Two New Sickle Cell Syndromes: Hbs, Hb Camden, and α-Thalassemia; and Hbs in Combination With Hb Tacoma

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Hemoglobin variants having electrophoretic mobility more rapid than that of HbA were identified in combination with sickle hemoglobin in two patients at the Cook County Hospital. Neither individual had symptomatic hematologic disease. In one patient, the rapidly migrating hemoglobin had the amino acid substitution characteristic of Hb Tacoma (β-40 arg → ser), a mildly unstable variant. In the other patient, Hb Camden (β-131 gin → glu) was identified, and the hematologic findings also indicated that he has α-thalassemia trait. In the patient with HbS-Camden-α-thalassemia, globin synthesis findings also indicated that he has α-thalassemia trait. In the patient with HbS-Camden-α-thalassemia, globin synthesis was unbalanced (α/β 0.66), and Hbs represented only 19.5% of the total hemoglobin. The latter finding suggests that under conditions of limited α-chain availability β Camden may combine with α subunits at least as efficiently as does βA. Hbs represented 56% of the hemoglobin of the patient with HbS Tacoma, although the rate of synthesis of β Tacoma by her reticulocytes was consistently greater than that of βA. A time-course synthesis study demonstrated a progressive increase in the specific activity of β Tacoma in relation to that of βA, suggesting that the unstable β-chains of Hb Tacoma underwent selective intracellular degradation. This process appears to explain the disparity between the rates of synthesis of the two β chains and the relative representation of Hbs and Hb Tacoma in the patient’s erythrocytes.

The hematologic expression and pathologic potential of sickle hemoglobin may be modified, in some cases substantially, by the presence of other abnormal hemoglobins or thalassemia. Studies of individuals with these coexisting genetic abnormalities have provided increased understanding of mechanisms that regulate hemoglobin gene expression, and have also helped to elucidate molecular properties of HbS that promote or inhibit gelation and sickle cell formation.

In this report we present the hematologic and laboratory findings from two patients of the Cook County Hospital with newly identified sickle cell syndromes.

Materials and Methods

Hematologic measurements were made with a model S Coulter counter that was standardized daily using a commercial standard. Tests for hemoglobin stability employed 17% solutions of isopropanol as described by Carrell and Kay. Hemoglobin separations for structural analysis were by DEAE-cellulose column chromatography using glycine-containing buffers. Other measurements as well as the structural studies of the abnormal globin chains were by methods as described in a recent report. Gelation studies of Hbs-containing hemoglobin mixtures were performed by the method of Singer and Singer as modified by Bookchin et al.

For studies of globin synthesis, washed red cells from peripheral blood were incubated with 1-leucine-14C in medium prepared as previously described. After completion of the incubations, globin was prepared from the stroma-free lysates, and the globin chains from these preparations were fractionated by CMC column chromatography with buffers made up in 8M urea. Determinations of incorporated radioactivity and specific activity calculations were as described in a previous report.

Patients

C.F., a 58-yr-old black male, was admitted to the Cook County Hospital because of urinary frequency and nocturia. He had previously been in good health. His examination demonstrated prostatic enlargement, but otherwise was unremarkable. A biopsy of the prostate showed adenocarcinoma, and a transurethral prostatic resection was performed.

The patient’s hemoglobin level was at the lower end of the normal range, with microcytic, hypochromic red cell indices (Table 1). His erythrocyte morphology was mildly abnormal, with anisopackilocytosis and occasional target forms. A bone marrow biopsy specimen demonstrated ample amounts of storable iron. There was no history of anemia or other hematologic disease in relatives of the patient, but family members were not available for study. A.C., a 16-yr-old black primigravida, was found to have a positive sickling test in the course of her prenatal obstetrical evaluation. She had been healthy, and her physical examination demonstrated no abnormality. The pregnancy was uneventful and she delivered a full-term male infant with a birth weight of 3.12 kg.

Her hematologic findings (Table 1) included normal erythrocyte indices and morphology. The patient’s parents and four siblings have been in good health without history of anemia. Efforts to study family members were unsuccessful.

Results

Hemoglobin Measurements and Structural Analyses

The hemoglobin composition of patient C.F. included 19.5% Hbs and normal levels of hemoglobins A2 and F, with the rapidly migrating hemoglobin making up the remainder (Table 1).

