Bone Marrow and Peripheral Blood Globin Synthesis in an American Black Family With Beta Thalassemia

By Shlomo Friedman, Frank A. Oski, and Elias Schwartz

Synthesis of globin chains in bone marrow and peripheral blood samples from a black family with mild beta thalassemia was compared with similar studies in white people. Blood and bone marrow were incubated with $^{14}$C-leucine, globin chains were isolated, and $\beta/\alpha$ and $\gamma/\alpha$ ratios were calculated. The results of studies of globin synthesis in homozygotes of different races were similar, despite the differences in severity of clinical disease. In the heterozygotes, there was a significant defect in beta synthesis in the peripheral blood of white subjects, while in two of three black patients the $\beta/\alpha$ ratio was in the normal range. Although there was no evidence of segregation of an alpha thalassemia gene in this black family to explain the unusual $\beta/\alpha$ ratios, the presence of such a gene in the heterozygotes could not be excluded.

THALASSEMIA occurs commonly in black populations in Africa, Jamaica, and the United States. Both beta and alpha thalassemia have been described in black people in this country. The heterozygous form of beta thalassemia most frequently found is associated with an elevated percentage of hemoglobin A2 (Hb A2), a normal or slightly increased amount of Hb F, microcytosis, hypochromia, and variability in red cell size and shape. The morphologic and hemoglobin abnormalities in black people with beta thalassemia trait are similar to those seen in Mediterranean and Oriental patients. Despite the high frequency of elevated Hb A2 thalassemia trait in black people, reflected by an incidence of 0.8% in St. Louis and 0.75% in Nigeria, only 20 cases of homozygous beta thalassemia in this group have been reported from the United States. The disease has been generally mild, with only four of the reported patients requiring regular blood transfusions.

Decreased synthesis of the beta chain of normal human hemoglobin (Hb A) has been found in the peripheral blood of patients with heterozygous and homozygous beta thalassemia. Recent reports have suggested that the moderate imbalance of globin synthesis found in reticulocytes of white people with beta thalassemia trait is more severe than that found in black people. Recent studies have also shown that the ratio of beta to alpha chain synthesis is closer to unity in the bone marrow cells of Italian patients with heterozygous or homozygous beta thalassemia than in their peripheral blood.

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Table 1. Hematologic Data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Hb (g/100 ml)</th>
<th>Hct (vol/100 ml)</th>
<th>RBC ($\times 10^{-1}$/cu mm)</th>
<th>Retic. (%</th>
<th>Mean Corpuscular Volume (cu µ)</th>
<th>Mean Corpuscular Hb (g/dl)</th>
<th>Mean Corpuscular Hb Concent. (g/100 ml)</th>
<th>Hb A₂ (%)</th>
<th>Hb F (%)</th>
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<tr>
<td>I-1</td>
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<td>12.9</td>
<td>42</td>
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<td>5.6</td>
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<td>3.0</td>
<td>74</td>
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<td>31.0</td>
<td>5.4</td>
<td>3.8</td>
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<td>10.0</td>
<td>34</td>
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<td>8.4</td>
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<td>17.1</td>
<td>29.4</td>
<td>7.5</td>
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<td>5.68</td>
<td>2.2</td>
<td>72.5</td>
<td>22.7</td>
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</table>
BETA THALASSEMIA

Similar studies are not available in black patients. We report here studies of globin synthesis in blood and bone marrow of two black sisters with homozygous beta thalassemia and similar studies of peripheral blood reticulocytes in three of their family members with beta thalassemia trait.

MATERIALS AND METHODS

The “H” family, of black descent, includes two sisters with Cooley’s anemia. The propositus (II-2), an 8-yr-old girl, has had chronic anemia that was first noted when she was 3 yr old. She received two blood transfusions at 3 and 4 yr of age for anemia. Her urine has frequently turned dark brown when she has had upper respiratory infections. On physical examination she appeared slightly pale, without jaundice. Her height was 114 cm, and weight was 16.6 kg, both values below the third percentile for her age. She had maxillary overgrowth and a facial configuration typical of that seen in Cooley’s anemia. The spleen was palpable at 5 cm below the left costal margin, and the liver at 2 cm below the right costal margin. Radiographs of the long bones showed increased recticulation suggestive of chronic marrow hypertrophy. There was thickening of the cranial vault due to widening of the diploic space. The patient’s hematologic values, as well as those of her family, are shown in Table 1. Her peripheral blood smear showed marked hypochromia, microcytosis, anisocytosis, and poikilocytosis. There was erythroid hyperplasia in the bone marrow, with a ratio of myeloid to erythroid cells of 1:4.1. Bone marrow iron stores were increased. A supravital stain of the marrow with 1% methyl violet showed large purple inclusions typical of those seen in Cooley’s anemia in numerous nucleated and nonnucleated red cells.

