Pathophysiology of alpha thalassemia

Author
Stanley L Schrier, MD

Section Editors
William C Mentzer, MD
Donald H Mahoney, Jr, MD

Deputy Editor
Stephen A Landaw, MD, PhD

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Literature review current through: Jan 2015. | **This topic last updated:** Oct 28, 2013.

INTRODUCTION — The major hemoglobin in adults is hemoglobin A (HbA), which is a tetramer consisting of two pairs of globin polypeptide chains: one pair of alpha chains and one pair of beta chains. In normal subjects, globin chain synthesis is very tightly controlled so that the ratio of production of alpha to non-alpha chains is 1.00 ± 0.05. There are two copies of the alpha globin gene on chromosome 16. A single beta globin gene resides on chromosome 11 adjacent to genes encoding the beta-like globin chains, delta and gamma (figure 1). (See "Structure and function of normal human hemoglobins".)

Thalassemia refers to a spectrum of diseases characterized by reduced or absent production usually of one of the globin chains.

- Alpha thalassemia is due to impaired production of alpha globin chains, which leads to a relative excess of gamma globin chains in the fetus and newborn, and beta globin chains in children and adults. While the excess beta globin chains are capable of forming soluble tetramers (beta-4, or HbH), they are unstable and some precipitate within the cell, leading to a variety of clinical manifestations. Since all normal hemoglobins of postnatal life contain alpha chains, homozygous alpha (0) thalassemia, in which no alpha globin chains can be produced, is incompatible with extrauterine life, leading to hydrops fetalis and/or death shortly after delivery.

- Beta thalassemia, in comparison, is due to impaired production of beta globin chains, which leads to a variable excess of alpha globin chains. For reasons that are not entirely clear, alpha globin chains cannot form soluble tetramers and thus precipitate. (See "Pathophysiology of beta thalassemia".) The excess alpha globin chains in beta thalassemia therefore begin aggregating as soon as they accumulate in erythroid precursors.

For these reasons and because of the unique biochemical composition of aggregates of alpha globin, the clinical manifestations are generally less severe in alpha compared with beta thalassemia of comparable genetic severity, except for homozygous alpha (0) thalassemia [1].

The net loss of alpha globin synthesis reflects not only the number of alpha globin genes affected by the mutation(s), but also which of the two alpha globin loci is affected, whether the mutation(s) involved are deletional or nondeletional, and whether the nondeletional mutation(s) partially or fully block gene expression. In addition, defects in alpha globin synthesis in alpha thalassemia can ameliorate beta thalassemia severity by rebalancing the alpha to beta globin chain ratio. Thus, understanding the basis for the broad spectrum of clinical severity (phenotypes) in individual patients with alpha thalassemia requires a detailed knowledge of the underlying genetic defect(s) and the impact of these defects on the overall levels and balance of globin chain synthesis.

The pathophysiology of the anemia in alpha thalassemia will be reviewed here. This discussion will focus on the mechanisms by which excess unmatched beta globin chain synthesis leads to hemolysis of red cells in the peripheral circulation. Less importantly than in beta thalassemia, the accumulation of excess beta chains in erythroid precursors within the bone marrow and in extramedullary sites, such as the liver and spleen, also leads to certain amount of ineffective erythropoiesis. The molecular pathology of the thalassemias is discussed separately. (See "Molecular pathology of the thalassemic syndromes".)

DEFINITIONS — While the thalassemias are profoundly heterogeneous from a genetic standpoint, certain clinical

terms are available to describe the phenotypic expression of the alpha thalassemias. Because the alpha gene locus is duplicated in humans, each individual normally carries four alpha chain genes (ie, aa/aa).

Alpha (0) thalassemia — Alpha (0) thalassemia refers to the more than 20 different genetic mutations of the alpha globin locus which result in the deletion of both alpha chain loci (ie, aa/--) (figure 1) on one chromosome 16. Patients who carry alpha (0) gene mutations on both chromosomes (ie, --/--) cannot make alpha chains and are therefore unable to make any hemoglobin A, F, or A2. This condition is incompatible with extrauterine life (see 'Hydrops fetalis and hemoglobin Bart's' below).

