Thalassemia Intermedia

Cases in Negro Siblings with Unusual Differences in Minor Hemoglobin Components

By Howard A. Pearson and Ward D. Noyes

Thalassemia is a hereditary defect of hemoglobin synthesis which results in production of microcytic hypochromic red cells. Most individuals with thalassemia can be clinically classified as having either thalassemia major or minor. The former, usually associated with homozygosity for thalassemia genes, is characterized by severe hemolytic anemia and markedly abnormal red cell morphology; whereas the latter, usually resulting from heterozygosity, is manifested by a mild microcytic, hypochromic anemia. Thalassemia intermedia is a clinical designation for hematologic disease which is intermediate in severity between the two more common clinical entities. Thalassemia intermedia is genetically heterogenous. Individual patients have been studied who appear to be homozygous for thalassemia while others are presumably heterozygous. Still others cases of thalassemia intermedia result from interaction of thalassemiia with abnormal hemoglobin genes or with variants such as the Lepore hemoglobin or hemoglobin H.

In addition to the degree of anemia, abnormal red cell morphology and symptomatology, characteristic changes in the proportion of minor hemoglobins occur in the thalassemia syndromes. Thalassemia major is accompanied by large amounts of fetal Hb (Hb F) in the red cells, whereas Hb A₂ is low or normal. The red cells of most individuals with thalassemia minor contain elevated levels of Hb A₂ while Hb F is slightly elevated in only half of these cases. A few families have been described in which heterozygous thalassemia is accompanied by normal Hb A₂ levels and pronounced elevations of Hb F (10-20 per cent). Indeed, a genetically determined reciprocal relationship between Hb A₂ and F levels in thalassemia has been hypothesized. In the American literature, detailed studies of minor hemoglobin components have not been reported in any substantial series of cases of thalassemia intermedia.

This report describes genetic and hematologic studies of two adult Negro brothers with clinical thalassemia intermedia. Despite nearly identical hematologic and clinical findings, the proportions of Hb A₂ and Hb F were

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*In this paper, the discussion of thalassemia will be restricted to the so-called beta-thalassemia, a hereditary defect in synthesis of beta polypeptide chains of hemoglobin. Since the patients in the pedigree have elevated levels of hemoglobins with α chains α₂ δ₂, α₂γ₂, so-called α thalassemia could not be present.
Table 1.—Levels of Hb F and Hb A₂ in Cases of Thalassemia Intermedia

<table>
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<tr>
<th>Author</th>
<th>Hb F (%)</th>
<th>Hb A₂ (%)</th>
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<td>Kunkel et al. 10</td>
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<td>Gabuzda et al. 24</td>
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<td></td>
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<td></td>
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*Probably heterozygous.
†Probably homozygous.

strikingly different. The rates of synthesis of these minor hemoglobin components are thought by some investigators to be primarily under genetic control. The findings in this pedigree might be interpreted as supporting recent suggestions that in thalassemia alterations in the proportions of the minor hemoglobin components may occur by non-genetic as well as genetic mechanisms 13, 14.

MATERIALS AND METHODS

Starch block electrophoresis was performed using barbital buffer, pH 8.6 to quantitate Hb A₂. Hb F was measured by a modification of the 1-minute alkali denaturation method of Singer et al. (normal < 2.0 per cent) and visually confirmed by agar gel electrophoresis. Blood groupings were done and in no instance were results incompatible with the stated relationship.

FAMILY STUDY

I-1. This 75-year-old female was asymptomatic. Spleen and liver were not enlarged. A mild hypochromic, microcytic anemia was present. Red cell morphology is illustrated in figure 2. Hb A₂ was increased; Hb F was slightly elevated.

I-2. This man died at age 65 of a heart attack. He reportedly had been healthy with no symptoms of anemia.

II-1.* This 46-year-old male was sickly and had chronic leg ulcerations as a child. However, he had been able to work as a laborer until about the age of 25, when a "stomach tumor" was removed. At this time he was found to be anemic and received blood transfusions at irregular but infrequent intervals. At the age of 34 he developed weakness and fatigue and had to

*The remarkable clinical details of cases II-1 and II-2 will be described elsewhere. 18
Fig. 1.—Family pedigree. The propositus is indicated by the arrow.

stop working. Transfusions were then given every 1–2 months for shortness of breath and fatigue.

Physical examination revealed an eunuchoid body habitus with scanty axillary and pubic hair. Scleral icterus was present. The liver was palpated 5 cm. and the spleen 3 cm. below the costal margins. He was bilaterally cryptorchid.

