalous features of the radioactivity incorporation pattern are apparent from the chromatogram. First, although hemoglobin F comprised less than 10% of the hemoglobin in the blood of this patient, approximately 40% of the radioactivity recovered from the non-α chain fractions (i.e., β + γ) corresponded to the γ-chain fraction. Second, incorporation of radioactivity into the total non-α chain fractions was substantially less than that incorporated into the α-chain globin. In peripheral blood from both parents as well as bone marrow from the mother a significant excess of α-chain synthesis occurred relative to β-chain synthesis (Table 4). Globin chain synthesis from the patient's sister appeared normal.

**DISCUSSION**

The patient described in this report demonstrated many of the characteristics of the congenital dyserythropoietic anemias. These included refractory anemia, ineffective erythropoiesis, and bone marrow findings of bi- and multi-nucleated cell types.
erythroblasts with karyorrhexis. Based on the morphologic criteria proposed by Heimpel and Wendt in 1968, the findings in the bone marrow of this patient are consistent with the classification of Type II CDA. In contrast, however, to previously described patients of this morphologic type who were found to have serologic abnormalities, the acidified-serum (Ham) test and i antigen agglutination titer were normal in this patient.

Table 4. Globin Chain Synthesis Ratios

<table>
<thead>
<tr>
<th></th>
<th>α/β</th>
<th>α/(β+γ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>2.11</td>
<td>1.27</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>1.32</td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>1.37</td>
<td></td>
</tr>
<tr>
<td>Bone marrow</td>
<td>1.26</td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>1.34</td>
<td></td>
</tr>
<tr>
<td>Sister</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>1.09</td>
<td></td>
</tr>
<tr>
<td>Normal controls (5)</td>
<td>1.04 ± 0.05</td>
<td></td>
</tr>
</tbody>
</table>

*Studied 8 wk after transfusion.
The study of globin chain synthesis by red cells from this patient demonstrated a clearly abnormal balance between the synthesis of the α and non-α globin components of hemoglobin, the α-chains being synthesized in significant excess. A similar imbalance of globin chain synthesis was demonstrated in both parents. Heretofore findings of this kind have been found only in β-thalassemia and related disorders including hemoglobin Lepore disease, and in certain unstable β-chain-variant hemoglobinopathies. The hematologic studies performed in this child and her parents do not substantiate a diagnosis of any of this group of disorders by usual criteria, but abnormal globin synthesis in both parents suggests a genetic relationship. The underlying abnormality of any form of β-thalassemia remains undefined, and recent evidence suggests that at least two different types of mechanisms may produce these pathologic conditions. At the present state of understanding of β-thalassemia, a deficiency of β-chain synthesis relative to the synthesis of α-chains is generally regarded as the primary basis for establishing this diagnosis, and in some forms of the disease studies of this kind may be the only means for determining its existence. More complete understanding of the abnormalities which underlie the thalassemia disorders as well as the dyserythropoietic anemias will be required to define any specific relationship between these groups of disorders.

It is also possible that the unbalanced synthesis of α and β globin observed in this patient may represent an acquired or secondary manifestation of the dyserythropoietic anemia. Several examples of a disorder hematologically indistinguishable from α-thalassemia ("hemoglobin H disease") have been reported as an acquired abnormality in patients having erythroleukemia and related diseases. In addition, in a group of sideroblastic anemias, which are classifiable as acquired dyserythropoietic conditions, globin chain synthesis studies by White et al. demonstrated α-thalassemia-like characteristics with an excess of β-chains being synthesized relative to α-chains.

To our knowledge a β-thalassemia-like state has not been observed as an acquired abnormality, although in certain of the genetically determined unstable hemoglobinopathies affecting the β-chain a relative excess of α-chain synthesis may be a discernable feature.

It may be of significance in this family that none of the individuals demonstrating globin chain synthesis imbalance exhibit erythrocyte hypochromia. It could be considered that the relative excess of α-chain synthesis in these individuals may represent an absolute increase in the rate of α-chain synthesis while β-chain synthesis may be normal.

Additional studies in both congenital and acquired dyserythropoietic states will be required to assess the extent and generality of these findings. Studies of this kind may be able to provide worthwhile insight into the underlying abnormalities of the dyserythropoietic anemias as well as further understanding of the factors which regulate the synthesis of the complementary globin chains of hemoglobin.

ADDENDUM

Since completion of this study a report by Weatherall and co-workers appeared describing similar findings of unbalanced globin chain synthesis with α
chain production exceeding that of β chains. These observations were seen in several members of a family having many of the hematologic features of dyserythropoietic anemia.

ACKNOWLEDGMENT

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REFERENCES


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