The Thalassemia Syndromes

By Arthur Bank

The thalassemia syndromes are one group of inherited anemias of man in which major advances have been made as a result of a wealth of biochemical, genetic, morphologic, and clinical investigations. In particular, a variety of defects in globin biosynthesis in these disorders have been characterized at the molecular level during the past decade. Clinical application of these studies has already resulted in the availability of prenatal diagnosis. In addition, new approaches to treatment of established disease with iron-chelating agents can soon be expected to have a major favorable impact on the clinical course of these patients.

This review will summarize (1) current knowledge of the molecular biology of the thalassemias; (2) the pathophysiology and clinical manifestations of these disorders; (3) current therapy of established disease; (4) the status of antenatal diagnosis; and (5) perspectives for future research in thalassemia.

The thalassemias are defined by decreased or absent production of a specific globin chain. In contrast to the hemoglobinopathies, there is a quantitative change in globin production and no structural change in the affected globin chain. In \( \beta \) thalassemia, \( \beta \) globin is either diminished or absent. Similarly, the \( \alpha \) thalassemias are associated with either decreased or absent \( \alpha \) globin. Alpha and beta thalassemia result in decreased adult hemoglobin or hemoglobin A (HbA, \( \alpha_2\beta_2 \)). Rarely, \( \delta \) or \( \gamma \) thalassemias have been reported.

Types of \( \beta \) Thalassemia

The heterozygous state of \( \beta^+ \) thalassemia, the most common form of thalassemia, is manifested as abnormal red cell morphology with microcytic, hypo-
Table 1. Heterozygous States of \( \beta \) Thalassemia and Variants

<table>
<thead>
<tr>
<th>Type</th>
<th>( \text{HbA}_2 )</th>
<th>( \text{HbF} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \beta^+ ) thalassemia</td>
<td>Increased</td>
<td>Normal to slightly increased</td>
</tr>
<tr>
<td>( \beta^0 ) thalassemia</td>
<td>Increased</td>
<td>Normal to slightly increased</td>
</tr>
<tr>
<td>( \delta \beta ) thalassemia</td>
<td>Normal</td>
<td>Increased (2%–10%)</td>
</tr>
<tr>
<td>HPFH</td>
<td>Normal</td>
<td>Increased (10%–40%)</td>
</tr>
</tbody>
</table>

chromic cells and target cells, an increase in the percent of \( \text{HbA}_2 \) (\( \alpha_2 \delta_2 \)), and a variable increase in \( \text{HbF} \) (\( \alpha_2 \gamma_2 \)) (Table 1).

When two \( \beta^+ \) thalassemia heterozygotes have offspring, the changes are one in four that a child will have homozygous \( \beta^+ \) thalassemia (Cooley anemia, thalassemia major). In the homozygous state of \( \beta^+ \) thalassemia, there is some detectable \( \beta \) globin and \( \text{HbA} \) present. By contrast, in homozygous \( \beta^0 \) thalassemia, a rarer form, there is no detectable \( \beta \) globin and no \( \text{HbA} \).

Heterozygotes for \( \beta^0 \) thalassemia have the same morphologic changes in red cells and elevation of \( \text{HbA}_2 \) seen in heterozygotes for \( \beta^+ \) thalassemia. The severity of the anemia in \( \beta^+ \) and \( \beta^0 \) thalassemia homozygotes is roughly comparable, suggesting that \( \beta \) globin synthesis in \( \beta^+ \) thalassemia is inadequate to decrease the clinical severity of this disorder. \( \text{HbF} \), although the major hemoglobin in both \( \beta^+ \) and \( \beta^0 \) thalassemia, is insufficient in amount to compensate for the decreased \( \text{HbA} \).

Two other disorders, \( \delta \beta \) thalassemia and hereditary persistence of fetal hemoglobin (HPFH), are related to thalassemia. In the few \( \delta \beta \) thalassemia homozygotes studied, there was no \( \text{HbA} \) or \( \text{HbA}_2 \); however, there was only a mild anemia, since \( \text{HbF} \) synthesis was markedly increased. In \( \delta \beta \) thalassemia heterozygotes, there is normal or decreased \( \text{HbA}_2 \) and a higher \( \text{HbF} \) level than in \( \beta^+ \) or \( \beta^0 \) thalassemia. In homozygotes for HPFH, there is no \( \text{HbA} \) or \( \text{HbA}_2 \), and yet there is no anemia. This disorder represents the extreme of essentially complete compensation by \( \text{HbF} \) production for the complete absence of \( \text{HbA} \) or \( \text{HbA}_2 \), although morphologic abnormalities and microcytosis may be present in the red cells. Black HPFH heterozygotes have elevations of \( \text{HbF} \) to between 15% and 40%. An intact \( \beta \) globin gene cis to the HPFH gene has been reported. In the Greek type of HPFH, there is less increase in \( \text{HbF} \), and no homozygotes have been reported.

