Absence of β mRNA in β<sup>0</sup>-Thalassemia in Kurdish Jews

By Francesco Ramirez, Deborah Starkman, Arthur Bank, Helene Kerem, Gabriel Cividalli, and Eliezer A. Rachmilewitz

We report the characterization of the amount of β mRNA in a Kurdish Jewish population with β<sup>0</sup>-thalassemia using the same methods employed for characterization of the Catania and Ferrara β<sup>0</sup> patients. We found very low amounts of β mRNA sequences, consistent with the presence of β<sup>0</sup>-thalassemia of the β mRNA-negative population type. In addition, no globin gene deletion was detected that could account for the absence of β mRNA.

THREE GENERAL TYPES of β-thalassemia have been described to date: β<sup>+</sup>-thalassemia, associated with the decreased production of structurally normal β globin; β<sup>δ</sup>-thalassemia, with total absence of β globin synthesis, and δβ-thalassemia, in which synthesis of both δ and β globin is absent. The use of complementary DNA (cDNA) specific for detecting α and β human mRNA sequences has permitted quantitation of the relative amounts of α and β mRNA in these disorders. Using this approach, it has been shown that the defect in β<sup>+</sup>-thalassemia is due to a decreased amount of globin mRNA, while no β mRNA is detectable in δβ-thalassemia. By contrast, at least three different types of molecular defects have been reported in β<sup>δ</sup>-thalassemia: (1) the presence of relatively high amounts of β mRNA sequences in patients of Southern Italian (Catania region) and Chinese extraction; (2) the presence of detectable amounts of abnormal incomplete β-like mRNA sequences or low amounts of complete β mRNA in patients from Northern Italy (Ferrara region); and (3) very low (less than 2%) β-like mRNA sequences in patients of different ethnic backgrounds.

We report the characterization of the amount of β mRNA in a Kurdish Jewish population with β<sup>0</sup>-thalassemia using the same methods employed for characterization of the Catania and Ferrara β<sup>0</sup> patients. We found very low amounts of β mRNA sequences, consistent with the presence of β<sup>δ</sup>-thalassemia of the β mRNA-negative population type. In addition, no globin gene deletion was detected that could account for the absence of β mRNA.
MATERIALS AND METHODS

Patients. Of 11 patients studied, 10 were Kurdish Jews (ages 3-24 yr), 7 with \( \beta^0 \)-thalassemia and 3 with \( \beta^+ \)-thalassemia. All 10 were splenectomized, and all required frequent transfusions. The clinical and hematologic parameters of this population have already been described extensively.\(^{10} \) The other patient, H.S., an Arab, had \( \beta^- \)-thalassemia, with the clinical syndrome of thalassemia intermedia.\(^{11} \) Globin chain synthesis was measured as described previously.\(^{10} \)

Preparation of thalassemia RNA. Washed whole blood cells from the thalassemia patients (collected approximately 2-4 wk after their last transfusions) were lysed with 10 vol 0.1 M NaCl, 0.01 M sodium acetate pH 5.2, 1 mM EDTA, and 1% SDS and extracted with cold phenol.\(^{12} \)

Separation of human globin \( \alpha \) and \( \beta \) mRNA and preparation of cDNA. Total poly A-containing RNA was prepared from reticulocytes of patients with autoimmune hemolytic anemias by phenol extraction and subsequent purification by oligoid T-cellulose chromatography.\(^{13} \) Then \( \alpha \) and \( \beta \) mRNA were separated on a linear gradient gel electrophoresis of 4%-6% acrylamide in 90% formamide as previously described.\(^{14} \) Full-size \( \alpha \) and \( \beta \) globin cDNA were synthesized using the purified \( \alpha \) and \( \beta \) mRNA as templates as previously described.\(^{15} \)

Hybridization of cDNA with cellular mRNA and DNA. Total RNA from \( \beta^0 \) and \( \beta^+ \)-thalassemia cells was hybridized to separated cDNA probes under conditions previously described.\(^{16} \) In all experiments the time of hybridization was usually 4 hr and never exceeded 12 hr. Cellular DNA was extracted from white blood cells as previously described.\(^{17} \) Hybridization with separated full-size \( \alpha \) and \( \beta \) cDNA was performed as previously described.\(^{18} \)

