Congenital Dyserythropoietic Anemia Type II: Ultrastructural and Radioautographic Studies of Blood and Bone Marrow

By Kwan Yuen Wong, George Hug, and Beatrice C. Lampkin

A 12-yr-old white girl with congenital dyserythropoietic anemia (CDA) type II was studied. Excessive cytoplasmic membranes (appearing like "double membranes") were found in the majority of the normoblasts. There was marked decrease in the uptake of tritiated thymidine in the binucleated normoblasts as demonstrated by radioautography. The results suggest that the cells with more severe structural cytoplasmic abnormalities and/or decreased DNA synthesis are destroyed within the bone marrow, and the circulating red cells are derived from a less abnormal population of precursors.

C ONGENITAL DYSERYTHROPOIETIC ANEMIA (CDA) is a rare hereditary form of anemia that is characterized by multinuclearity of the normoblasts. Heimpel and Wendt1 have classified the anemia into three types on a morphologic basis. Type I is characterized by megaloblastic changes, inter-nuclear chromatin bridges, and macrocytosis. In type II, the red cell precursors are normoblastic with bi- and multinuclearity, pluripolar mitoses, and karyorrhexis. The red cells are normocytic. Type III is characterized by multinuclearity with up to 12 nuclei, gigantoblasts, and macrocytosis. Crookston and his co-workers2 further delineated CDA type II by demonstrating a positive acidified serum (Ham) test, a negative sucrose lysis test, a high agglutination score with anti-i, and an unusual susceptibility to lysis by anti-i- and anti-I. It is the purpose of this paper to report the results of studies done in a patient with CDA type II, demonstrating an ultrastructural abnormality of the cytoplasmic membranes of uni- and multinucleated erythroid precursors and a small number of mature erythrocytes and impaired DNA synthesis in the multinucleated erythroblasts.

MATERIALS AND METHODS

All routine hematologic studies, autohemolysis and osmotic fragility of the red cells, blood smears for Heinz bodies, and urine examination for hemosiderin were done by methods described by Cartwright.3 The level of vitamin E in the plasma was done by the method of Quaife and co-workers.4 Chromosomal analysis of phytohemagglutinin-stimu-
Table 1. Bone Marrow Picture

<table>
<thead>
<tr>
<th>Normoblast type</th>
<th>Uni- (%)</th>
<th>Bi- (%)</th>
<th>Multi- (%)</th>
<th>Karyorrhexis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pronormoblast</td>
<td>0.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Basophilic</td>
<td>8.6</td>
<td>0.1</td>
<td>3.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Polychromatophilic</td>
<td>31.4</td>
<td>6.9</td>
<td>3.9</td>
<td>2.3</td>
</tr>
<tr>
<td>Orthochromatic</td>
<td>42.1</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
</tr>
</tbody>
</table>

*Karyorrhexis.

lated lymphocytes from the blood sample was done using the method of Moorhead.\textsuperscript{5} Urinary amino acids were quantitated by paper chromatography.\textsuperscript{6} Hemoglobin electrophoresis was done on cellulose acetate, and the quantity of fetal hemoglobin was determined by alkali denaturation.\textsuperscript{7,8} The amount of glucose-6-phosphate dehydrogenase (G-6-PD) in the red cells was determined by a method described by Zinkham, and the level of pyruvate kinase was done by a method described by Tanaka in the red cells was determined by a method described by Michel.\textsuperscript{9,10} The presence of a heat precipitable hemoglobin was checked for by a method described by Dacie and associates.\textsuperscript{11} The quantity of acetylcholinesterase in the red cells was determined by a method reported by Michel.\textsuperscript{12} The technique described by Ebaugh and co-workers was used to determine the red cell survival time, and the technique of Huff and associates was used for the determination of ferrokinetic studies.\textsuperscript{13,14}