The so-called Kleihauer-Betke technique is useful in distinguishing thalassemia and HPFH. In this procedure, acid elution is used to remove \( \text{HbA} \) and permits evaluation of \( \text{HbF} \) in individual cells on a stained peripheral blood smear. In \( \beta^0 \), \( \beta^+ \), and \( \delta \beta \) thalassemia relatively few cells have most of the \( \text{HbF} \), and there is variation in the amount of \( \text{HbF} \) in different cells, a "heterogeneous" distribution; by contrast, in HPFH all cells have relatively similar staining, indicating a "homogeneous" distribution of \( \text{HbF} \). These findings support the concept that in HPFH, \( \text{HbF} \) biosynthesis continues postnatally in all cells, while in \( \beta \) thalassemia, \( \text{HbF} \) synthesis is restricted to fewer erythroid precursors and varies markedly from cell to cell.

**TYPES OF \( \alpha \) THALASSEMIA**

Genetic and biochemical evidence suggests that there are four \( \alpha \) globin loci per diploid genome in most populations, although some individuals may have
**Table 2. Types of α Thalassemia**

<table>
<thead>
<tr>
<th>Type</th>
<th>Morphology</th>
<th>α/β Synthesis Ratio</th>
<th>Number of α Genes* (Genotype)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silent carrier state</td>
<td>Normal</td>
<td>0.8-0.9</td>
<td>3 (α2α2)</td>
</tr>
<tr>
<td>α Thalassemia trait</td>
<td>Abnormal</td>
<td>0.7-0.8</td>
<td>2 (α2α2 or α1α1)</td>
</tr>
<tr>
<td>HbH disease</td>
<td>Abnormal</td>
<td>0.3-0.6</td>
<td>1 (α1α2)</td>
</tr>
<tr>
<td>Hydrops fetalis</td>
<td>Abnormal</td>
<td>0</td>
<td>0 (α1α1)</td>
</tr>
</tbody>
</table>

*The presumed number of α genes is four per individual. Two α thalassemia genotypes were designated by Wasi (α1 and α2). The α2 gene designation is compatible with the loss of one of four α loci or with decreased but not absent function of one of two α loci. The α1 gene indicates either the loss of two of four α loci or the absent function of one of two α loci.

only two or three α globin genes. There are four types of α thalassemia believed to be related to the loss of function of one to four of these loci (Table 2). The mildest form, the silent carrier state, is associated with no hematologic abnormalities. A second form, α thalassemia trait, is associated with morphologic abnormalities in red cells, including hypochromia and microcytosis, but no anemia. The loss of function of three or four α loci leads to hemoglobin H disease. HbH is an unstable tetramer of β globin chains, which accumulates because of the lack of adequate numbers of α globin chains. HbH is identifiable by electrophoresis as a rapidly moving hemoglobin and represents 5%-30% of the total hemoglobin. HbA2 is usually reduced in amount in HbH disease. In the most severe form of thalassemia, hydrops fetalis, no α globin is present, and only Hb Barts (γ4) and HbH are found. Severe morphologic abnormalities of red cells are present, and the disease is incompatible with postnatal survival.

**MOLECULAR DEFECTS IN β THALASSEMIA SYNDROMES AND HPFH**

Globin synthesis is measured by incubating either peripheral blood (containing reticulocytes) or bone marrow samples with a radioactive amino acid (usually 3H-leucine) and quantitating the amount of radioactivity in α, β, and γ globin chains by column chromatography. More recently, a method permitting analysis of more samples at one time has been reported using 35S-methionine as the labeled amino acid and separation and quantitation of these chains by cellulose acetate electrophoresis and subsequent radioautography. In nonthalassemic patients, the α/β ratio is close to 1.0. In reticulocytes of β thalassemia heterozygotes of nonblack origin the ratio of α globin synthesis to that of β globin (α/β ratio) is close to 2.0, consistent with the decreased or absent function of one β globin allele. In some black patients, α/β ratios of close to 1.0 have been reported. In thalassemia homoygotes, α/β ratios vary between 5 and 25. It is still unclear whether this variation is due to different levels of β globin production by different β thalassemia genes or to double heterozygosity for β+ and β0 thalassemia. Similar α/β ratios have been found in siblings with homozygous β+ thalassemia.