RESULTS

Whole-cell incubations were performed on peripheral blood samples and separation of globin chains on carboxymethylcellulose columns.\(^{11} \) No \( \beta \) chain synthesis was detectable in the eight \( \beta^0 \) patients studied. The \( \alpha/\beta \) synthetic ratios in the three \( \beta^+ \) patients were 0.25, 0.10, and 0.025 (Table 1). The last, in patient R.S., is an unusually low value, as previously reported.\(^{10} \)

In the three \( \beta^+ \)-thalassemia patients, the relative amounts of \( \beta \) as compared to \( \alpha \) mRNA (percent \( \beta \) mRNA content) were 7%, 12.5%, and 2.5% (Fig. 1A, Table 1). These values are roughly comparable to the relative amounts of \( \beta \) to \( \alpha \) globin synthesized, although discrepancies are present (Table 1).

In the eight \( \beta^0 \) patients, the \( \beta \) mRNA content was \( \leq 2\% \) of that of \( \alpha \) mRNA (Figs. 1B-1D, Table 1). The values varied between 0.5% and 2%. In some cases, insufficient material was available to completely hybridize the \( \beta \) cDNA probe (Figs. 1B-1D).

<table>
<thead>
<tr>
<th>Patients</th>
<th>Diagnosis</th>
<th>( \beta ) Chain Content(^* )</th>
<th>( \beta ) mRNA Content(^† )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z.O.</td>
<td>( \beta^+ )</td>
<td>10%</td>
<td>7%</td>
</tr>
<tr>
<td>N.Z.</td>
<td>( \beta^+ )</td>
<td>25%</td>
<td>12.5%</td>
</tr>
<tr>
<td>R.S.</td>
<td>( \beta^+ (?) )</td>
<td>2.5%</td>
<td>2.5%</td>
</tr>
<tr>
<td>H.K.</td>
<td>( \beta^0 )</td>
<td>-</td>
<td>( \leq 2% )</td>
</tr>
<tr>
<td>M.A.</td>
<td>( \beta^0 )</td>
<td>-</td>
<td>( \leq 0.5% )</td>
</tr>
<tr>
<td>M.B.</td>
<td>( \beta^0 )</td>
<td>-</td>
<td>( \leq 1% )</td>
</tr>
<tr>
<td>Mi.O.</td>
<td>( \beta^0 )</td>
<td>-</td>
<td>( \leq 1.6% )</td>
</tr>
<tr>
<td>Mo.O.</td>
<td>( \beta^0 )</td>
<td>-</td>
<td>( \leq 2% )</td>
</tr>
<tr>
<td>I.D.</td>
<td>( \beta^0 )</td>
<td>-</td>
<td>( \leq 1.7% )</td>
</tr>
<tr>
<td>C.S.</td>
<td>( \beta^0 )</td>
<td>-</td>
<td>( \leq 1% )</td>
</tr>
<tr>
<td>H.S.(^‡ )</td>
<td>( \beta^0 )</td>
<td>-</td>
<td>( \leq 1.8% )</td>
</tr>
</tbody>
</table>

\(^*\)Estimated by CMC column (percent of \( \alpha \) globin).

\(^†\)Estimated by molecular hybridization (percent of \( \alpha \) globin mRNA).

\(^‡\)Arab.
In order to determine if the molecular defect in these \( \beta^0 \) patients was due to deletion of the \( \beta \) globin genes, cellular DNA from one of the \( \beta^0 \) patients (M.B.) was hybridized to \( \alpha \) and \( \beta \) cDNA. The extent of hybridization was found to be similar to that of nonthalassemic patients (Table 2).

**DISCUSSION**

Globin cDNA is capable of detecting as little as ten molecules of globin mRNA per cell and provides a very sensitive probe for the presence of globin mRNA sequences.\(^6\) The specificity of \( \alpha \) and \( \beta \) cDNA probes for their respective mRNA has also been shown.\(^{3,14}\) These purified \( \alpha \) and \( \beta \) cDNA have been used

<table>
<thead>
<tr>
<th>Sources of DNA</th>
<th>( \alpha ) cDNA</th>
<th>( \beta ) cDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonthalassemic</td>
<td>46</td>
<td>47</td>
</tr>
<tr>
<td>( \beta^0 ) thalassemia (M.B.)</td>
<td>42</td>
<td>45</td>
</tr>
</tbody>
</table>

