A New Alkali-resistant Hemoglobin $\alpha^J_{2Oxford} \gamma^F_2$ in a Sicilian Baby Girl With Homozygous $\beta^0$ Thalassemia

By G. Schiliro, S. Musumeci, G. Pizzarelli, A. Russo, M. Marinucci, E. Bruni, and G. Russo

A 10-mo-old baby girl with homozygous $\beta^0$ thalassemia and $\alpha^J_{Oxford}$ presenting the clinical picture of homozygous $\beta$ thalassemia is described. Hemoglobin electrophoresis showed three bands: the first two with the mobilities of hemoglobin Hb $A_2$ (1%) and Hb F (69%), respectively, the third migrating a little faster than Hb A (30%). About 30% of her $\alpha$ chains were $J_{Oxford}$ which, bound to her $\gamma$ chains, produced a new alkali-resistant hemoglobin, $\alpha^J_{2Oxford} \gamma^F_2$, which has not been described previously. Hemoglobin synthesis in vitro showed the absence of $\beta$ chain synthesis and an $\alpha/\alpha' /non-\alpha$ ratio of 2. The patient's father was heterozygous for both the Hb J Oxford and $\beta^0$ thalassemia genes, the mother a carrier of $\beta^0$ thalassemia; four other relatives were carriers of Hb J Oxford, and one was a carrier of $\beta$ thalassemia.

In 1964 LIDDELL et al. described hemoglobin J in an English family for the first time. This hemoglobin has an electrophoretic mobility at alkaline pH between Hb A and Hb H, and is characterized by an amino acid substitution at position 15 of the $\alpha$ chain with glycine replaced by aspartic acid. Up to now, ten other families with Hb J have been found: nine were in southern Italy and Sicily. The remaining family was discovered by Barclay et al. in the Manyika tribe from southern Tanzania. Lehmann has considered these to be independent mutations, as they have been discovered in different racial groups. All were discovered on routine electrophoresis and were in heterozygous subjects who presented a normal clinical and hematologic picture. One exception, however, was a subject heterozygous for both Hb J and Hb Lepore. Our paper deals with a Sicilian family with two genetic anomalies, $\beta^0$ thalassemia and Hb J Oxford.

MATERIALS AND METHODS

Routine hematologic studies were performed using standard methods. Estimation of Hb F was made by the method of Betke et al. Hemoglobins were analyzed by electrophoresis on cellulose acetate in glycine buffer at pH 9.0. Quantitative analysis was carried out by elution of the various hemoglobins from cellulose acetate strips. Hemoglobin fractions were separated on DEAE-Sephadex A-50 using a pH gradient buffered from 8.4 (Tris-HCl 0.05 M) to 6.8. Analytic separation of globin chains was performed on cellulose acetate by the method of Komarny and Barnes. The abnormal polypeptide chain was separated by chromatography of the globin on CM-cellulose according to Clegg et al., and was digested with trypsin for fingerprinting. The abnormal peptide was eluted from four fingerprints by 5.7 N HCl and then concentrated by...
vacuum for 16 hr at 110°C. Amino acid analysis was carried out by the method of Spackmann et al.17

Globin chain synthesis was studied by directly incubating peripheral blood for 2 hr at 37°C with 10 μCi of 14C leucine (specific activity 343 mCi/mM). The red cells were then washed and hemolyzed. The total radioactivity and specific activity of each chain were determined after separation of α, β, and γ chains by column chromatography according to the method originally described by Clegg et al.15

Red cell survival was calculated by labeling red cells with 51Cr according to the method of Mollison and Veall.18

RESULTS

A 10-mo-old baby girl from Catania was admitted into our clinic because of pallor and failure to gain weight over several months. Physical examination and routine laboratory tests revealed clinical and hematologic characteristics of Cooley's disease. The family pedigree is shown in Fig. 1. The hematologic data of the child and her family are summarized in Table 1, while the results of hemoglobin electrophoresis on cellulose acetate can be seen in Fig. 2. The Hb pattern of the patient comprised three bands: the first corresponded to Hb A2; the second migrated as Hb F; the third was a little faster than Hb A. It must be noted that almost all of the baby's hemoglobin was alkali resistant (92%).

Chromatography of the hemoglobin on DEAE-Sephadex A-50 allowed the separation and measurement of the three fractions: Hb A2 (1%), Hb F (69%), and a fraction which emerged at pH 7.2 (30%). The latter was seen to move a
### Table 1. Hematologic Data on Family Members