The electrophoretically rapid hemoglobin from the patient was isolated in pure form and its α and β chains were separated by CMC-column chromatography. The α-chain emerged earlier than normal. A peptide map prepared from a trypsin digest of the aminoethylated β-chain demon-
The value for valine fromTacoma T-3,4 was obtained from a 72-hr hydrolysate. Trp was identified by spot testing on paper.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Hb (g/dl)</th>
<th>PCV (%)</th>
<th>MCV (f)</th>
<th>MCH (pg)</th>
<th>Pc</th>
<th>HbA1 (%)</th>
<th>HbF (%)</th>
<th>HbS (%)</th>
<th>Hb Tacoma (%)</th>
<th>Hb Camden (%)</th>
<th>2,3-DPG (μmol/g Hb)</th>
<th>θ°2O2 (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.F. (S-Camden)</td>
<td>13.7</td>
<td>0.457</td>
<td>69</td>
<td>20.5</td>
<td>0.6</td>
<td>2.7</td>
<td>1.0</td>
<td>19.5</td>
<td>—</td>
<td>76.8</td>
<td>17.8</td>
<td>(27.4-29.2)</td>
</tr>
<tr>
<td>A.C. (S-Tacoma)</td>
<td>11.9</td>
<td>0.387</td>
<td>99</td>
<td>29.7</td>
<td>2.2</td>
<td>3.5</td>
<td>1.3</td>
<td>56.2</td>
<td>39.0</td>
<td>—</td>
<td>20.1</td>
<td>29.0</td>
</tr>
</tbody>
</table>

Proteins, as well as the isolated T-3 peptide, had normal amino acid compositions (Table 2), indicating that one of the two glutaminyl residues of the peptide was replaced by a glutamyl residue. To identify the site of the substitution, the purified peptide as well as a sample of normal β T-13 were further digested with chymotrypsin. High-voltage electrophoresis of the peptides and their chymotryptic cleavage products (Fig. 1) demonstrated that the difference in electrophoretic mobility of the T-13 peptides was relatable to the fragment representing β 131-132. The amino acid substitution, therefore, is β 131 gln → glu, and the rapidly migrating hemoglobin in the patient is Hb Camden.

In patient A.C., HbS represented more than half of the total hemoglobin, with the rapidly migrating fraction making up 39% (Table 1). The latter was isolated in pure form by DEAE-cellulose chromatography, and an abnormal β-chain was prepared as described above. The elution position of the variant β-chain from the CMC column was similar to that of β3 Camden. Tryptic peptides of the aminoethylated β-chain were subjected to peptide mapping, which demonstrated an absence of T-3 (β 18-30) and T-4 (β 31-40) and the presence of a new peptide that gave positive staining reactions for arginine, tryosine, and tryptophan. The abnormal peptide was isolated by Sephadex G-50 gel filtration of a trypsin digest (Fig. 2) followed by chromatography on Dowex 50-X2. The amino acid composition of the abnormal peptide (Table 2) was consistent with an arg → ser substitution at β 30, and the variant hemoglobin is therefore Hb Tacoma.

**Hemoglobin Function and Stability Studies**

Oxygen affinity measurements of fresh whole blood samples from both of the patients (Table 1) showed each to be approximately normal (AA: N = 27.2 ± 0.6 mm Hg; AS: N = 26.7 ± 1.8) with concentrations of 2,3-diphosphoglycerate that were normal or slightly elevated (N = 15.0 ± 2.4 μmol/b Hb).