In β0 and δβ thalassemia and HPFH homozygotes, only α and γ globin chains are synthesized. Measurements of globin chain biosynthesis in reticulocytes permit distinguishing individuals with homozygous sickle cell disease (two β+ genes) from those with sickle β0 thalassemia (one β+ gene). The α/β+ ratio in homozygous sickle cell disease is 1.0, while in sickle β0 thalassemia the ratios are closer to 2.0.
There is unbalanced \( \alpha/\beta \) globin synthesis in total bone marrow erythroblasts of thalassemia homozygotes as well as in the earliest erythroid precursors, indicating that \( \beta \) globin biosynthesis is decreased at all stages of erythroid maturation. In \( \beta^0 \) thalassemia homozygotes, no \( \beta \) globin synthesis is present in nucleated red cells in bone marrow. One anomalous finding is that in the bone marrow of patients heterozygous for \( \beta^+ \) or \( \beta^0 \) thalassemia there is apparently equal \( \alpha \) and \( \beta \) globin synthesis (\( \alpha/\beta \) ratio close to 1.0). At the same time, there is a reduced amount of \( \beta \) globin mRNA in these bone marrow cells (see below). The reason for the balanced \( \alpha \) and \( \beta \) globin synthesis in intact bone marrow cells is unclear; posttranslational events such as \( \alpha \) globin proteolysis or feedback inhibition of \( \alpha \) globin translation by excess \( \alpha \) globin could account for these findings. The possibility of contamination of the \( \beta \) globin region of the chromatogram with nonglobin proteins has also been reported.

In the \( \alpha \) thalassemias, there is a decreased \( \alpha/\beta \) ratio in reticulocytes (Table 2). The \( \alpha/\beta \) ratios in silent carrier state are 0.8–0.9; in \( \alpha \) thalassemia trait, 0.7–0.8; in HbH disease, 0.3–0.6; in hydrops fetalis, no \( \alpha \) globin is synthesized. There is decreased \( \alpha \) globin synthesis in bone marrow precursors in these conditions as well.

**Globin mRNA**

The \( \beta \) globin synthesized in \( \beta^+ \) thalassemia is structurally identical to normal \( \beta \) globin. In addition, the time required to translate a \( \beta \) globin chain in \( \beta^+ \) thalassemia is normal. These data suggest that \( \beta \) globin mRNA in \( \beta^+ \) thalassemia is qualitatively normal within the nucleotide sequence required to code for globin.

Two technical advances have permitted the characterization of globin mRNA in thalassemia, (1) the isolation and subsequent translation of globin mRNA in cell-free systems and (2) the synthesis of a radioactive probe, globin complementary DNA (cDNA), which has permitted measurement of actual globin mRNA content. Studies in crude cell-free systems showing a defect in the ability of ribosomes from \( \beta^+ \) thalassemia cells to support normal globin synthesis indicate that the underlying defect is associated with either decreased or abnormal \( \beta \) globin mRNA. The isolation and faithful translation of human globin mRNA in cell-free systems led to the direct demonstration that the defect in \( \beta \) globin synthesis in intact cells could be reproduced by isolated globin mRNA. In cell-free systems using rabbit reticulocyte fractions, Krebs ascites tumor lysates, and wheat germ preparations, isolated mRNA from homozygous \( \beta^+ \) thalassemia cells yield \( \alpha/\beta \) ratios comparable to those obtained in intact cells. Bone marrow mRNA also directs decreased \( \beta \) globin synthesis in these cell-free systems. There is reduced translatable \( \beta \) globin mRNA in heterozygotes as well. The mRNA from patients with homozygous \( \beta^0 \), \( \delta\beta \), and HPFH directs no \( \beta \) globin synthesis in cell-free translational systems. Stimulation of \( \beta \) globin synthesis by the addition of normal reticulocyte supernatant to ribosomes of \( \beta^0 \) thalassemia patients from Ferrara has been reported. Similar stimulation of \( \beta \) globin synthesis in vivo by transfusion has also been reported in these patients. However, no globin translation has been seen using isolated mRNA from these patients.

The preparation of globin cDNA has made quantitation of the actual globin
mRNA content feasible. Globin mRNA is an excellent substrate for the viral enzyme reverse transcriptase. This reaction results in the formation of cDNA with very high specific activities capable of detecting 10-100 pg of their complementary sequence in mRNA or globin genes.

Aside from its sensitivity, globin cDNA has an extremely high degree of nucleotide sequence specificity. Human globin cDNA is not hybridized to viral, bacterial, or ribosomal RNA; in addition, α cDNA does not hybridize with β mRNA (and vice versa), and β cDNA does not crosshybridize with γ mRNA (and vice versa).