<table>
<thead>
<tr>
<th>Subjects*</th>
<th>Age (yr)</th>
<th>Hemoglobin (g/100 ml)</th>
<th>RBC (x 10³ µm)</th>
<th>MCHC (g/100 ml)</th>
<th>MCH γγ</th>
<th>MCV (cu µm)</th>
<th>Erythrocyte Morphological Changes</th>
<th>Osmotic Fragility</th>
<th>α/α-α ratio</th>
<th>hCr 1/2 (days)</th>
<th>Hemoglobin (%)</th>
<th>Bette et al.</th>
<th>J. Oxford</th>
<th>a2T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>I 1</td>
<td>70</td>
<td>14.5</td>
<td>5.5</td>
<td>30</td>
<td>23.5</td>
<td>80</td>
<td>- - - decreased</td>
<td>Normal</td>
<td>4.6</td>
<td>0.96</td>
<td>2.3</td>
<td>1.5</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>I 2</td>
<td>66</td>
<td>15</td>
<td>5.4</td>
<td>22</td>
<td>27</td>
<td>90</td>
<td>+ - - Normal</td>
<td></td>
<td>2.5</td>
<td>1.6</td>
<td>5.6</td>
<td>2.4</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>II 1</td>
<td>30</td>
<td>15</td>
<td>5.2</td>
<td>32</td>
<td>26.5</td>
<td>89</td>
<td>+ - - Normal</td>
<td></td>
<td>6</td>
<td>0.90</td>
<td>2</td>
<td>1.9</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>II 2</td>
<td>36</td>
<td>13</td>
<td>4.9</td>
<td>28</td>
<td>23</td>
<td>79</td>
<td>+ - - Decreased</td>
<td></td>
<td>0.98</td>
<td>Normal</td>
<td>2.8</td>
<td>1.80</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>II 3</td>
<td>30</td>
<td>11</td>
<td>4.9</td>
<td>29</td>
<td>22</td>
<td>79</td>
<td>+ - - Decreased</td>
<td></td>
<td>0.99</td>
<td>Normal</td>
<td>1.96</td>
<td>1.87</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>II 4</td>
<td>40</td>
<td>15</td>
<td>5</td>
<td>29</td>
<td>27</td>
<td>90</td>
<td>- - - Normal</td>
<td></td>
<td>1</td>
<td>92</td>
<td>1(e)</td>
<td>69(c)</td>
<td>30(c)</td>
<td></td>
</tr>
<tr>
<td>II 5</td>
<td>48</td>
<td>14.5</td>
<td>5.2</td>
<td>29</td>
<td>27</td>
<td>85</td>
<td>- - - Normal</td>
<td></td>
<td>1(e)</td>
<td>69(c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III 1</td>
<td>6</td>
<td>10.5</td>
<td>4.8</td>
<td>32</td>
<td>27</td>
<td>90</td>
<td>- - - Normal</td>
<td></td>
<td>1(e)</td>
<td>69(c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III 2</td>
<td>10</td>
<td>7.2</td>
<td>3.6</td>
<td>26</td>
<td>19</td>
<td>71</td>
<td>+ + + Decreased</td>
<td></td>
<td>1(e)</td>
<td>69(c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*See Fig. 1.

(+ Values obtained by elution from cellulose acetate strips: normal values 2.3 ÷ 0.6%.)

*(c) Values obtained by chromatography on DEAE-Sephadex A-50.
Table 2. Amino Acid Composition of α Tp III After Acid Hydrolysis

<table>
<thead>
<tr>
<th></th>
<th>α⁺ Tp III</th>
<th>α⁻ Tp III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Asp</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Gly</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Ala</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Trp*</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Ehrlich positive.
NEW ALKALI-RESISTANT HEMOGLOBIN

DISCUSSION

β thalassemia trait is present in 7.7% of Sicilians, and there are also numerous abnormal structural genes for α and β chain mutants. It is not uncommon to find heterozygotes for β thalassemia trait and an α or β globin variant. The β thalassemia gene interacts with the β variants, causing a considerable increase in the amount of the abnormal hemoglobin. In fact, the heterozygote for both Hb J Oxford and β thalassemia (II-2) is clinically normal and presents with 25% Hb J, the percentage usually found in carriers of α-chain abnormalities. The only hematologic abnormalities are those of β thalassemia trait. The patient who is homozygous for β thalassemia shows severe signs of Cooley’s anemia. About 30% of her α chains are α1Oxford, bound to γ chains because of the unavailability of β chains. This union produces a new alkali-resistant hemoglobin formed by α1Oxfordγ3, with an electrophoretic mobility a little faster than Hb A. This abnormal hemoglobin should be present in heterozygous carriers of Hb J at birth and should represent 25% of their Hb F. It has not been reported previously, probably because it is present in small amounts, and its electrophoretic migration is similar to that of Hb A.

Since Hb 1Oxford represents 30% of the total hemoglobin in the propositus, while α1Oxford is 25% of the total α globin synthesized (Fig. 4), α1Oxford and αA appear to have equal affinity for γ chains. It will be of interest to determine whether or not the clinical severity of this patient’s anemia continues to be similar to that of other patients with Cooley’s anemia as she grows.

Four other members of the family were carriers of Hb J Oxford and had about 25% Hb J in their total hemoglobin. This percentage agrees with the hypothesis put forward by Lehmann and Carrell, according to which there are four structural genes for the α chains. Hemoglobin synthesis in these four subjects was balanced, which showed that the structural abnormality did not influence the activity of the affected α gene. None of them showed either clini-
cal or peripheral blood abnormalities. In fact, the Hb J was stable and had a normal oxygen affinity.\textsuperscript{5}

**ACKNOWLEDGMENT**

We wish to thank Dr. A. Massa and G. Pecchi for their technical assistance.

**REFERENCES**


A new alkali-resistant hemoglobin alpha2J Oxford gammaF2 in a Sicilian baby girl with homozygous beta0 thalassemia

G Schiliro, S Musumeci, G Pizzarelli, A Russo, M Marinucci, E Bruni and G Russo

Updated information and services can be found at:
http://www.bloodjournal.org/content/48/5/639.full.html

Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml