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Similarities of the Erythrocytes in Juvenile Chronic Myelogenous Leukemia to Fetal Erythrocytes

By Helen S. Maurer, Loyda N. Vida, and George R. Honig

A 4-yr-old boy was studied who showed typical findings of juvenile chronic myelogenous leukemia, including massive hepatosplenomegaly, thrombocytopenia, low leukocyte alkaline phosphatase, and absence of a Philadelphia chromosome. The erythrocytes of the patient exhibited many characteristic features of erythrocytes of newborn infants: the fetal hemoglobin concentration was greatly elevated (72%); the oxygen dissociation curve of the whole blood was displaced to the left of the curve from normal adult blood; the hemoglobin A_2 level and the erythrocyte I antigen titer were reduced; and a structural analysis of the γ-chain of the fetal hemoglobin showed the glycine to alanine ratio in γ-136 to be typical of the neonatal pattern. These findings support the suggestion that juvenile chronic myelogenous leukemia is accompanied by reversion to a fetal pattern of erythropoiesis.

ELEVATED LEVELS of fetal hemoglobin accompany a variety of congenital hematologic disorders. These include hemoglobinopathies, thalassemias, hypoplastic anemias, and the syndromes of hereditary persistence of fetal hemoglobin. The high fetal hemoglobin levels present in the last condition, and to some extent in the others, may represent a congenital abnormality in which the normal switchover from fetal to adult hemoglobin synthesis is incomplete or absent.[1]

Fetal hemoglobin levels may also be higher than normal in a number of acquired disorders,[2] including pernicious anemia, aplastic anemia, myelofibrosis, sideroblastic anemia, paroxysmal nocturnal hemoglobinuria, and several types of leukemia.[3] Usually, however, the fetal hemoglobin concentration in acquired hematologic disorders is only moderately increased, falling within the range of 2–12%. A notable exception is the juvenile form of chronic myelogenous leukemia, in which hemoglobin F comprises 30–70% of the total hemoglobin. This form of leukemia is distinguished from the more common adult type by a younger age of onset, the presence of early thrombocytopenia, the absence of a Philadelphia chromosome, and a generally poor response to therapy. In addition to the increased percentages of fetal hemo-

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globin, a number of other changes in the erythrocytes have also been identified in juvenile chronic myelogenous leukemia. These include a subnormal level of hemoglobin A_2,[4,5] an increase in glucose-6-phosphate dehydrogenase activity,[6] reduced activity of carbonic anhydrase,[4-6] and a decreased titer of the erythrocyte I antigen.[4] Erythrocytes of the fetus and newborn share all of these characteristics, and this has led to the suggestion that juvenile chronic myelogenous leukemia may be a congenital disorder accompanied by failure of normal erythrocyte differentiation.[4,7]

The patient described in this report provided an opportunity to confirm a number of these findings in juvenile chronic myelogenous leukemia and to examine some other properties of the erythrocytes in this disorder, including oxygen affinity and some structural features of the fetal hemoglobin.

CASE REPORT

J. G. (born November 9, 1965) was a 4-yr-old boy who had appeared well until April 1969 when he developed anorexia with weight loss, abdominal swelling, and a skin eruption consisting of raised pruritic lesions over his lower extremities. After an episode of rectal bleeding he was brought for examination. His liver and spleen were found to be enlarged, each extending 2 cm below the costal margins. His hematocrit was 36%, white blood cell count 28,000/cu mm, and platelet count 36,000/cu mm. The white cell differential consisted of 19% segmented neutrophils, 11% band forms, 4% metamyelocytes, 4% blast cells, 6% basophils, 3% eosinophils, 8% monocytes, and 45% lymphocytes. Myeloid hyperplasia was found on examination of a specimen of aspirated bone marrow. A 2-wk course of corticosteroid therapy was given, and the rectal bleeding promptly ceased. Widespread purpura subsequently developed, however, involving his face, neck, and extremities.

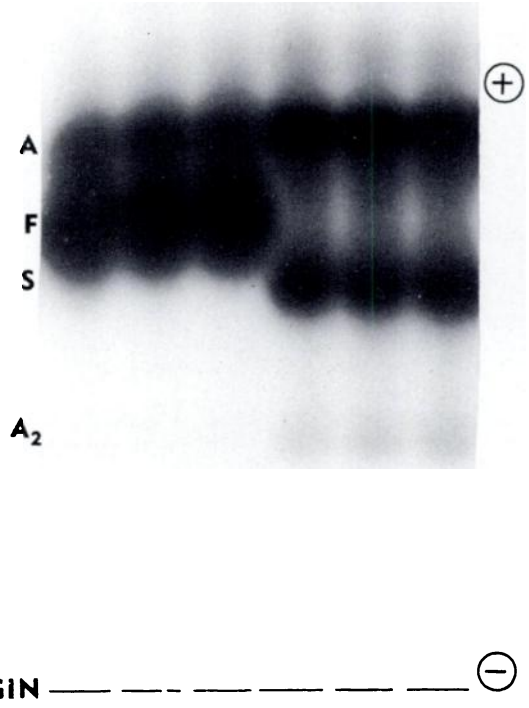

Fig. 1. Hemoglobin electrophoresis in starch gel Tris-EDTA-boric acid buffer, pH 8.6. Three patterns on the left represent hemolysates from patient J.G., and those on the right hemolysates from individual with sickle trait.

