HYPOTHESIS

Refractory Anemia, Preleukemic Conditions, and Fetal Erythropoiesis

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REFRACTORY ANEMIA (R.A.) is a convenient term to describe those acquired idiopathic chronic disease characterized by a failure of erythropoiesis with or without changes in granulopoiesis and/or thrombopoiesis. Patients with R.A. are noteworthy for the normal or increased cellularity of the bone marrow. All patients with R.A. may be considered as possibly preleukemic. Of particular interest is the fact that R.A. erythropoiesis has many striking similarities to that normally seen in the fetus.

BIOLOGICAL CHARACTERISTICS OF REFRACTORY ANEMIA

The pathophysiology of the anemia is complex. Effective erythropoiesis is strikingly decreased, and most frequently there is an intramedullary destruction of red cell precursors together with disturbed hemoglobinogenesis. There may also be peripheral hemolysis, particularly of young red blood cells. The anemia is macrocytic and often hypochromic. It is rarely dimorphous. There are typical or intermediate megaloblasts and frequently pathologic sideroblasts in the bone marrow.

A moderate increase to about 5% in fetal hemoglobin (Hb F) is frequently seen, and sometimes values as high as 37% are seen. The distribution of Hb F as appreciated by Kleihauer and Betke's technique is heterogenous. Some red cells have no Hb F. Others are similar to cord blood and others have intermediate contents. The glycine gamma chain/alanine gamma chain ratio is 3:4. Hemoglobin H (β4) and Bart's (γ4) have been found in some cases; increased as well as decreased enzymatic activities of red cells are usual. The average factor of variation is 2 for aldolase (ALD); 1.5 for hexokinase (HK), enolase (ENOL), glucose-6-phosphate dehydrogenase (G-6-PD), 6-phosphogluconate dehydrogenase (6-PGD), and triosephosphate isomerase (TPI); 1.2 for glycoldehyde-3-phosphate dehydrogenase (G-3-PD) and adenylyl kinase (AK); and 0.9 for phosphoglucose isomerase (PGI). We found pyruvate kinase (PK) to be slightly decreased or normal or even increased, while others have reported increases in some varieties of R.A. RBC separation by the differential flotation method isolates a layer of very light cells that have very high G-6-PD and HK activities, whereas adenosine triphosphate (ATP) and glutamic oxalate transaminase (GOT) activities are normal. These facts allow distinction between R.A. light RBC and reticulocytes with their high ATP as GOT activities.

Isozymic pattern variations have also been identified, particularly an excess of LDH-5 over LDH-1, whereas normal adult RBC are practically devoided of LDH-5. In the latter two bands of HK, I and III slowly appear in starch gel electrophoresis; band II,
which is not seen in normal adults, comes out in R.A.\textsuperscript{17} There is often an increase of I antigen, a decrease or even a loss of A\textsubscript{1}, and less frequently a decrease of antigens H and B. A decrease of antigen A is seldom found.\textsuperscript{6,18-20} Antigen I may be decreased, normal, or increased.\textsuperscript{21-23}

THE CHARACTERISTICS OF FETAL ERYTHROPOIESIS

Initially, erythropoiesis in the embryo is characterized by megaloblasts reminiscent of those seen in pernicious anemia.\textsuperscript{24} The hepatic phase of erythropoiesis is characterized by macrocytosis and hypochromia, and the red cell life span is shortened.\textsuperscript{25,27} During the embryonal and fetal development, several different types of hemoglobin were found.\textsuperscript{28} Initially, hemoglobin Gower is seen in two forms, Gower 1 (c\textsuperscript{4}) and Gower 2 (a\textsuperscript{2} and r\textsuperscript{2}). Fetal hemoglobin (a\textsuperscript{2}y\textsuperscript{2}) develops shortly thereafter and rapidly becomes the dominant type of hemoglobin until birth. Its synthesis decreases during the first months of extrauterine life, and after 6 mo insignificant levels are seen. There are two types of gamma chains in Hb F; one has a glycine and the other an alanine residue at position 136. During fetal life and in cord blood, the ratio of glycine to alanine is 3:1, but in the adult Hb F the proportions are equal and occasionally may be reversed.\textsuperscript{28} Adult hemoglobin (a\textsuperscript{2}y\textsuperscript{2}) is negligible until the 8th mo of fetal life. At birth there is 15-40% of Hb A, 0-17% of Hb A\textsubscript{2}, (a\textsuperscript{2}y\textsuperscript{2}), and less than 0.5% of Bart's (y\textsuperscript{4}). The RBC enzymes at birth are threefold greater than those in the adult for HK, ENOL, PGM, PGK, G-6-PD, PK, GR, and 1.2 times for G-6-PD. PFK is decreased to 0.8,\textsuperscript{31} and AC is decreased to 0.6.\textsuperscript{32}