The coexistence of the second structural hemoglobin variant with HbS in these patients provided an opportunity to examine the effects of the abnormal hemoglobins on HbS gelation. The minimum gelling concentration (MGC) of a stroma-free lysate of washed erythrocytes from the patient with hemoglobins S and Camden was 39.5 ± 1.5 g/dl; the MGC of a corresponding percentage of HbS in a mixture with HbA was 43.5 ± 1.2 g/dl. A lysate from the HbS-Tacoma patient had a MGC of 37.9 ± 0.6 g/dl.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>β T-13</th>
<th>β3 Camden T-13</th>
<th>β T-3 + 4</th>
<th>β T-3.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>1.99</td>
</tr>
<tr>
<td>Threonine</td>
<td>1</td>
<td>1.00</td>
<td>1</td>
<td>0.84</td>
</tr>
<tr>
<td>Serine</td>
<td>—</td>
<td>—</td>
<td>0</td>
<td>0.99</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>3</td>
<td>2.68</td>
<td>3</td>
<td>2.99</td>
</tr>
<tr>
<td>Proline</td>
<td>2</td>
<td>2.21</td>
<td>1</td>
<td>0.95</td>
</tr>
<tr>
<td>Glycine</td>
<td>—</td>
<td>—</td>
<td>3</td>
<td>3.06</td>
</tr>
<tr>
<td>Alanine</td>
<td>2</td>
<td>1.72</td>
<td>1</td>
<td>1.02</td>
</tr>
<tr>
<td>Valine</td>
<td>1</td>
<td>0.96</td>
<td>5</td>
<td>4.76*</td>
</tr>
<tr>
<td>Leucine</td>
<td>—</td>
<td>—</td>
<td>3</td>
<td>2.62</td>
</tr>
<tr>
<td>Tryptosine</td>
<td>1</td>
<td>1.21</td>
<td>1</td>
<td>1.21</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Lysine</td>
<td>1</td>
<td>1.07</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Arginine</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>0.92</td>
</tr>
<tr>
<td>Tryptophan†</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>+</td>
</tr>
</tbody>
</table>

*The value for valine from β Tacoma T-3.4 was obtained from a 72-hr hydrolysate.
†Tryptophan was identified by spot testing on paper.
whereas the result from its corresponding control was 29.2 ± 0.8 g/dl.

Incubation of a stroma-free hemolysate from the patient with HbS-Tacoma with isopropanol resulted in the appearance of turbidity after 4 min, and by 16 min approximately 6% of the hemoglobin had undergone precipitation (Fig. 3). To examine the possibility that HbS might have coprecipitated with the Hb Tacoma, a larger volume of hemolysate was incubated in the isopropanol solution, and after collecting and washing the precipitate, the globin chains were separated by CMC chromatography. The recovered globin chains included α and βTacoma, but βS was absent.

Globin Synthesis Studies

The pattern of incorporation of l-leucine-14C into globin chains by reticulocytes from patient C.F. was unbalanced (Fig. 4) with a calculated α/β chain synthesis ratio of 0.66. Synthesis of the βCamden chain accounted for 77% of the total β-chain incorporation, with βS synthesis representing only 23%; the latter, however, corresponded approximately to the percentage of HbS in the patient’s erythrocytes.

The pattern of globin chain synthesis by reticulocytes from patient A.C. is shown in Fig. 5. Radioactivit
panel), consistent with loss of unstable $\beta^{\text{Tacoma}}$ chains as an ongoing process during the course of the incubations. A parallel experiment was carried out in which globin synthesis was allowed to proceed for 2 hr to label the globin chains, after which a large excess of nonradioactive L-leucine was added as a "chase," and the incubation was then continued for an additional 10-hr period to look for evidence of selective globin chain degradation. At the end of the 10-hr incubation period the specific activity of the $\beta^{\text{Tacoma}}$ chains remained unchanged (Fig. 6), suggesting that the apparent loss or degradation of the $\beta^{\text{Tacoma}}$ chains occurred in a nonselective manner, affecting both newly synthesized and preexisting $\beta^{\text{Tacoma}}$ chains in approximately equal proportions.

**DISCUSSION**

Hb Camden has previously been identified in families of African extraction in England and subsequently in the United States. This hemoglobin variant has been shown to have normal stability and functional properties, and none of the affected individuals has shown evidence of hematologic disease.