Each hybridization contained 210 \( \mu \)g cellular DNA and 70 pg (about 1000 cpm) \( \alpha \) or \( \beta \) cDNA probe in 70 \( \mu \)l reaction mixture. After hybridization for 72 or 144 hr the percentage of \( \alpha \) or \( \beta \) cDNA hybridized was measured by hydroxylapatite chromatography.\(^{17}\) The values at the two time points were comparable.
to show that in the erythroid cells of $\beta^0$ patients from the Catania region of Italy significant amounts of intact $\beta$ mRNA sequences are present by hybridization to $\beta$ cDNA, suggesting the presence of untranslated $\beta$ mRNA. Similar results have also been reported in Chinese and Northern Italian patients by others. The large relative amount of $\beta$-like mRNA sequences in these cases is not well explained by the presence of $\delta$ mRNA, since $\delta$ globin represents less than 5% of the total $\beta$-like globin present. Evidence for intact $\beta$ globin mRNA sequences in some of these patients was presented recently using RNA sequencing. The $\beta^0$-thalassemia patients in this study (Table 1) showed reduced $\beta$ as compared to $\alpha$ mRNA roughly in proportion to the $\beta/\alpha$ globin synthesis in intact cells, and levels were similar to those reported previously in Greek and Italian populations.

The $\beta^0$-thalassemia Kurdish Jews in this study had little, if any, intact $\beta$ globin mRNA sequences. The small amounts of $\beta$-like mRNA sequences detected in these patients is best explained by the presence of small amounts of $\delta$ globin mRNA and the extensive amino acid homology between $\delta$ and $\beta$ globin. We previously showed that no hybridization above 20% occurs at these same mRNA concentrations and $C_0t$ values using mRNA from a patient homozygous for $\delta\beta$ thalassemia who lacked HbA2 and $\delta$ globin synthesis. Since the $\beta^0$ patients studied had HbA2, it is most likely that the $\beta$ cDNA was hybridizing to $\delta$ mRNA sequences in these cells. In addition, we recently showed that $\beta$ cDNA is fully hybridized by RNA from a patient homozygous for Hb Lepore, indicating complete homology of 5'-ended $\delta$ sequences present in this RNA with $\beta$ cDNA.

In most cases, insufficient $\beta^0$-thalassemic RNA was available to reach saturation with the $\beta$ cDNA probe. Thus we cannot exclude the possibility that incomplete $\beta$ mRNA sequences are present in these patients similar to those described in $\beta^0$-thalassemia patients from the Ferrara region of Italy. However, several findings make this possibility less likely. First, much less $\beta$-like mRNA was present in the Kurdish $\beta^0$ patients than in those from Ferrara. Second, the hybridization of $\beta$ cDNA in all five of the Ferrara patients never exceeded 56%, and reached a hybridization plateau at that level. By contrast, in the present study no such hybridization plateau was reached (Fig. 1), and in two of the seven patients more than 65% of the $\beta$ cDNA was hybridized in RNA excess. Third, none of the hybridization curves (Fig. 1) appeared to be reaching a plateau; these data are consistent with the interpretation that only the amount of mRNA available limits the extent of hybridization.

In these patients, as in other $\beta^0$ patients reported to date, no detectable deletion of $\beta$-like globin genes has been found by liquid hybridization of purified $\alpha$ and $\beta$ cDNAs to cellular DNA. Thus to date three groups of $\beta^0$-thalassemia patients have been described: (1) $\beta^0$ patients without detectable $\beta$ globin mRNA, such as those reported here and elsewhere; (2) $\beta^0$ patients with detectable $\beta$ globin mRNA in significant amounts; and (3) $\beta^0$ patients described from Ferrara in whom either very small amounts of $\beta$ mRNA are present or no intact $\beta$ mRNA is present. Further studies on the structure of the $\beta$ globin genes and $\beta$ mRNA and its precursors in $\beta^0$-thalassemia of all of these types should be of interest in order to understand the genetic defect underlying these disorders.
REFERENCES


Absence of beta mRNA in beta0-thalassemia in Kurdish Jews

F Ramirez, D Starkman, A Bank, H Kerem, G Cividalli and EA Rachmilewitz

Updated information and services can be found at: http://www.bloodjournal.org/content/52/4/735.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