A decrease in the content of β mRNA sequences as compared to α mRNA sequences in β⁺ thalassemia was first demonstrated by using α and β globin cDNA prepared from purified rabbit α and β globin mRNA. These cDNA crossreact with the analogous human mRNA. Methods for isolating specific human α and β globin cDNA have been developed and used to confirm the decreased amount of β globin mRNA in β⁺ thalassemia.

The RNA from homozygous δβ thalassemia and HPFH patients have no sequences complementary to β cDNA. In β⁰ thalassemia, the situation is more complicated, and results in different patients vary. In some Italian β⁰ thalassemia homozygotes, no β mRNA is demonstrable. In other β⁰ patients of Sicilian and Chinese extraction, the presence of structural β globin mRNA sequences can be demonstrated. This suggests the presence of an abnormal, untranslatable β globin sequence with considerable homology to normal β mRNA in these patients. The defect in mRNA in β⁰ patients from the Ferrara region of Italy is still unresolved. In one study, only partial hybridization of the β cDNA probe in a vast excess of β Ferrara mRNA was achieved, indicating either the β-like mRNA was grossly abnormal or that there was no β mRNA and the hybridization observed was due to homology between β cDNA and δ mRNA. In another study of these same patients intact β mRNA-like sequences were reported. Some of the conflicting results in these studies may be due to differences in the conditions of hybridization and possible instability of β mRNA in β⁰ thalassemia.

In the α thalassemias, deficiency of α globin mRNA has been demonstrated both by assay of isolated globin mRNA in cell-free systems and by hybridization to α and β cDNA. In hemoglobin H disease, the decrease in the α/β ratio is greater using isolated mRNA than in intact cells. These findings suggest translational or posttranslational regulation of α and β globin synthesis in intact cells.

Globin Gene Deletions

The specificity and sensitivity of globin cDNA probes has been most dramatically shown by the detection of specific deletions of α- or β-like globin genes in the DNA from patients with α and β thalassemia and HPFH. In these experiments, cellular DNA was sonicated to a size approximating that of the cDNA; the two strands of cellular DNA and cDNA were denatured together to single strands and then allowed to rehybridize. If there is a deletion of part or all of the globin genes in a sample of DNA, less cDNA than normal will hybridize. Deletion of α globin genes in α thalassemia–hydrops fetalis DNA was demonstrated using this method. No detectable gene deletions
were observed using this technology in \( \beta^+ \) or \( \beta^0 \) thalassemia.\textsuperscript{29,40,42,54} By contrast, deletion of \( \beta \)-like genes is detectable in both \( \delta \beta \) thalassemia\textsuperscript{29,53} and HPFH.\textsuperscript{4,29,52} In one study,\textsuperscript{29} the extent of deletion of \( \beta \)-like genes in homozygous HPFH DNA was greater than in homozygous \( \delta \beta \) thalassemia DNA. When the HPFH and \( \delta \beta \) thalassemia DNA were mixed, the results were consistent with overlapping \( \beta \)-like gene deletions in HPFH and \( \delta \beta \) thalassemia and more extensive deletion in HPFH.

It has been postulated that the \( \delta \) gene region (R\textsubscript{3} in Fig. 1) is involved in the regulation of \( \gamma \) globin gene expression,\textsuperscript{55} since individuals with HPFH and \( \delta \beta \) thalassemia who lack structural \( \delta \) genes have greater \( \gamma \) globin biosynthesis than patients with \( \beta^+ \) and \( \beta^0 \) thalassemia (who have intact \( \delta \) structural genes). The more extensive hybridization of \( \beta \) cDNA to \( \delta \beta \) thalassemia DNA than to HPFH DNA suggests that sequences remain in \( \delta \beta \) thalassemia DNA that are responsible for \( \gamma \) globin gene repression. The extent of these sequences and their relation to structural gene sequences is completely unknown. In experiments in which the relative amounts of cDNA and DNA used established a competition between one cellular DNA strand and the cDNA for the complementary strand of cellular DNA containing the globin genes, decreased numbers of globin genes in heterozygotes for \( \alpha \) thalassemia, \( \delta \beta \) thalassemia, and HPFH were detected.\textsuperscript{29,56,57}