A severe anemia with markedly abnormal red cell morphology, as shown in figure 3, was present. No circulating nucleated red cells were seen and reticulocyte count was only 0.8 per cent. Serum iron level was normal. RBC Cr$^{31} T_{1/2}$ was 18 days (normal 25–35 days). Excretion of 17 keto-steroids was very low at 1.0 mg./day. Radiological examinations showed widening of the medullary cavities and a generalized coarse trabecular pattern of the skeleton. Paraspinous masses presumed to be extramedullary hematopoietic tissue were seen.

The hematologic values listed in table 2 were obtained 8 weeks after transfusion and comparable values were obtained on one other occasion. Hb F and Hb A$\varepsilon$ were both elevated. Acid elution study of peripheral blood for Hb F-containing red cells showed only a rare cell which contained appreciable quantities of resistant hemoglobin.

II-2. This 42-year-old male had been in good health working as a laborer until 35 years of age when he became weak and developed palpitations. He stopped working because of weakness and for the next 7 years monthly transfusions were given because of anemia. He developed an episode of obstructive jaundice for which he was first admitted to the hospital.

Physical examination revealed marked icterus. The liver was palpable 3 cm. and the spleen 2 cm. below the costal margins. A severe anemia with markedly abnormal red cell morphology was present as illustrated in figure 4. No circulating nucleated red cells were seen and the reticulocyte count was 2.0 per cent. Serum iron was elevated, RBC Cr$^{31} T_{1/2}$ was 16 days. Radio-
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<th>Hct (%)</th>
<th>MCV (cu. µ)</th>
<th>MCH (pg)</th>
<th>MCHC (%)</th>
<th>Hb A₂ (%)</th>
<th>Hb F (%)</th>
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logical examinations showed a generalized coarse bony trabecular pattern and a paravertebral mass presumed to be extramedullary hematopoietic tissue. The jaundice cleared rapidly; however, 2 months later he developed signs of acute spinal cord compression. Decompressive laminectomy revealed extradural masses of hematopoietic tissue.

Hematologic studies listed on table 2 were performed 8 weeks after transfusion. Hb A₂ was normal while Hb F was markedly elevated, and similar values were obtained on two other occasions. Acid elution studies of peripheral blood showed a mixed population of Hb F-containing red cells; some appeared to contain considerable amounts of Hb F and others were normal.

II-3, II-4, and II-6 were normal.

II-7. This 35-year-old woman had received transfusions during two pregnancies because of anemia. Physical examination was normal. Red cell morphology showed slight hypochromia and microcytosis similar to that of I-I. The level of Hb A₂ was increased, whereas Hb F was normal.

III-1. Because of her importance in the pedigree, this child was studied on two occasions. Red cell morphology (figure 5) and Hb A₂ and Hb F values were normal.

III-2 and III-3. These children had no symptoms and normal physical examinations. There was a moderate hypochromic anemia in each, and their red cell morphology was similar to that of their grandmother I-I and aunt II-7. Both had elevated Hb A₂ and Hb F levels.

III-4 and III-5 were normal children.
Fig. 3.—Peripheral blood smear of II-1. Note the extreme microcytosis poikilocytosis and hypochromia.

Fig. 4.—Peripheral blood smear of II-2. The red cell morphology is almost identical with that illustrated in figure 3.

DISCUSSION

The family described in this report raises two possibly inter-related problems. The first concerns factors producing the hematologic syndrome of thalassemia intermedia while the second involves mechanisms for the radically different proportions of Hb A₂ and F in these otherwise similar patients. The late onset of symptomatic anemia, slight splenomegaly, and absence of cir-
culating nucleated red cells, differentiate these patients from the usual case of thalassemia major. However, the markedly abnormal red cell morphology, skeletal changes, and transfusion requirements can hardly be considered as manifestations of “minor” disease. Their disease is certainly more severe than that of their mother, sibling, or children, who have the hematologic and clinical criteria of thalassemia minor resulting from heterozygous thalassemia.

In explaining this modification of disease, it must be assumed that a gene or genes were present in the deceased father (I-2) which interacted with the mother’s (I-1) thalassemia gene augmenting the hematologic severity of disease in II-1 and II-2. The exact nature of this interacting gene is uncertain since it does not appear phenotypically distinct in any of the members of generation II. For this reason, it is not possible to state with complete confidence whether the two brothers are heterozygous or homozygous for thalassemia. However, the completely normal findings in the child, III-1, is strong evidence against II-2 being homozygous. In this instance, unless non-paternity is invoked for which there is no evidence, the interacting gene either has no recognizable phenotypic expression or is non-allelic to the thalassemia locus.

