Failure of the α-Thalassemia Gene to Decrease the Severity of Sickle Cell Anemia

By Clayton Natta

A 15-yr-old black male with homozygous sickle cell disease was severely growth retarded and had a chronic hemolytic anemia requiring transfusions. Globin chain synthetic studies of both peripheral blood reticulocytes and bone marrow cells revealed a ratio of α to β\textsuperscript{A} globin synthesis (α/β\textsuperscript{A} ratio) of 0.5, indicating the presence of an α-thalassemia gene. Messenger RNA isolated from the bone marrow of the patient was translated in the wheat germ cell-free system, and the globin synthesized had an α/β\textsuperscript{A} ratio of 0.7. The hemolysate prepared from incubated bone marrow cells was fractionated on a Sephadex G100 column. The results showed that there was a peak of radioactivity that eluted after the hemoglobin peak. When this pooled peak was analyzed by CMC chromatography, the α/β\textsuperscript{A} ratio was 0.9. These globin intermediates, probably dimers, may have contributed to the hemolysis in this patient.

Sickle cell anemia is a chronic hemolytic anemia often characterized by severe repeated painful crises. There has been some documentation that certain factors in addition to the inheritance of hemoglobin S may lessen the clinical severity of sickle cell anemia. One such factor is the presence of an α-chain mutant Hb Memphis.\textsuperscript{1} There have been occasional reports of patients with SS-α-thalassemia genotype and mild disease. In two patients described by van Enk,\textsuperscript{2} there were clinical pictures of mild disease and uncomplicated pregnancies. In these patients the β-chain loci produced only hemoglobin S.

The studies reported in this paper document a case of homozygous S disease with an α-thalassemia gene occurring in a black patient. In this case the clinical course was characterized by chronic anemia resulting in severe growth retardation and requiring transfusions regularly.

Materials and Methods

Hemoglobin levels and red cell indices were obtained with a Model S Coulter Counter (Coulter Electronics, Hialeah, Fla.). Cellulose acetate electrophoresis in Tris-EDTA-borate buffer at pH 8.6 was done on peripheral blood samples.\textsuperscript{3} Citrate agar electrophoresis was done at pH 6.2.\textsuperscript{3} HbA\textsubscript{2} level was determined by the microchromatography method.\textsuperscript{4} Fetal Hb was determined by alkali denaturation.\textsuperscript{5}

Measurement of globin synthesis. Heparinized blood was obtained from both the patient and his mother. A bone marrow specimen was aspirated from the posterior iliac crest of the patient. Peripheral blood cells were washed thrice with isotonic saline by centrifugation at 1500 g for 10 min and the buffy coat removed. One volume of packed cells (1 ml) was incubated with an equal volume of Krebs-Ringer bicarbonate solution, containing all the amino acids except leucine, to which 0.1 volume \textsuperscript{3}H-leucine (New England Nuclear, Boston, Mass.), 39 Ci/mmol,
had been added, and the pH was adjusted to 7.4. Cells were incubated for 1 h at 37°C. After incubation the cells were washed three times with a large excess of cold isotonic saline and then lysed with 4 volumes of water. Stroma were removed by centrifugation at 15,000 rpm for 10 min. The supernatant was then analyzed on carboxymethylcellulose (CMC) chromatography at pH 6.7, using 14C-labeled sickle hemolysate and cold sickle hemolysate to aid in the recovery of chains. The 3H incorporation in βα/α were corrected using the relative recovery of 14C incorporation in βα/α, which was 1. The bone marrow specimen was similarly analyzed. Samples were counted using a Packard Tricarb liquid scintillation counter (Packard Instrument, Downers Grove, Ill.). The efficiency of counting with double-label settings was 105 for 3H and 30% for 14C. The counting error was less than 1%.

Analysis of globin mRNA. Total RNA was isolated from the bone marrow cells as described previously. The total RNA was then fractionated on sucrose density gradients and the 6-16 S RNA (mRNA) was isolated. The isolated mRNA was tested for biologic activity in a wheat germ cell-free system using rate-limiting amounts of mRNA.