The 11-yr-old sister (II-1) of the propositus was first noted to be anemic at age 10. She has never been transfused. Her urine has been dark on many occasions. On examination, her height was 144 cm, and weight was 28.3 kg, both values within the normal range for her age. Her facial characteristics were similar to those of her sister. Her spleen was palpable at 4 cm below the left costal margin. The liver was not palpable. There were no signs of early puberty. Abnormalities of her skull x-ray were not as marked as those of her sister. Hematologic values are shown in Table 1. Bone marrow examination showed erythroid hyperplasia, a myeloid to erythroid ratio of 1:4.4, an excess of iron stores, and inclusions in the red cells after incubation with methyl violet.

The 6-yr-old brother (II-3) and 4-yr-old sister (II-4) of the propositus were in good health. The mother (I-1) received blood transfusions after each delivery but had otherwise been well. The father (I-2), who lived in Virginia, was healthy. His blood sample was sent to us for analysis. Hematologic values of these family members are in Table 1. Two Italian boys and one Greek boy with Cooley’s anemia were also studied at the same time in order to compare the findings with those of the black family.

Hematologic studies were done by routine methods. Hb A, levels were determined by starch granule electrophoresis, and Hb F levels by alkali denaturation. Globin synthesis was studied by methods previously described. Four milliliters of peripheral blood or bone marrow were incubated with 10 μCi of L-leucine-U-14C for 2 hr at 37°C. The red cells were washed and hemolyzed, and globin was precipitated in cold acid-acetone. Globin chains were separated by carboxymethyl cellulose chromatography in 8 M urea, with a sodium phosphate gradient at pH 6.7. Results were expressed as β/α ratios. In the homozygotes, the counts in the tubes containing each globin chain were totaled to calculate radioactivity incorporated into the chains. The β/α ratio was determined on the basis of total radioactivity in each chain. In the heterozygotes, where less radioactivity was incorporated, the specific activities (cpm/OD) of the tubes containing the height of each peak were calculated. The average of the specific activities for each chain was used as the specific activity of that chain, and a β/α specific activity ratio was calculated. The absorption of beta chains exceeds that of alpha chains by a factor of 1.52 at pH 6.7, necessitating an appropriate correction in the calculation of specific activities. The counting error was 3% or less for the samples used for determinations of specific activities.
The diagnosis of Cooley's anemia in the propositus (II-2) and her sister (II-1) was based on the morphology of the red cells, hematologic values (Table 1), and the presence of purple inclusion bodies in erythroid cells of the bone marrows. The parents of the propositus (I-1, I-2), her brother (II-3), and one sister (II-4) had beta thalassemia trait, as evidenced by low mean corpuscular volumes (MCV) and mean corpuscular hemoglobins (MCH), and elevated levels of Hb A₂ and Hb F (Table 1).

The mean β/α specific activity ratios found after incubation of peripheral blood samples of 21 control subjects was 0.99 ± 0.05 (1 SD) (Fig. 1). The mean β/α ratios in ten patients of Italian, English, or Arab extraction with high A₂ heterozygous beta thalassemia studied in our laboratory was 0.57 ± 0.08 (1 SD). Two Italian children and one Greek child with homozygous beta thalassemia studied simultaneously with the H family and β/α radioactivity ratios in their peripheral blood of 0.22, 0.23, and 0.23 (Fig. 1). Reported ratios for larger groups of homozygotes range from zero to 0.24.12-14.53

The β/α ratios of the members of the H family are shown in Fig. 1 and 2. Incubation studies were not performed on the father because of his distance from the laboratory. The propositus (II-2) and her sister (II-1), who are homozygous for beta thalassemia, had peripheral blood β/α radioactivity

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Fig. 2. Pedigree. Numbers below symbols refer to β/α ratios in peripheral blood.
Fig. 3. Chromatograms of separations of globin chains on carboxymethyl cellulose in 8 M urea at pH 6.7 after incubation of peripheral blood and bone marrow of propositus (II-2) with $^{14}$C-leucine.

ratios of 0.21 and 0.24, respectively. These ratios are within the range of values observed in white patients with homozygous beta thalassemia (Fig. 1). The mother of the propositus and the remaining sister (II-4) and brother (II-3), who are heterozygotes for beta thalassemia, had $\beta/\alpha$ specific activity ratios above the range found in heterozygous beta thalassemia in white subjects (Fig. 1). Moreover, the ratios of the mother (I-1) and one sister (II-1), 0.94 and 1.00, respectively, are within the range of normal values, while the ratios obtained on the brother (II-3), 0.86, is within 3 SD of the mean of the control group.