Despite the very similar clinical and morphologic changes in II-1 and II-2, the proportions of Hb A2 and F were strikingly different. One brother had elevated Hb A2 and moderately elevated Hb F, whereas the other showed a normal Hb A2 and markedly elevated Hb F. The ratios of Hb A2 to A1 in the two brothers were also dissimilar. If the differences observed in II-1 and II-2 are to be explained on a genetic basis, an improbable assumption must be made about I-2; namely, that he possessed two different genes to interact with I-1’s thalassemia gene. Each of these, interacting with the thalassemia gene, would result in similar clinical and hematologic findings, and yet also result in the observed differences of proportions in minor hemoglobin components.
Another recently described condition might also be considered in this connection; namely so-called $\delta$-thalassemia.\textsuperscript{23} In the heterozygous state, the thalassemia gene results in decreased $\delta$ polypeptide chain synthesis, and hence, depressed levels of Hb A\textsubscript{2}. No other hematologic abnormalities are seen in this syndrome. It is possible that such a gene might be present in this family; as suggested by the low levels (1.6 per cent) of Hb A\textsubscript{2} in II-6. If II-2 possessed a $\delta$-thalassemia gene, a low level of Hb A\textsubscript{2} might result. However, such a hypothesis would not explain the considerable hematologic disease despite apparent $\beta$-thalassemia heterozygosity or high level of Hb F of this man.

Alternative, and to us more plausible, explanations require that I-2 possessed only one type of interacting gene. This was transmitted to both II-1 and II-2, and its interaction with the thalassemia gene derived from I-1 resulted in accentuation of the hematologic disease. The observed differences in the proportions of minor hemoglobins would then be due to environmental or individual modifying factors. This type of modification has been observed in other clinical situations. Elevated levels of Hb A\textsubscript{2} have been documented in some cases of pernicious anemia while depressed levels occur in iron deficiency.\textsuperscript{11} On the other hand, Hb F levels may rise in aplastic anemia\textsuperscript{20} and in normal or molar pregnancy.\textsuperscript{21} It is possible that the endocrinologic differences observed between II-1 and II-2, or other more subtle differences could have modified the relative rates of synthesis of Hb A\textsubscript{2} and F.

Environmental factors might operate in a different way. Nance\textsuperscript{14} has suggested that the elevation of fetal hemoglobin in thalassemia may be due in part to persistence of a clone of fetal hemoglobin-producing cells. This clone is postulated to result from intrachromosomal crossing over in a region of triplication ($\gamma$, $\beta$ and $\delta$ loci) with subsequent deletion of adjacent $\beta$ and $\delta$ loci. The factors governing selection of such clones are presumably environmental and individual, and could differ in two siblings who possessed otherwise identical genes regulating hemoglobin synthesis.

A number of theories recently advanced concerning thalassemia have rightfully emphasized genetic mechanisms.\textsuperscript{8,14,22} If, as suggested by this pedigree, individual or environmental factors may modify the biochemical expression of these genes, then thalassemia may be even more complex than generally considered.

**SUMMARY AND CONCLUSION**

Biochemical, genetic, and clinical studies of two adult Negro brothers with clinical thalassemia intermedia are presented. Their hematologic disease appears to have been caused by interactions of thalassemia with another gene which had no phenotypic expression or was non-allelic to the thalassemia locus. These men had striking differences in the proportions of the minor components Hb A\textsubscript{2} and F. It is considered unlikely that these differences were genetically determined. Rather, it is suggested that environmental or individual factors can modify this biochemical expression of thalassemia genes.
**THALASSEMIA INTERMEDIA**

**Summary in Interlingua**

Es presentate studios biochimic, genetic, e clinic in duo adulte fratres negre con clinic thalassemia intermedia. Lor morbo hematologic pare haber essite causate per interaiones de thalassemia con un altere gen que habeva nulle expressiun phenotypic o que esseva non-allelic pro le loco de thalassemia. Iste homines exhibiva frappante differentias in le proportiones del minor componentes hemoglobiniic A₂ e F. Es reguardate Como pauc probabil que iste differentias esseva determinate geneticamente. Es suggestionate plus tosto que factores ambiental o individual es capace a modificar iste expression biochimic del genes de thalassemia.

**Acknowledgments**

We wish to express our thanks to Dr. Edgar Strange, McIntosh, Fla., for referring these patients, and Dr. John Henry, Director of Laboratories, University of Florida College of Medicine, for performing the blood grouping studies.

**References**


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The suggestion by Sirs (Lancet 1:971, 1963) that the presence of a low level of carboxyhemoglobin in the circulation would reduce sickle-cell formation was explored in two boys with sickle-cell anemia. No benefit was noted either in the percentage of sickles in the circulation or in the mean cell life of the cells, which was 8 days before CO-administration and 5.5 days after 7 days of treatment.—I. C.
Thalassemia Intermedia: Cases in Negro Siblings with Unusual Differences in Minor Hemoglobin Components

HOWARD A. PEARSON and WARD D. NOYES