Sephadex chromatography of hemolysate. An aliquot of bone marrow cells of the patient was incubated with 3H amino acids for 1 hr at 37°C. After the incubation the cells were washed with a large excess of isotonic saline and centrifuged at 31,000 g for 20 min. The supernatant (stroma free) was analyzed by fractionation on a Sephadex G-100 column and elution with a buffer consisting of 0.05 M Tris and 0.1 M KCl at pH 7.5. Fractions were collected and analyzed for both absorbancy and counts. The fractions under each peak were pooled. There were two peaks that were then analyzed by CMC chromatography after adding 14C sickle hemolysate to correct for recovery of chains. The third peak consisted of low molecular weight compounds such as amino acids and incomplete nascent chains.

Patient Data
A 15-yr-old black male of West Indian extraction was seen at the Harlem Sickle Cell Center because of sickle cell crises, nausea, and growth retardation. He showed stunted growth as well as frontal bossing, protruding teeth, and hepatomegaly. There was no splenomegaly. Sclerae were icteric. Peripheral smear revealed moderate anisocytosis with many microcytes, polychromasia, and moderate hypochromia. Sickle cells were seen on smear, as well as nucleated RBC. Reticulocyte count was 16%. Indices were MCV 86 cu µm, MCH 28 pg, RBC 1.84 x 106/cu mm, 6.4 g/dl, Hct 18.6%, WBC 16.6 x 103/cu mm. Cellulose acetate and citrate agar electrophoresis revealed mainly HbS and no HbA. HbA2 was 3.2%, HbF was 1.2%. Serum iron was 116 µg/dl; TIBC 276 µg/dl; saturation 42%; T4 RIA 7.6 µg/dl; T3 RU 1.06 µg/dl.

Family studies were restricted to the mother, whose Hb was 12.6 g/dl and Hct 36%. MCV was 82 cu µg, MCH 28.7 pg, RBC 4.37 x 106/cu mm, WBC 6.4 x 103/cu mm. Smear was hypochromic and microcytic. Electrophoresis of blood on cellulose acetate revealed an AS pattern. HbA2 and HbF levels were normal. The percentage of HbS was 31%.

RESULTS

Hematologic values. The electrophoretic data and persistent reticulocytosis suggested homozygous sickle cell disease. The low MCV in the presence of
persistent reticulocytosis, a normal serum iron that was 42\% saturated, and microcytosis on smear suggested the presence of \(\alpha\)-thalassemia gene.

**Globin chain synthesis.** The patient had decreased \(\alpha\) compared to \(\beta\) synthesis (\(\alpha/\beta\) ratio) in both reticulocytes and bone marrow cells, the \(\alpha/\beta\) ratio being 0.5 in each case (Figs. 1 and 2, Table 1). Unlike the patient, however, the mother’s reticulocytes synthesized \(\beta\), but \(\alpha\)-chain synthesis was also diminished (\(\alpha/\beta^a + \beta^s = 0.6\)) (Table 1). These results are similar to those reported elsewhere.\(^1\)

Bone marrow mRNA added to a wheat germ cell-free system in rate-limiting amounts resulted in the synthesis of globin with an \(\alpha/\beta^s\) of 0.7 (Fig. 3). This result is consistent with the presence of a decreased amount of biologically active \(\alpha\)-globin mRNA compared to \(\beta\)-globin mRNA, characteristic of \(\alpha\)-thalassemia.