The pattern of isoenzymes in fetal red cells is characteristic. Hexokinase has two bands, II and III, with band II showing the greatest activity.\textsuperscript{33} LDH is composed of nearly equal parts of LDH-5 and LDH-1.\textsuperscript{34-36} At birth, antigen I predominates but then disappears progressively during the first year of life, whereas antigen I increases and achieves its highest level at about the 18th mo.\textsuperscript{37} ABO antigens appear early in life. These antigens are detected by the fifth or sixth wk but have not fully developed at birth.\textsuperscript{38-40} The red cells of a genetically A\textsubscript{1} newborn are not agglutinated by an anti-A\textsubscript{1} serum.\textsuperscript{41-42} It has been demonstrated with isotopic methods that the proportion of A antigenic sites in the red cells of cord blood is 0.3 of that of adult red cells for groups A\textsubscript{1}, A\textsubscript{1}B, and 0.5 for groups A\textsubscript{2}.\textsuperscript{43} Antigen H is incompletely developed at birth.\textsuperscript{38,44}

DISCUSSION

In comparing the characteristics of R. A. and fetal erythropoiesis, a number of similarities are noted. (1.) In both there is Hb F production, and the proportion of glycine to alanine in gamma chains is identical.\textsuperscript{10} (2.) There are similarities in the isoenzymes patterns of hexokinase\textsuperscript{17} and LDH.\textsuperscript{15} These need to be confirmed in a greater number of patients. (3.) Although not identical, most enzyme activities are changed, either increased or decreased in the same proportion. PK appears to be an exception, but this awaits further confirmation. (4.) Quantitative modifications in the red cell antigens, particularly the increase of I and decreases of A\textsubscript{1}, A, B, and H, are similar, although the methods of measurement are different. The excess of antigen I is not characteristic of fetal erythropoiesis. (5.) Macrocytosis, hypochromia, and megaloblastosis cannot be considered as significant analogies.

Thus, even though some of the changes in R.A. may be considered as embryonal characteristics, there are always features of adult erythropoiesis. We are faced with the coexistence of both fetal and adult characteristics. It is important therefore, to consider whether these changes exist in a single cell or represent mixtures of adult and fetal cells.
The first assumption is physiologic prematurity of the red cells that are released into the circulation. The bone marrow transit time of red cells is shortened when erythropoiesis is stimulated by increased production of erythropoietin. Hemoglobin synthesis is accelerated, the maturation time is shortened, but the intermitotic interval is unchanged. The intramedullary transit time of $^{59}$Fe is shortened. For example, in the rat the transit time is decreased from a range of 60–72 hr to 36–48 hr. It is probably that the terminal mitosis is skipped, and immature macrocytes are released into the peripheral blood. The hemoglobin concentration of this macrocytes is normal or slightly reduced, their life span is shortened, and glycolytic enzymes are increased. It might be suggested that a similar mechanism may exist in R.A.

In the more immature stages of red cell maturation of the normal bone marrow, features similar to those of fetal red cells may be demonstrated, e.g., the isoenzyme pattern of LDH. In circumstances in which there is more rapid maturation of erythroblasts, i.e., after repeated phlebotomies in man, there will be an increase in antigen i of the red cells. Those red cells that are prematurely released will have antigen i throughout their life span. The persistence of i is, however, not accompanied by a decrease in antigen I. Some of the early erythroblasts may synthesize Hemoglobin F, and gamma chain synthesis stops as erythroblasts mature, being replaced with beta chain synthesis.

One might assume, therefore, that the synthetic capabilities during the early stages of maturation for fetal type products exist, but as maturation proceeds these are switched off in favor of adult-type enzymes and the like. It is possible that this switching mechanism is defective in R.A., and hence, cells are released that still demonstrate embryonal characteristics. Prematurity, however, is not a completely satisfactory explanation. Reticulocytosis is the usual sign of physiologic increase in erythropoiesis but is not seen in R.A. PK, which is always in excess in young cells, is sometimes low in R.A. An increase in HK and G-6-PD contrasts with the normal levels of GOT and ATP.

Another possibility would be that of a partial restoration of embryonal protein synthesis. In acute leukemia a mild increase of Hb F, about 2–5%, is usual. Marked increases in Hb F are usually observed in juvenile myeloid leukemia and in erythroleukemia. In the latter case, red cell HK is of the fetal type. The immerge of specific embryonal characteristics in malignant cells of diverse tissues and induced by various agents seems to be a common phenomenon and not related to leukemia alone.

In human hepatoma, the embryonal protein alpha I-F may be detected in both serum and tumor cells. The same fetoprotein is found in some patients with teratoblastomas. In the membrane of malignant cells from the colon, neoantigens identical to those of normal fetal colonic cells may be seen. In human hepatoma the isoenzyme of aldolase is identical to that of
the fetus. An isoenzyme of alkaline phosphatase identical to that found in the placenta has been described in a number of malignancies. Finally, patients with polycythemia vera may have an abnormal vitamin B₁₂ binding protein similar to that of the normal fetus.

The reappearance of some embryonal characteristics seen in patients with R.A. raises the question of their relationship to the etiology and to the malignant transformation that is common in this category of disease. Some hemopoietic stem cells may have a mutation leading to altered genetic function, perhaps induced by chemicals, radiation, or viruses. Cytogenetic studies indicating chromosomal abnormalities would be in keeping with such an idea. Whatever the mechanism, there appears to be a reactivation of normally repressed fetal genes; a significant number would appear to be involved, since numerous fetal characteristics have been detected. It would be of interest, therefore, to determine whether some of these embryonal characteristics play a role in the pathophysiology of R.A.

REFERENCES

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