Alpha (+) thalassemia — Alpha (+) thalassemia refers to the more than 15 different genetic mutations which result in decreased production of alpha globin, usually due to deletion of one of the two alpha chain loci in the affected chromosome. As a result, there are three general forms of alpha (+) thalassemia based upon the number of inherited alpha genes:

- Inheritance of three normal alpha genes (aa/a-) has been termed alpha thalassemia minima, silent carrier of alpha thalassemia, alpha thalassemia-2 trait, or heterozygosity for alpha (+) thalassemia. Affected subjects are clinically normal and may also be hematologically normal; the diagnosis can be reliably made only via DNA analysis.
- Inheritance of two normal alpha genes has been termed alpha thalassemia minor or alpha thalassemia-1 trait, and is due either to heterozygosity for alpha (0) thalassemia (aa/--) or homozygosity for alpha (+) thalassemia (a-/a-). These subjects are clinically normal but may have minimal anemia along with reductions in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH).
- Inheritance of one normal alpha gene (a-/--) is termed hemoglobin H (HbH) disease, because of the formation of HbH, which is composed of tetramers of the resulting excess beta chains (beta 4). These patients have moderate to severe degrees of lifelong hemolytic anemia, very modest degrees of ineffective erythropoiesis, splenomegaly, and variable bony changes [2]. (See "Clinical manifestations and diagnosis of the thalassemias", section on 'Hemoglobin H disease'.)
- As mentioned above, inheritance of no alpha genes (--/--) is incompatible with extrauterine life, since the affected fetus will be unable to make any of the hemoglobins normally produced after birth (eg, hemoglobins A, F, and A2), all of which require the ability to produce alpha globin chains (see 'Hydrops fetalis and hemoglobin Bart's' below).

Complex alpha thalassemia variants — The above definitions are useful as a starting point but the classification of the alpha thalassemias can become extremely complex. For example, two general forms of HbH disease have been recognized, the deletional and non-deletional forms. In the former, the patient has inherited only a single alpha globin gene (ie, a-/--). In the non-deletional form, the patient has inherited two alpha globin genes from one parent, but one of these carries a non-deletional defect, such as a point mutation (ie, aa*/--, where a* represents the mutated alpha chain, such as hemoglobin Constant Spring). HbH disease tends to be more severe in patients with the non-deletional form [3], due, at least in part, to interference with transcription of the normal alpha chain gene by the abnormal one [4]. (See "Clinical manifestations and diagnosis of the thalassemias", section on 'Hemoglobin H disease' and "Clinical manifestations and diagnosis of the thalassemias", section on 'Hemoglobin Constant Spring'.)

As another example of the potential complexity of these disorders, individual patients may have combinations of alpha and beta globin gene mutations of varying nature and severity (eg, combinations or variants of alpha (0) from one parent and alpha (+) from the other with one or more beta chain abnormalities).

Anemia — The anemia in the alpha thalassemic syndromes has at least four components:

- Underproduction of hemoglobin HbA
- Production of two nonfunctional hemoglobins because of insufficient numbers of alpha globin chains: HbBart's (gamma globin tetramers) in newborns before the switch to beta globin production and HbH (beta globin tetramers) at all ages

- Intracorpuscular hemolysis caused by the accumulation within red blood cells (RBCs) of excess unmatched non-alpha globin chains
- Ineffective erythropoiesis, or intramedullary hemolysis, also resulting from the globin chain imbalance

Protection against severe malaria — The alpha thalassemia genes affect up to 90 percent of some populations and appear to offer a selection advantage by protecting against severe malaria (due to Plasmodium falciparum) and perhaps other infections [5-8]. In Papua New Guinea, for example, there is intense malaria transmission and alpha thalassemia is present in more than 90 percent of the population. In one study, the relative risk of severe malaria, compared with normal children, was 0.40 in homozygotes and 0.66 in heterozygotes; similar values were seen for hospitalization for infections other than malaria [8]. Similar finding were also reported from Ghana, where heterozygosity for alpha (+) thalassemia was noted in 32.6 percent of controls but in only 26.2 percent of cases of P. Falciparum infection (odds ratio 0.74) [9].