**Sephadex G-100 chromatography.** Fractionation of \(^3\)H-labeled bone marrow hemolysate under the conditions described demonstrated three peaks (I, II, and III, Fig. 4). Peak I, which was under the A\(_{s40}\) peak, when fractionated on CMC chromatography had an \(\alpha/\beta^s\) ratio of 0.5. Peak II when similarly analyzed gave an \(\alpha/\beta^s\) ratio of 0.9. Peak III was not analyzed further.

| Table 1. Comparison of Globin Synthesis in Bone Marrow (BM) and Peripheral Blood (PB) of Normal Subjects and Patients with Sickle Cell Anemia and Sickle Cell \(\alpha\)-Thalassemia |
|---------------------------------|-----------------|-----------------
| **Cells**                       | **No. of Patients** | **\(\alpha/\beta\) Ratio (Mean ± SD)** |
| Normal                          |                   |                   |
| BM                              | 3                | 1.0 ± 0.1         |
| PB                              | 5                | 1.0 ± 0.1         |
| Sickle cell anemia              |                   |                   |
| BM                              | 5                | 1.0 ± 0.1         |
| PB                              | 5                | 1.0 ± 0.2         |
| Patient                         |                   |                   |
| BM                              |                 | 0.5*              |
| PB                              |                 | 0.5*              |
| Mother                          |                   |                   |
| BM                              |                 | 0.6†              |
| (S.S. with \(\alpha\)-thalassemia)|                 |                   |
| PB                              |                 |                   |

\(^*\)\(\alpha/\beta^s\) ratio.

\(^†\)\(\alpha/\beta^a + \beta^s\) ratio.
DISCUSSION

The patient had a severe clinical course requiring repeated blood transfusions. The high HbS level, 95.6%, with the low MCV, low MCH, normal HbA2 level of 3.2, and normal serum iron, and hypochromia and microcytosis are consistent with homozygosity of HbS and heterozygosity for α-thalassemia gene.

That this thalassemia gene was α-thalassemia is supported by decreased α-chain synthesis as shown by decreased α/β4 ratios of intact bone marrow and peripheral blood cells and the ratios in cell-free studies of bone marrow mRNA. The mother’s reticulocyte α/βs + βt ratio was diagnostic of sickle α-thalassemia in that there was diminished α-chain synthesis and βs + βt > α.

The problem of severe hemolysis in this patient is most intriguing. Even though the α/β4 ratios of bone marrow and peripheral blood and the cell free bone marrow ratio were similar to those of HbH patients we previously described,12 we were unable to find a fast-moving hemoglobin variant on repeated electrophoresis. This may be a reflection of the instability of βt, if it is found.13

Aksoy14 described a 4-yr-old male Turkish child with homozygous S and
α-thalassemia, confirmed by family studies. The child’s course was relatively mild, probably because of HbF level of 27%. The findings in our patient are also different from those of the 4-yr-old Shite Arab girl described by Weatherall et al.¹³ because of the high HbF level (43.7%) and mild clinical course of the Arab girl. The differences in the clinical courses may be related to the HbF levels. Another factor that may have determined increased clinical severity in our case may be the presence of a pool of β⁺ chains. These chains may associate with membranes in an as yet unspecified way and may contribute to increased hemolysis.

It was previously reported that β⁺ globin and HbS are preferentially bound to stroma and could contribute to the hemolytic process.¹⁵,¹⁶ In this report we have identified low molecular weight aggregates of α and β⁺ globin chains that are probably dimers. Although the role of these globin intermediates is not known, it is possible that they may contribute to the decreased life span of the RBC, which may be removed prematurely from the circulation.

The nature of the α-thalassemia gene in this family is unclear. The mother has sickle cell trait and α-thalassemia as shown by the hypochromia and microcytosis of the smear, HbS level of 31%, and an α/β⁺ + β⁺ ratio of 0.6. The number of α-globin loci in blacks may vary.¹⁷ One may speculate that the synthetic data of the proband are consistent with the presence of one active α-chain gene on one chromosome in cis and two in trans. However, whether or not α-thalassemia in blacks can be classified, as it has been in oriental populations, remains unresolved.

ACKNOWLEDGMENT

I would like to express my thanks to Joan Nicholson and Yolanda Ashby for expert technical assistance and to Dr. A. Bank and Dr. U. Nudel for their helpful comments during the preparation of this manuscript. I would like also to thank Dr. Jeanne Smith for helping to obtain peripheral blood from the mother.

REFERENCES

Failure of the alpha-thalassemia gene to decrease the severity of sickle cell anemia

C Natta