The acid thrombin test was performed as described by Crosby,\textsuperscript{15} and a sucrose lysis test was done as described by Hartmann and Jenkins.\textsuperscript{16} DNA synthesis of the normoblasts was studied by testing their ability to incorporate tritiated thymidine. One milliliter of heparinized sample of bone marrow was incubated with 1 \muCi tritiated thymidine (specific activity 1.9 Ci/m mole) for 50 min at 37\textdegree C with constant shaking. The sample was then centrifuged at 1200 g for 10 min. Cover slip smears were made of the nucleated cell layer, and radioautographs prepared with Kodak AR-10 stripping film. After an exposure period of 13 days, the films were developed, and the slides stained with Wright's stain. The percent of pronormoblasts, basophilic normoblasts, and polychromatophilic normoblasts labeled with tritiated thymidine (labeling index) was recorded. For electron microscopic studies, bone marrow was aspirated into a heparinized syringe and transferred into Win-trobe tubes that were centrifuged for 10 min in a PR2 International Centrifuge at 0\textdegree C and 1500 rpm. The supernatant plasma was discarded. The buffy coat was fixed in cold (0-5\textdegree C) buffered 3% gluteraldehyde (pH 7.2, 0.1 M phosphate buffer) and then processed as previously reported for specimens of liver tissue.\textsuperscript{17} Thin sections were examined in a Philips 300 electron microscope. Bone marrow specimens from both parents and two siblings were studied by both light and electron microscopy.

CASE REPORT

A 12-yr-old white girl was referred to The Children's Hospital because of episodic jaundice and mild anemia since 2 yr of age. There was no family history of jaundice or anemia. The parents are first cousins. Mild pallor and hepatosplenomegaly were found on physical examination. On admission her hemoglobin was 9.7/100 ml, reticulocyte count was 5.2%, and white blood cell count 4000/cu mm with a normal differential count. Platelets were normal on smear. The red blood cells were normochromic, with anisocytosis and poikilocytosis. MCV was 95 c.u.; MCH, 34.5 \mu g; and MCHC, 35.5%. An occasional nucleated red blood cell was present. The bone marrow was hyperplastic, with a myeloid:erythroid ratio of 0.6:1. There was no erythrophagocytosis. The presence of multinucleated normoblasts was striking (Fig. 1). The distribution of the various erythroid precursors is shown in Table 1.

Liver function studies were normal (SGOT, prothrombin time, alkaline phosphatase, total protein, and A/G ratio). BUN was normal, but the serum uric acid level varied from
5.6 to 8.4 mg/100 ml, and the indirect bilirubin ranged from 0.7 to 3.5 mg/100 ml. Serum vitamin E level was normal (1.01 mcg/100 ml), and there was no amino aciduria. The patient had a normal 46 XX karyotype. Normal adult hemoglobin was found by electrophoresis, and the quantity of fetal hemoglobin present in the red cells was normal (0.25%). Autohemolysis and osmotic fragility tests were normal, as were the quantities of G-6-PD, pyruvate kinase, and acetylcholinesterase within the red cells. The Heinz body preparation was negative, as was the heat precipitable test for unstable hemoglobin. Urobilinogen was present in the urine, but hemosiderin was not. The serum iron was 243 µg/100 ml, with total iron binding capacity of 280 µg/100 ml and per cent saturation of 87.

There has been no change in the patient's clinical and laboratory findings over a 2-yr period of observation.

RESULTS

Special Studies

The patient's blood type was B, Rh positive. The acid thrombin test was positive with three out of five group compatible sera, while the sucrose lysis test was consistently negative. No antibodies against the patient's red cells were demonstrated in her serum or on the surface of her red cells when tested with specific Coombs' antisera. Normal immunoglobulins were present by immunoelectrophoretic analysis, but haptoglobin was absent. The complement system was normal as assayed by CH50 titer with a modified method of Kabat and Meyer, as reported by Gewurz et al.18
Erythrokinetic studies are shown in Table 2. The red cell survival was only minimally shorter than normal. There was rapid clearance of iron from the plasma, and the plasma iron turnover was increased. The iron utilization by red cells was low. There was no abnormal loss of iron from the body as determined by whole body scan.

The results of radioautographic studies of normoblasts are shown in Table 3. A normal per cent of the uninnuclear normoblasts of all stages labeled with tritiated thymidine. However, only 2% of the binucleated polychromatophilic and none of the multinucleated polychromatophilic normoblasts were labeled.