Over the following 5 mo, the child gradually became anemic with concomitant enlargement of his liver and spleen. Reticulocyte counts of up to 15% were obtained on several occasions, and nucleated red cells were seen in peripheral blood smears. By September 1969, his hemoglobin concentration had decreased to 6.1 g/100 ml, and hematocrit to 18%. His liver edge was palpated 10 cm below the costal margin, and his spleen was found to be massively enlarged, extending across the midline and into his pelvis. A red cell survival study with ^{51}Cr tagging showed a survival half-life of 7 days, indicating a markedly accelerated rate of red cell destruction. A splenectomy was performed in October 1969, followed by improvement of his symptoms and partial clearing of his pulpuric rash.

The child was first seen at the University of Illinois Hospital in March 1970. His hemoglobin concentrations was 14.0 g/100 ml, hematocrit 37%, platelet count 21,000/cu mm, white cell count 50,000/cu mm, and reticulocyte count 9%. The white cell differential count had remained virtually unchanged. Leukocyte alkaline phosphatase activity of cells from peripheral blood[8] was found to be markedly reduced. Chromosomal studies were performed with cells from peripheral blood. Leukocytes were cultured in medium 199[9] in the presence of autologous serum. Cultures were harvested at varying times ranging from 3 to 4½ days. In some cultures phytohemagglutinin was added initially or 4 hr prior to harvesting; in other cutures phytohemagglutinin was omitted. More than 100 satisfactory mitotic figures were examined, and only normal karyotypes were produced. A Philadelphia chromosome could not be detected in any of the preparations. A specimen of aspirated bone marrow was hypercellular with increased numbers of myeloid precursors and reduced megakaryocytes. Treatment with 6-mercaptopurine was begun and produced temporary symptomatic improvement and a decrease in the size of the liver. The child's treatment was subsquently continued at another hospital. His clinical course deteriorated, and a change to vincristine and prednisone therapy produced no improvement. He died in August 1970.

RESULTS

Electrophoresis of stroma-free red cell hemolysates in starch gel at pH 8.6 (Tris-EDTA-borate buffer)[10] showed a predominance of hemoglobin F, with hemoglobin A making up the remainder (Fig. 1). Hemoglobin A_2 was greatly

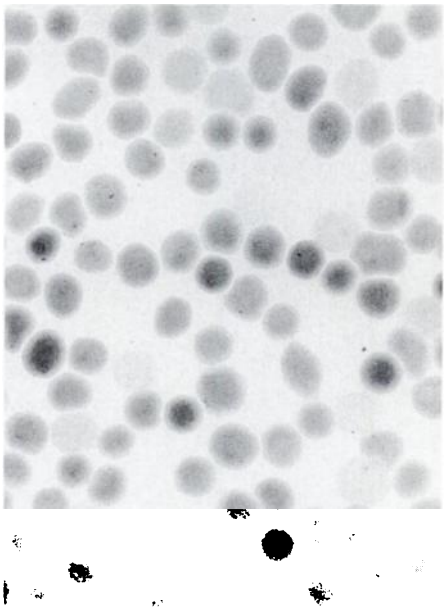

Fig. 2. Peripheral blood smears of patient J.G. stained for hemoglobin F by the Kleihauer and Betke technique.

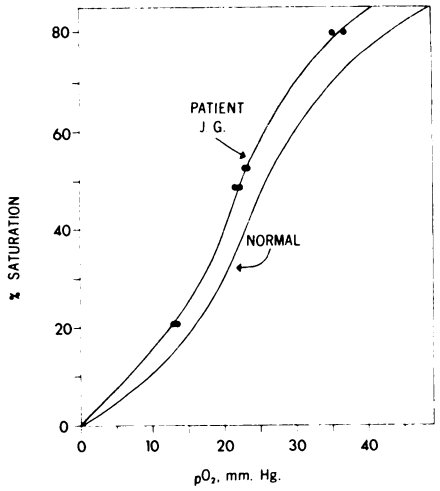

Fig. 3. Oxygen dissociation curve of whole blood from patient J.G. and of normal adult controls. Values were all corrected to pH 7.4.

reduced. Quantitation of hemoglobin A_2 by DEAE-Sephadex column chromatography[11] indicated a concentration of 0.23%. Hemoglobin F in the blood ranged from 70 to 73%, as determined by two methods.[12,13] The slide elution test of Kleihauer et al.[14] confirmed the presence of hemoglobin F in a major fraction of the erythrocytes by demonstration of only a small number of unstained cells containing only hemoglobin A (Fig. 2). An erythrocyte I antigen titer of 1:64 was obtained that was in the range normally seen in blood of newborn infants. A sample of blood from a normal adult control showed a titer of 1:2560.