In one of the reported families with Hb Camden, a child doubly heterozygous with Hbs was also identified. The proportion of Hbs in relation to Hb Camden in this child (34/66) contrasts with the corresponding...
ratio of 20/80 in the patient described in this report. A lower than usual percentage of HbS is a consistent finding in individuals with sickle cell trait in combination with \( \alpha \)-thalassemia trait,\(^{13-16} \) and a similar relationship appears to be represented by the findings in this patient. The morphological erythrocyte changes of patient C.F. as well as his microcytic, hypochromic erythrocyte indices (Table 1) are also consistent with the presence of \( \alpha \)-thalassemia trait, and his \( \alpha/\beta \) globin chain synthesis ratio of 0.66 is within the range of 0.65 ± 0.10, which we previously obtained from a group of adults and children with sickle cell trait combined with \( \alpha \)-thalassemia trait.\(^{17} \)

Hemoglobin tetramer formation involving \( \alpha \) and \( \beta^A \) chains appears to proceed more efficiently than that involving \( \alpha \) and \( \beta^S \),\(^{18} \) suggesting that under conditions of limiting \( \alpha \)-chain availability, HbA formation will take precedence over that of HbS. This difference may account for the lower than usual percentage of HbS in sickle cell trait individuals having concomitant \( \alpha \)-thalassemia. Consistent with a mechanism of this kind, Shaeffer et al.\(^{14} \) have shown that a fraction of newly synthesized \( \beta^S \) chains is rapidly degraded in erythroid cells of sickle cell trait subjects with \( \alpha \)-thalassemia. The distribution of hemoglobins in patient C.F., with his fraction of HbS making up even less than the 25.8% ± 1.4% that we have previously seen in sickle cell trait–\( \alpha \)-thalassemia trait individuals,\(^{17} \) suggests that \( \beta^C_{\text{Camden}} \) chains may compete at least as effectively for \( \alpha \) chains as does \( \beta^A \). Also consistent with this hypothesis was the finding that the percentage of Hb Camden in a heterozygous individual with concomitant \( \alpha \)-thalassemia trait was unchanged from that seen in nontalassemic heterozygotes.\(^{12} \)

The gelation of HbS did not appear to differ significantly when comparable percentages of HbA or Hb Camden were present, a finding similar to that reported by Blackwell et al.\(^{10} \)

Hb Tacoma was first identified in an American family of European extraction\(^{19} \) and subsequently was found in individuals from Russia\(^{20} \) and from Finland\(^{21} \) and from a family of unstated ancestry in Vancouver.\(^{22} \) To our knowledge, patient A.C. is the first individual of African ancestry in whom Hb Tacoma was identified and may therefore represent an independent mutation.

The arg → ser substitution in \( \beta^{\text{Tacoma}} \) appears to have a destabilizing effect on the \( \alpha_i\beta_i \) interchain contact, due to loss of the van der Waals interaction between the \( \beta \) 30 arginyl residue and the \( \alpha \) 122 histidyl, as well as the hydrogen bonding with the \( \alpha \) 117 phenylalanyl residue.\(^{8} \) Although Hb Tacoma is unstable, as demonstrated by precipitation from heating or denaturation with exposure to urea\(^{19} \) or by isopropanol test,\(^{21} \) none of the reported individuals with this hemoglobin variant has shown evidence of hemolytic disease. Studies of erythrocyte survival in carriers of this hemoglobin have not been reported, however. The hematologic findings of patient A.C., in whom hemoglobin S and Tacoma were found, had hemoglobin and packed cell volume measurements at the lower limits of the normal range and a normal or minimally elevated reticulocyte count (Table 1).

The globin synthesis studies with reticulocytes from patient A.C. (Figs. 5 and 6) demonstrated a rate of synthesis of the \( \beta \)-chain of Hb Tacoma that exceeded that of \( \beta^S \), but because of an ongoing process of degradation of \( \beta^{\text{Tacoma}} \) globin chains, the percentage of HbS in the patient’s red cells was greater than that of Hb Tacoma. The finding by Deacon-Smith and Lee-Potter of stainable inclusions in erythrocytes of a patient with Hb Tacoma\(^{21} \) is also consistent with a process of ongoing intracellular degradation and precipitation of Hb Tacoma.

The 56% level of HbS in patient A.C. may be sufficient to produce symptomatic sickle cell manifestations under appropriate circumstances, but the patient has not had any apparent sickle-related problem. The finding that a stroma-free lysate of her erythrocytes had a substantially higher MGC than did a corresponding control in which HbA was substituted for Hb Tacoma may indicate that Hb Tacoma exerts an inhibitory effect on the gelation of HbS and may therefore suppress sickle cell transformation and its associated clinical consequences. Further studies will be required to substantiate this relationship.

ACKNOWLEDGMENT

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