**SUMMARY OF THE MOLECULAR BIOLOGY OF THE THALASSEMIAS (TABLE 3)**

In \( \delta \beta \) thalassemia and HPFH, deletions of the \( \beta \) and probably the \( \delta \) structural genes are responsible for the absent \( \beta \) and \( \delta \) mRNA and globins. In \( \beta^+ \) and \( \beta^0 \) thalassemia, no detectable deletion of \( \beta \) structural genes is observed. In some cases of \( \beta^0 \) thalassemia, there are detectable \( \beta \) globin mRNA sequences despite complete suppression of \( \beta \) globin synthesis, indicating that an abnormal, untranslatable \( \beta \) mRNA is present. The gene defect in these cases might be a nucleotide change in DNA leading to a \( \beta \) mRNA incapable of normal binding to ribosomes or normal translation. It is known that globin mRNA has a methylated nucleotide structure as well as approximately 50 nucleotides at its 5′ untranslated end. In addition, there are 100 untranslated nucleotides at the

**Table 3.** Homozygous States of \( \beta \) Thalassemia and Variants

<table>
<thead>
<tr>
<th>Type</th>
<th>Anemia</th>
<th>( \delta ) Globin</th>
<th>( \beta ) Globin Synthesis</th>
<th>( \beta ) Globin mRNA</th>
<th>( \beta ) Globin Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \beta^+ ) thalassemia</td>
<td>Severe</td>
<td>Present</td>
<td>Decreased</td>
<td>Decreased</td>
<td>Present</td>
</tr>
<tr>
<td>( \beta^0 ) thalassemia</td>
<td>Severe</td>
<td>Present</td>
<td>Absent</td>
<td>Absent or Abnormal</td>
<td>Present</td>
</tr>
<tr>
<td>( \delta \beta ) thalassemia</td>
<td>Mild</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Deleted</td>
</tr>
<tr>
<td>HPFH</td>
<td>None</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Deleted</td>
</tr>
</tbody>
</table>
3' end of the structural nucleotide sequence prior to the 100 or so poly (A) residues. Defects in either of these sequences could lead to an untranslatable and perhaps unstable mRNA.

In β⁺ thalassemia, the amount of β globin synthesized in both intact cells and by isolated mRNA is roughly proportional to the amount of β mRNA measured by hybridization to cDNA. The two possible defects at the gene level are (1) decreased transcription of a normal β globin "structural" gene (defined here as the sequences in DNA transcribed into β globin mRNA and its precursors in the nucleus), and (2) decreased stability of β globin mRNA or its precursors due to nucleotide changes in the β globin "structural" gene. In the case of decreased transcription, the genetic defect in β⁺ thalassemia would involve an abnormality in a "regulatory" gene sequence modulating RNA production by the β globin gene; in the case of decreased stability of a β globin mRNA or its precursors, no such "regulatory" gene activity need be postulated.

Leder and Flavell (personal communication) recently found that mouse and rabbit β globin cellular genes have a 600–1000-nucleotide sequence inserted between the 5' and 3' ends of the structural gene; this is not found in mature β globin mRNA. This extra inserted nucleotide sequence may be transcribed into the globin mRNA precursors in the nucleus, and this extra piece may subsequently be excised during mRNA processing. If a normal β globin mRNA precursor is required for nuclear stability of β globin mRNA sequences, then a nucleotide defect in this inserted midstructural gene sequence in β⁺ thalassemia could result in decreased stability of the β globin mRNA precursor, decreased β globin mRNA, and decreased β globin. If, as postulated, the abnormal inserted midgene sequences are subsequently excised during the formation of mature globin mRNA, structurally normal β globin mRNA in β⁺ thalassemia would be explained. A recent study comparing α and β globin mRNA content in nuclei and cytoplasm of homozygous β⁺ thalassemia patients is consistent with the presence of relatively unstable β globin mRNA precursors in these disorders. 58

**RELATIONSHIP OF MOLECULAR DEFECTS TO CLINICAL DISEASE**

Although the basic defect in the thalassemias is reduced or absent biosynthesis of a specific globin chain, the relative excess of the unaffected globin chain also contributes to major pathophysiologic complications.

**β Thalassemia**

In β thalassemia, the decreased or absent β globin synthesis leads to a vast relative excess of α globin chains. 10 These α chains, when not incorporated into HbF or HbA, are relatively unstable in solution and tend to aggregate and precipitate at increasing concentrations. Free α chains are also found in solution in relatively small amounts and can be reproducibly detected only by radioactive labeling of cells and analysis of hemolysates. Thus free α globin leaves the soluble portion of cells either as precipitated material 59 or by proteolysis. 60 Aggregated α globin material can be seen by electron microscopy in both nuclei and cytoplasm of nucleated erythroid precursors and reticulocytes. 61 Particulate inclusion bodies have been collected and shown to contain α globin peptides. 62
Nuclear dysfunction has been noted in erythroblasts soon after globin biosynthesis begins, consistent with a role for free α globin chains in the profound ineffective erythropoiesis in thalassemia. Abnormal erythroblast maturation is further evidenced by the discrepancy between massive erythroid hyperplasia in bone marrow and the inappropriately low reticulocyte count in homozygous β thalassemia. Destruction of cells in bone marrow and to a lesser extent of reticulocytes and red cells in the circulation by the spleen leads to profound anemia. Extramedullary hematopoiesis occurs in liver, spleen, all bones, and lymph nodes as well. Abnormal growth and development accompany the bone changes and chronic anemia.