The protection against severe malaria may be mediated by increased susceptibility to infection with the nonlethal P. vivax, particularly in young children, thereby inducing limited cross-species protection against subsequent P. falciparum infection [5,7]. In addition, thalassemic red cells may remain susceptible to P. falciparum invasion but are associated with significantly reduced parasite multiplication [10]. (See "Protection against malaria in the hemoglobinopathies", section on 'Alpha thalassemia'.)

HISTORICAL PERSPECTIVE — In 1955, two groups of investigators virtually simultaneously described the syndrome of HbH disease [11,12]. Affected patients had a hypochromic microcytic anemia of variable severity, target cells on peripheral smear, reticulocytosis, inclusions in a few RBCs, many more of which could be produced by incubating RBC with mild oxidant dyes like brilliant cresyl blue, resistance to lysis by hypotonic solutions, and signs of hemolysis, including a shortened 51Cr RBC survival, splenomegaly, and elevated indirect reacting bilirubin. (See "Red blood cell survival: Normal values and measurement" and "Approach to the diagnosis of hemolytic anemia in the adult".)

A key finding was an abnormal hemoglobin, accounting for up to 40 percent of the total hemoglobin, which had more rapid electrophoretic mobility at pH 8.6 than HbA. When this hemoglobin, now called HbH, was allowed to stand it turned brown and precipitated, forming aggregates that looked somewhat like the RBC inclusions. This phenomenon was accentuated by addition of mild oxidants.

Consequences of impaired alpha globin synthesis — Subsequent studies identified this disorder as a moderately severe form of alpha thalassemia with the deletion or inactivation of three of the four alpha genes, leading to a clinically significant unbalanced globin chain synthesis [13]. The alpha/beta synthesis ratio was found to range from 0.3 to 0.6 (normal: 1.0 ± 0.05) [14].

The reduced synthesis of alpha globin chains leads to an accumulation of otherwise normal beta globin chains in adults and gamma chains in the fetus. Instead of forming alpha/beta dimers which then form normal HbA tetramers ($\alpha_2\beta_2$), the excess beta globin chains assemble into beta-4 tetramers, called HbH [15,16]. In the neonatal period, before beta globin chain synthesis reaches adult levels, excess unmatched gamma globin chains accumulate and form gamma-4 tetramers, called HbBart's [17]. These two homotetramers are susceptible to oxidant injury [15,18], and are functionally useless as oxygen delivery pigments because their affinity for oxygen is at least 10 times greater than HbA. They demonstrate no Bohr effect, and the oxyhemoglobin dissociation curve is not sigmoidal because there is no heme-heme interaction. The oxyhemoglobin dissociation curves for HbH and HbBart's resemble that of myoglobin, a compound that holds but cannot deliver oxygen [19]. (See "Structure and function of normal human hemoglobins".)

A critical factor underlying the difference in pathophysiology between alpha and beta thalassemia is that, in the alpha thalassemias, the excess beta or gamma globin chains can form partially soluble but ineffective hemoglobin homotetramers. These homotetramers do not precipitate extensively until they are exposed to damaging effects, mostly oxidant in nature, in the circulation. In contrast, in the beta thalassemias the excess unmatched alpha chains that accumulate cannot form alpha-4 tetramers; instead, they produce insoluble alpha aggregates even in

very early marrow erythroid precursors, affecting membrane assembly and accelerating programmed cell death. (See "Pathophysiology of beta thalassemia".)

MORPHOLOGY OF ALPHA THALASSEMIC RED BLOOD CELLS — The RBCs in alpha thalassemia are hypochromic and microcytic. The hypochromia is due both to less hemoglobin per cell and to hyperhydration of the cell.