The results of simultaneous incubations of bone marrow and peripheral blood of the propositus (II-2) and her sister (II-1) are shown in Table 2 and Fig. 3. For comparison, bone marrow and peripheral blood of two patients of Greek (C.G.) and Italian (L.C.) extraction with Cooley's anemia were incubated in a similar fashion (Table 2). In control subjects, the $\beta/\alpha$ ratios in the bone marrow are the same as those in the peripheral blood.$^{15,16}$ In each patient

<table>
<thead>
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<th>Patient</th>
<th>$\beta/\alpha$</th>
<th>$\gamma/\alpha$</th>
<th>$(\beta + \gamma)/\alpha$</th>
<th>$\beta/\alpha$</th>
<th>$\gamma/\alpha$</th>
<th>$(\beta + \gamma)/\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>II-1</td>
<td>0.24</td>
<td>0.30</td>
<td>0.54</td>
<td>0.43</td>
<td>0.30</td>
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</tr>
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<td>0.41</td>
<td>0.43</td>
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<td>0.68</td>
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<tr>
<td>C.G.</td>
<td>0.22</td>
<td>0.27</td>
<td>0.49</td>
<td>0.34</td>
<td>0.31</td>
<td>0.65</td>
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<tr>
<td>L.C.</td>
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<td>0.33</td>
<td>0.56</td>
<td>0.42</td>
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</table>
with homozygous beta thalassemia, the $\beta/\alpha$ radioactivity ratio in the peripheral blood was less than that in the bone marrow. The difference ranged from 49 to 65%, with the average peripheral blood $\beta/\alpha$ ratio being 56% of the bone marrow ratio. The $(\beta + \gamma)/\alpha$ ratios in the peripheral blood and bone marrow demonstrated unbalanced globin synthesis in each patient, with ratios well below one. The comparisons between $\gamma/\alpha$ ratios in bone marrow and peripheral blood were similar in II-1, II-2, and C.G., where there was little change in relative synthesis. None of these three patients needed regular transfusions. In L.C., a 16-yr-old boy who had been transfused frequently since infancy, the $\gamma/\alpha$ ratio in the peripheral blood was 1.7 times that in the bone marrow.

In the simultaneous incubations of bone marrow and peripheral blood, the total radioactivity in the marrow samples ranged from 4.8 to 34.8 times that of the peripheral blood.

**DISCUSSION**

Homozygous beta thalassemia in the black patient usually has a much milder clinical course than the thalassemia major in patients of Mediterranean ancestry. Most patients do not need regular transfusions, and they may live into the fourth decade and beyond without the need for frequent medical care. Some of the black patients have had children, an uncommon occurrence in white patients requiring frequent transfusions. The hemoglobin concentration is usually decreased below 10 g/100 ml, the liver and spleen are enlarged, and there are findings of expansion of the marrow spaces. $\mathrm{Hb~F}$ is elevated, usually 40–80%, as in most Mediterranean patients. $\mathrm{Hb~A_2}$ may be normal or elevated. Black heterozygotes have laboratory findings that are similar to those of white heterozygotes, including abnormalities of red cell morphology and elevated levels of $\mathrm{Hb~A_2}$. In heterozygotes with elevated $\mathrm{Hb~A_2}$, $\mathrm{Hb~F}$ is frequently slightly elevated. In the present report, the two sisters with $\text{thaI}$-assemia major have had little clinical difficulty from their disease. The short stature of the propositus may not be related to her chronic anemia, since her similarly affected sister has grown normally. Both girls lead an active life and are able to attend school regularly. Their siblings and parents have thalassemia minor that is indistinguishable by clinical laboratory studies from that found in other racial groups.

The globin synthesis ratios in the peripheral blood of heterozygotes in this family differ strikingly from those found in white patients. The values are in the normal range or slightly below it, in contrast to $\beta/\alpha$ ratios of 0.41–0.69 in white subjects studied in our laboratory and by others. Similar findings in some members of three other black families have been reported in recent abstracts. The lesser degree of imbalance in these black individuals with beta thalassemia trait, including those reported here, suggests that expression of the beta thalassemia gene in this group differs from that previously studied.