There is a good correlation between the amount of α globin excess and degree of anemia. As noted earlier, there is heterogeneity in the HbF level of cells in thalassemia. Those cells with the greatest amount of HbF preferentially survive and are present in peripheral blood presumably because of their lesser imbalance of α as compared to non-α globin. In δβ thalassemia and HPFH, the lack of β globin synthesis has no physiologic sequelae, since γ globin production is either equal or nearly equal to that of the α globin and no significant α globin excess accumulates. In some patients, presumably heterozygotes for β thalassemia, anemia is moderate and tolerable without transfusions. These individuals with so-called thalassemia intermedia may have an increased ability to degrade free α globin chains and prevent their adverse consequences. Furthermore, heterozygotes for β thalassemia have been described with α globin precipitates and anemia. These individuals may have a decreased ability to degrade free α globin. In most thalassemia heterozygotes, not enough excess α globin chains accumulate to lead to either anemia or bony abnormalities because of erythroid hyperplasia, although intramedullary death of nucleated red cell precursors is moderately accelerated and α globin proteolysis is present.

In α thalassemia, globin chain imbalance is also associated with morphologic abnormalities and anemia. In α thalassemia–silent carrier state, the excess of β chains is apparently insufficient to result in hematologic changes. While α globin has no preferred state of aggregation, excess β globin is usually found in tetrameric form as HbH. Cells containing HbH are preferentially removed from the circulation by the spleen. Although HbH is not demonstrable in α thalassemia trait, it is presumed that β4 tetramers are responsible for the morphologic changes. In HbH disease, a mild to moderate anemia is present owing to splenic sequestration of cells containing precipitates of HbH. In hydrops fetalis, the absence of HbA, HbF, and HbA2 results in death in utero or at birth due to anoxia and congestive heart failure.

HEMOGLOBINOPATHIES RESULTING IN THALASSEMIALIKE DISORDERS

In homozygous hemoglobin Lepore disease, the Lepore fusion product (δβ Lepore), presumably stemming from unequal crossing over between the δ and β globin genes, is the only nonfetal Hb present. In heterozygotes for hemoglobin Lepore, Lepore Hb constitutes only about 15% of the total hemoglobin. In homozygotes, there is a marked excess of α globin over non-α globin synthesis similar to that seen in homozygous β thalassemia; the clinical severity of the two disorders is similar. Since Lepore globin has the same N-terminal amino...
acid sequence as δ globin, it has been postulated that Lepore globin biosynthesis is under the same genetic control as δ globin and is therefore reduced. The reduction in δ globin synthesis could be due either to decreased δ mRNA production or decreased stability of nuclear δ globin mRNA precursors or to δ globin mRNA itself. Reports of low or undetectable δ globin synthesis in reticulocytes as compared to bone marrow favor decreased δ mRNA stability. We recently found a reduced amount of Lepore globin mRNA in reticulocytes of a patient homozygous for Hb Lepore proportional to the decrease in Lepore globin synthesis. Hb Miyada, the anti-Lepore chain (β-like at NH₂ end and δ-like at COOH end) was also found to be synthesized and present in low amounts in heterozygotes. This result suggests that the δ-like end of the mRNA for this abnormal globin also influences either the transcription or processing of Miyada mRNA.

The recent findings of inserted sequences within the β globin genes of mouse and rabbit suggest another alternative for the reduced stability of δ, Lepore, and Miyada mRNA. Perhaps the δ globin structural gene has an inserted sequence that results in decreased stability of nuclear δ mRNA precursors. Lepore and Miyada genes may also contain part or all of this sequence as part of the fusion gene products. This altered inserted sequence could result in unstable globin mRNA precursors and explain the decreased production of δ, Lepore, and Miyada globins by a common mechanism.