The oxygen affinity of whole blood from the patient was determined by previously described procedures.[15] The oxygen dissociation curve was displaced to the left of that of blood from normal adults (Fig. 3), in the pattern typical of that seen in cord blood of newborn infants. The P_{O_2} at pH 7.4 at 50% oxygen saturation was 22.4 mm Hg in the blood of the patient, as compared to a normal adult value of 26 mm Hg. The 2,3-diphosphoglycerate (2,3 DPG) concentration[16] in the red cells of the patient was 4.6 μmoles/ml, which did not differ significantly from the normal mean of 4.3 μmoles/ml of cells. Erythropoietin levels in plasma and urine from the patient were within the normal range of values.

Analysis of the amino acid composition of position 136 of the γ-chain of the fetal hemoglobin was carried out by Dr. T. H. J. Huisman and Dr. W. A. Schroeder. The ratio of glycine to alanine in the γ-CB3 peptide was 0.66/2.40, which was similar to that observed in fetal hemoglobin from normal newborn infants and differed from the mean ratio of 0.4/2.6 observed in normal individuals 6 mo of age or older.[17]

DISCUSSION

The patient described in this report demonstrated the typical clinical and laboratory features of juvenile chronic myelogenous leukemia, including massive hepatosplenomegaly, thrombocytopenia, low leukocyte alkaline phos-

phatase, absence of a Philadelphia chromosome, and a markedly elevated level of hemoglobin F. The poor response to 6-mercaptopurine was similar to other reported experiences with this disorder.[7]

The finding of a leftward shift in the oxygen dissociation curve of blood from this patient demonstrates further that erythrocytes containing primarily fetal hemoglobin have oxygen affinity properties similar to those of cord blood, in spite of normal intracellular levels of 2,3 DPG.[15] This characteristic is attributable to the low affinity of fetal hemoglobin for 2,3 DPG and other intracellular phosphorylated compounds[18,19] that interact with hemoglobin A to produce a decrease in the oxygen affinity.[20]

It can be predicted that the increased oxygen affinity of the blood in juvenile chronic myleogenous leukemia may result in a significant degree of tissue hypoxia, even if the hemoglobin concentration of the blood is not greatly reduced. This may explain in part the elevated hemoglobin concentration and reticulocytosis that were present in this patient at a time when his leukemia was nevertheless poorly controlled.

Schroeder et al.[21] have recently demonstrated that two γ-chain subtypes can be identified in human fetal hemoglobin. The two species of γ-chains differ at γ-136, with one chain type containing alanine at this position and the other containing glycine. The γ-chain subtypes are designated Aγ and Gγ, respectively. This heterogeneity has been interpreted to indicate the existence of at least two pair of structural gene loci for the γ-chains.[21] Analysis of Gγ:Aγ ratios in individuals of different ages[17] has demonstrated that the relative quantities of the two subtypes undergo a significant change beyond infancy. In the newborn period, a Gγ:Aγ ratio of approximately 3:1 was obtained, whereas beyond 6 mo of age the ratio became reversed, reaching approximately 2:3.

Ratios of the γ-chain subtypes have been determined in a variety of abnormal states in which the fetal hemoglobin is affected. Analysis of the fetal hemoglobin of patients heterozygous for hereditary persistance of fetal hemoglobin indicated two modes of γ-chain gene expression. In some families, only a single γ subtype could be identified, whereas in others both Gγ and Aγ chains were present in approximately equal quantities.[22-25] In no instance was a ratio of the two types obtained that was similar to that found in normal newborn infants. Examination of black individuals heterozygous for β-thalassemia[26] also indicated two patterns of γ-chain subtype ratios, both of which, however, differed from those seen in the patients with hereditary persistance of fetal hemoglobin.[23] In some of the β-thalassemia families studied, a Gγ:Aγ ratio of 3:1 was obtained, which is the pattern seen in normal newborns; in other families the ratio was approximately 2:3, as occurs in normal individuals beyond 6 mo of age.

The finding in the fetal hemoglobin of our patient of a Gγ:Aγ ratio of approximately 3:1, thus, provides a further point of similarity between the erythrocytes in juvenile chronic myelogenous leukemia and those produced during fetal life. As with many of the other criteria with which these comparisons have been made, however, this property cannot be considered to be

unique to the newborn period, particularly in view of the finding by Schroeder et al.[26] of a similar ratio in the fetal hemoglobin of certain individuals with β-thalassemia. It is notable, however, that none of the findings reported thus far has indicated any significant difference between the red cells in juvenile chronic myelogenous leukemia and those of the newborn.

The significance of the fetal-like erythrocytes in juvenile chronic myelogenous leukemia remains obscure. Weatherall et al.[4] have suggested that this finding may represent the abnormal proliferation of a stem cell line in which normal differentiation processes have not occurred. It was demonstrated by these authors that progression of this form of leukemia from its early stages is accompanied by gradual replacement of the normal erythrocytes by the fetal-like cells, in a manner resembling a reversal of the normal switchover from fetal to adult erythrocytes. Further study of the red cell changes in juvenile chronic myelogenous leukemia may yield valuable insight into the normal processes of hemoglobin switchover and erythrocyte maturation.

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