The finding of an unexpectedly elevated $\beta/\alpha$ ratio in association with high $\mathrm{Hb~A_2}$ has been previously reported in an Italian patient heterozygous for alpha and beta thalassemia. In the double heterozygote, the $\beta/\alpha$ ratio was 0.86. A family member heterozygous for beta thalassemia alone had a ratio of 0.59, while in a related alpha thalassemia heterozygote the ratio was 1.36. In
the four black families studied, there has not been segregation of an alpha thalassemia gene. In a clinically mild Italian patient homozygous for beta thalassemia and heterozygous for alpha thalassemia, the $\beta/\alpha$ ratio was 0.45, a higher value than those found in other Italian homozygotes. The $\beta/\alpha$ ratios in heterozygotes in the same family were 0.55, 0.58, and 0.62, in the range of Mediterranean beta thalassemia trait. In the family reported here and in the one previously studied in our laboratory, the $\beta/\alpha$ ratios of the four black homozygotes were low and did not indicate the presence of an alpha thalassemia gene.

The unbalanced globin synthesis found in the peripheral blood of heterozygotes has been interpreted as a direct expression of the thalassemic defect, but recent evidence indicates that it may be a secondary rather than primary expression of the disorder. Nucleated red cells of white subjects heterozygous for beta thalassemia produce equal amounts of alpha and beta chains. As the cells matures, beta chain synthesis decreases more rapidly than alpha chain synthesis, resulting in a relative deficiency of beta chain production during the final stages of globin synthesis in the reticulocyte. In some patients, the balanced synthesis in the marrow may be the result of increased output of the nonthalassemic beta chain allele or augmented protein synthetic activity on nonthalassemic polyribosomes, in compensation for the thalassemia trait.

The hypochromia observed in many patients with beta thalassemia trait may be the result solely of the relative decrease of beta chain synthesis in the reticulocyte, with resultant precipitation and proteolysis of the small excess of alpha chain. In some black patients with beta thalassemia trait, such as II-4 in the present study, there is balanced globin synthesis in the reticulocyte. The hypochromia in these patients cannot be explained by the mechanism proposed for white heterozygotes. Either a relative deficiency of beta chain production is present in the nucleated cell but not in the reticulocyte, or there is decreased total production of both alpha and beta chain during red cell maturation. In one black heterozygote, the $\beta/\alpha$ ratios in both bone marrow and peripheral blood were close to one, indicating that the suggestion of a decreased $\beta/\alpha$ ratio in the marrow as an explanation for hypochromia is not correct. The control of hemoglobin synthesis in cells with a thalassemia gene, whether in black or white patients, may be such as to reduce total globin production in the nucleated cell.

In the homozygote, there is no normal beta gene to compensate for decreased nonalpha chain production, but the gamma loci act in this capacity to a variable extent. The response differs among the cells in individual patients, as indicated by the heterogeneous distribution of Hb F. Cells that synthesize relatively large amounts of gamma chain appear to survive the longest, presumably because of the combination of gamma chains with excess alpha chains to form Hb F, thus preventing precipitation of alpha chains and membrane damage. The intramedullary and splenic destruction of the cells with the greatest total imbalance of globin synthesis does not allow a simple comparison of relative synthesis of globin chains in bone marrow and peripheral blood in homozygous beta thalassemia. It is not known if there is heterogeneity of beta or even alpha chain synthesis in the cells of patients with thalassemia.
major, similar to the heterogeneity of gamma chain synthesis. The relatively rapid decay of delta chain synthesis in normal persons\textsuperscript{32,33} and of beta chain synthesis compared to alpha chain in thalassemia trait\textsuperscript{15,27} may also be present in thalassemia major. The type of experiments described here do not provide adequate evidence for differential decay of gamma and beta synthesis in relation to alpha synthesis because of lack of a representative sample in the peripheral blood of the cells that mature in the marrow. However, some speculation on the basis of the data is possible. Since the cells that contain the least gamma chain synthesis are preferentially destroyed, the $\gamma/\alpha$ ratio should be higher in the peripheral blood than in the marrow. The absence of this difference in three patients in this study suggests that gamma chain synthesis decays more rapidly than that of alpha chain in these patients. In the fourth patient (L.D.), who had the most severe clinical disease of the group, the magnitude of intramedullary destruction may have been so large as to result in an increased $\gamma/\alpha$ ratio in the peripheral blood, despite a rapid rate of decay of gamma chain synthesis.

The decreased $\beta/\alpha$ ratio in the peripheral blood compared to bone marrow of all patients in this study, and in four of five patients in a previous study,\textsuperscript{18} may indicate a relatively rapid decay of the rate of beta chain synthesis, but this conclusion cannot be made in the absence of data on the possibility of heterogeneity of beta and alpha synthesis in cells of beta thalassemia. The studies of globin synthesis reported here do not reveal a major difference between black and white patients with thalassemia major, despite the differences in clinical severity of the two groups. The molecular basis of these puzzling observations must await further studies.

REFERENCES

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