Hemoglobin Constant Spring (HbCS) contains an elongated α globin chain including 31 extra amino acids at its carboxyl-terminal end. This results from a single base change in the normal termination codon for α globin. In heterozygotes, HbCS comprises 10% of the total hemoglobin and acts like an α thalassemia gene. Biosynthetic studies indicate that αCS synthesis and content are reduced proportionally. There appears to be no decreased rate of translation of αCS. It is possible that the mutation in α leads to either decreased transcription or abnormal processing of αCS mRNA.

HETEROCELLULAR HEMOGLOBIN F

Recent investigations in several systems suggest that changes in specific globin biosynthesis may occur at early stages of erythroid cell development. In sheep, with the stress of anemia, β-like globin genes, which are usually inactive, are reactivated. In man, it has been shown that three β-like Hbs (β", β", and γ) can be activated in a single clone of cells derived from a single erythroid cell precursor and that cells surviving in long-term culture from bone marrow produce predominantly HbF. Evidence has recently been presented suggesting a genetic basis for increasing the percentage of HbF-producing cells in human erythroid populations. Most interestingly, the gene involved, termed the gene for heterocellular HPFH, has been found linked and in cis to either the β thalassemia or the δ gene. Detection of this gene relies on the sensitivity of immunofluorescent measurements of HbF in cells using an anti-HbF antibody. In heterozygotes for β thalassemia or δ and the gene for heterocellular HPFH, there is a small increase in HbF. However, homozygotes for sickle cell anemia, β thalassemia, or sickle β thalassemia and the heterocellular HPFH gene are capable of markedly increased HbF production.
Another finding of interest is that pregnant women have a higher proportion of HbF-producing cells than normal individuals, suggesting that the population of cells capable of producing HbF can be increased by hormones. The implications of these findings are obvious for patients with thalassemia.

PRENATAL DIAGNOSIS OF THALASSEMIA

The availability of methods to accurately measure α, β, and γ globin biosynthesis has provided the biochemical basis for the prenatal diagnosis of homozygous β thalassemia. However, it was not until improved techniques for fetal blood sampling became available that prenatal diagnosis proved feasible. Two approaches to obtaining fetal blood have been used, (1) multiple blind placental aspiration and (2) fetoscopy to obtain fetal blood samples under direct visualization. Both methods can cause placental hemorrhage, and the overall rate of fetal loss is 10%-15%; the fetoscopy method has obvious advantages when it is available. The systematic development of nomograms of normal values for γ and β globin synthesis at various gestational ages has provided the necessary control data required for prenatal diagnosis. Fetal blood sampling is usually performed at 18-20 wk gestation.

The mother may be transfused 1 wk prior to the procedure to suppress her reticulocyte count. Although this is unnecessary if a pure fetal blood sample is obtained (fetal blood contains up to 20% reticulocytes), in cases in which a mixture of fetal and maternal blood is aspirated the transfusion ensures that fetal reticulocyte globin biosynthesis is being measured. Methods are available for rapidly quantitating the percentage of fetal cells. A Coulter particle counter can be used to distinguish larger fetal cells from smaller maternal cells. Samples containing a low percentage of fetal cells can be further purified by agglutination with anti-i immunoglobulin. Alternatively, a mathematical correction can be made by using measurements of β and γ globin synthesis in maternal blood and calculating the effect of this synthesis on the β/γ ratios of the fetal blood sample.

Over 75 pregnancies have been studied, and the antenatal diagnosis was compared with findings in the newborn or from abortuses in the majority of the cases. Inadequate samples were obtained in 10%-15% of the cases. In all cases analyzed, the antenatal diagnosis of homozygous β thalassemia was correctly made. There is overlap of normal values with those in β thalassemia trait. At 18-20 wk gestation the normal β/γ ratio is above 0.07 with a mean of 0.11, while the ratio in β thalassemia trait is 0.058 ± 0.020 (±2 SD). In the nine reported fetuses with homozygous β thalassemia, the β/γ ratio was below 0.03; and in seven cases no β globin synthesis was seen by chromatographic analysis.

Prenatal diagnosis requires a clear separation of γ and β globin chains by chromatography, since any significant overlap of the two globins results in an uninterpretable chromatogram. Although the vast majority of prenatal diagnoses have been reported by two groups of investigators, increased experience with methods of obtaining fetal blood samples and for performing the biochemical analyses can be expected to encourage more widespread use of this procedure.
THERAPY OF THALASSEMIA

Patients with homozygous β thalassemia require transfusions to prevent severe, symptomatic anemia. Two types of transfusion programs have been used, (1) moderate transfusions to maintain Hb levels at 7–9 g/dl, and (2) “hypertransfusion” programs to maintain the Hb level above 10 g/dl in an attempt to decrease the effects of anemia and prevent development of orthodontic problems and other bony abnormalities. Both approaches have resulted in more normal growth and development, fewer pathologic fractures of bone, and increased psychologic well-being.