Under the electron microscope excessive intracellular membranes were seen in the majority (80%-90%) of the normoblasts of all stages of maturation. These excessive membranes, which looked like double cytoplasmic membranes, were present in uni-, bi-, and multinucleated red cell precursors (Fig. 2). These membranes were not the result of invagination of the plasma membrane for at least two reasons: continuity between the plasma membrane and intracellular membranes was not demonstrated on serial sections, and there was continuity between the excessive membranes and the outer membrane of the nuclear envelope. This latter observation indicated that the excessive membranes are part of the endoplasmic reticulum. Rarely, isolated excessive membranes were seen in 1%-2% of mature erythrocytes (Fig. 3). Various stages of nuclear extrusion were observed in the normoblasts with the membrane abnormality. No abnormality was present in the white cell precursors or the megakaryocytes.

Family Studies

The complete blood count and red cell morphology were normal in both parents and the patient's two siblings. In addition, the red cell precursors were normal by light and electron microscopy.
CONGENITAL DYSERYTHROPOIETIC ANEMIA

Fig. 2. Two orthochromatic normoblasts, one with imminent nuclear extrusion (upper), showing "double membrane" appearance. N, nucleus; M, mitochondria; arrows indicating native ferritin. × 24,800. Insert illustrates cisternae along inner surface of cytoplasmic membrane. N, nucleus. × 102,500.

DISCUSSION

Hemolysis was considered initially as the cause of the intermittent jaundice and anemia in our patient but was excluded by the normal 51Cr red cell survival
The marked increase in the erythroid precursors in the marrow, but with minimal reticulocytosis, suggested ineffective erythropoiesis that was confirmed by a rapid plasma clearance of $^{59}$Fe and a low uptake of $^{59}$Fe by the circulating red cells. Ineffective erythropoiesis is a common finding in patients with congenital dyserythropoietic anemia (CDA) type II. This type of anemia was documented in our patient by the presence of bi- and multinuclearity of the erythroblasts in the marrow and a positive acidified serum test but negative sucrose lysis test.

CDA type II is an inherited disease, but its pattern of inheritance has not been established. A recessive inheritance has been suggested. The presence of parental consanguinity along with the lack of abnormal findings in the bone
marrow of the parents and the siblings of our patient also indicate an autosomal recessive mode of inheritance.

The pathophysiology of CDA type II has not been elucidated. A “double membrane” was found in the majority of the red cell precursors. The only other instance of this abnormality was reported by Heimpel and co-workers at the XIII International Congress of Hematology in a patient with CDA type II. Although this abnormality was most evident in the polychromatophilic and orthochromatic normoblasts, the pronormoblasts were also involved. This ultrastructural abnormality was not necessarily more severe in the bi- or multinucleated normoblasts. Although nuclear extrusion was observed, the presence of these excessive membrane structures may impair denucleation to some degree and red cell egress from the bone marrow as suggested by the marked accumulation of the orthochromatic normoblasts in the marrow. With variable involvement from cell to cell, it is possible that the less affected cells may be able to undergo relatively normal maturation and enter the circulation.

Only 1%-2% of the red cells demonstrated isolated cisternae along the inner surface of the cell membrane. Portions of the excessive membrane structures may have been lost during the process of denucleation. Thus the membrane abnormality in the red cells appeared much less severe in extent. Since only very small segments of the red cells can be evaluated by electron microscopy, it is conceivable that more than 1%-2% of the red cells escaped demonstration of an ultrastructural abnormality. Therefore, the abnormal acid thrombin test seen in this type of anemia may be, indeed, a reflection of the membrane abnormality of the red cells.

Only a small per cent of the binucleated polychromatophilic normoblasts was found to label with tritiated thymidine, a specific indicator of DNA synthesis. This decrease in uptake may indicate a defect in incorporation of thymidine, a defect in DNA synthesis, or a loss in the ability to undergo further mitosis.

It is not possible in this study to correlate the electron microscopic findings with the radioautographic results. Despite the universal structural abnormality in all stages of maturation of the normoblasts, the labeling indices were not uniformly abnormal. Thus, it seems unlikely that the presence of excessive membrane structures can interfere with the incorporation of nucleotides for DNA synthesis. The bi- and multinucleated cells are probably out of the mitotic cycle. Our results suggest that the cells with the more severe structural abnormality and/or decreased DNA synthesis are destroyed within the marrow and that the circulating red cells are derived from a less abnormal population of precursors.

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REFERENCES


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