The concurrent use of iron-chelating agents, however, has not until recently prevented the death of most patients in the second or third decades of life due to cardiac hemochromatosis with congestive heart failure or fatal cardiac arrhythmias. Daily intramuscular injection of 0.5–1.0 g deferoxamine was, until recently, the standard therapy in thalassemic patients even though it failed to achieve negative iron balance in the face of ongoing transfusion therapy. More recently, Propper and co-workers showed that 1.5–2.0 g deferoxamine given either intravenously or by continuous subcutaneous infusion could sustain a higher level of urinary iron excretion. Since iron excretion by the two routes of administration is comparable, subcutaneous administration is favored because of its greater convenience. The success of continuous intravenous or subcutaneous deferoxamine is presumed to be due to the constant exposure of a “labile” iron pool to the chelating agent.

A portable pump, which can be worn easily by the patient, delivers the total deferoxamine dose in controlled fashion over 12 hr (at night) without significant inconvenience. Using this new mode of therapy, overall negative iron balance can be achieved in most, if not all, patients with thalassemia while maintaining their transfusion programs. The optimal drug dose required for maximal iron excretion must be determined for each patient. Although it will require a long-term trial of this therapy to evaluate its effectiveness in preventing cardiac hemochromatosis, this program holds great promise for forestalling or preventing complications due to iron overload. A large clinical trial of this regimen is currently underway.

Other iron-chelating agents are being evaluated in addition to deferoxamine, but none has yet proved as effective. Ascorbic acid increases iron excretion when used with deferoxamine; however, the possibility that ascorbic acid may have detrimental effects on cardiac function in patients with already established hemochromatosis has been raised.

Splenectomy, in homozygous β thalassemia, is usually reserved for those patients who develop an increasing transfusion requirement due to antibody-mediated hemolysis as a result of multiple transfusions. However, splenectomy may be performed as well for hypersplenism. Splenectomy should be delayed until at least age 3 yr, if possible, because of the increased incidence of fatal postsplenectomy septicemia in young children. Folic acid therapy should be used to prevent the development of megaloblastic anemia.

Heterozygotes for β thalassemia usually require no therapy, although folic acid deficiency can occur. As mentioned earlier, hemoglobin H disease is usually associated with mild or moderate anemia. If hypersplenism develops and is severe, splenectomy may be of benefit.
FUTURE RESEARCH IN THALASSEMIA

At the molecular level, analysis of the abnormalities of DNA structure in the thalassemias, using recently developed techniques for the isolation and characterization of specific genes, should provide new insights into the regulation of globin biosynthesis. The long-range expectations of these studies include reactivation of γ and/or β globin synthesis, suppression of excess α chain synthesis, and gene replacement using purified genes. In addition, the recent finding of subpopulations of erythroid cells capable of preferentially synthesizing HbF may result in the discovery of drugs or hormones that can expand HbF-synthesizing cell populations.

At the clinical level, the development of oral iron-chelating agents would provide more convenient therapy for iron overload. Improvements in bone marrow transplantation techniques would provide an alternative approach with the potential of cure. Improved methods of prenatal diagnosis not requiring fetal blood sampling would be a significant advance. If methods can be developed to determine the presence of homozygosity for thalassemia using DNA from fibroblasts from amniocentesis fluid, more extensive use of prenatal diagnosis with less risk than at present can be anticipated. This goal could be accomplished either by defining a characteristic defect in the cellular DNA of thalassemia patients or by hybridizing fibroblasts to other cells in the hope of obtaining globin gene expression by the fibroblasts in the hybrids.

Specific cellular DNA fragments containing globin genes can be identified by cleaving DNA with site-specific restriction enzymes, separating the DNA fragments on agarose gels, transferring the DNA to nitrocellulose filters, and hybridizing to 32P-labeled cDNA. With this method we recently found seven or eight EcoRI enzyme-restricted DNA fragments containing globin genes in normal human cellular DNA. Between one and ten picograms of cellular globin gene is detectable with this technique. Deletion of specific DNA restriction enzyme fragments is seen with homozygous β-thalassemia and HPFH DNA with greater deletion in HPFH than β-thalassemia DNA. If consistent differences from normal are found using β+ and β0 thalassemia DNA, this technique may permit prenatal diagnosis of these disorders by analysis of DNA from amniocentesis fluid cells.

Future research in thalassemia holds the promise of providing a more precise understanding of gene regulation in the human globin system and leading to the discovery of more general principles that can be applied to the study of other genetic diseases of man.

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