Red blood cell size and shape — The unbalanced globin chain synthesis leads to a deficiency in the amount of hemoglobin per cell (low MCH) and, since hemoglobin accounts for 30 to 35 percent of the RBC content, the RBC volume (MCV) will be low, producing the well known hypochromic target cells and microcytosis. However, in addition to these well characterized morphologic findings, there are frequently fragmented RBCs and bizarre shapes.

The introduction of sophisticated technology such as the Technicon Bayer H* Analyzer has allowed more precise identification of RBC indices. The MCH tracings show that, as a consequence of time in the circulation, RBC fragmentation occurs in HbH and the alpha thalassemia variant, HbH/Constant Spring (HbH/CS), producing populations of tiny hemoglobin-containing fragments. It is highly likely these fragments have shortened survival time in the circulation. Both the intact RBCs and the cell fragments with a markedly reduced surface area-to-volume ratio have great difficulty in undergoing the elongated elliptical deformation that normally allows 7 to 8 micron diameter RBCs to pass through 3 micron diameter capillaries and 2 to 3 micron slits in reticuloendothelial sinusoids. (See "Red blood cell survival: Normal values and measurement", section on 'Senescence'.)

Several early observations noted that while both beta- and alpha-thalassemic RBCs were hypochromic and microcytic, the decrease in hemoglobin concentration within the RBC (ie, reduced MCHC) was more prominent in the Constant Spring thalassemic variants [20]. These observations lead to further studies of RBC hydration.

Red blood cell hydration — One approach to assessing the extent of RBC hydration is to perform density gradient analyses on peripheral blood. In patients with severe alpha thalassemia, the RBC (not only the reticulocytes and younger RBC) are uniformly of lower than normal density, are found at the top of a discontinuous Stractan gradient, and are hyperhydrated [21]. This contrasts with the RBCs of comparably anemic patients with beta thalassemia in which a few of the younger RBCs are light, but other populations are found throughout the gradient, including populations of RBCs as dense and dehydrated as those seen in severe sickle cell anemia (MCHC >45) [21]. (See "Pathophysiology of beta thalassemia", section on 'Red blood cell dehydration'.)

The Technicon Bayer H*3 Analyzer allows one to make quantitative assessments of the levels of hydration. As an example, the percent of RBCs with MCHC levels below 28, (ie, definite hypochromia) is approximately 50, 70, and 20 for HbH disease, HbH/CS, and HbCS/CS, respectively [22,23]. The use of this instrument also showed that reticulocytes, as they left the marrow, were already well hydrated (increased MCV and decreased MCHC).

The cause of the hyperhydration in alpha thalassemic RBCs is not clear. The K-Cl cotransporter is a pH and volume activated transporter that functions in the normal remodeling of reticulocytes to mature RBC. This transporter controls the loss of KCl and water, leading to a reduction in the MCV. It closes down in normal RBC but stays open in beta thalassemic and sickle cell RBCs in which it is partly responsible for the RBC dehydration in these disorders. (See "Control of red blood cell hydration", section on 'K-Cl cotransport'.) It is an unproven hypothesis that, in alpha thalassemia, the K-Cl cotransporter closes down very early, thereby preventing the usual loss of K-Cl and water that is part of the remodeling process.

HEMOLYTIC ANEMIA — Studies with ^{51}Cr-labeled RBCs in HbH disease have shown a shortened survival halftime (12 to 19 days versus 28 to 37 days in normals) [11,16,18,20,24] and evidence for increased splenic sequestration [18,24,25]. A potential error with this technique is that HbH binds more ^{51}Cr than HbA and has a higher rate of label elution [20,24]. (See "Red blood cell survival: Normal values and measurement".) However, studies using the noneluting cohort label glycine-2-^{14}C showed that RBC survival in HbH disease was one-third of normal [20].

There are other signs of uncompensated hemolysis. Patients are mildly jaundiced with indirect reacting

hyperbilirubinemia and palpable splenomegaly. These results are consistent with the presence of an intracorpuscular abnormality, leading to extravascular RBC destruction by the macrophages of the reticuloendothelial system. (See "Approach to the diagnosis of hemolytic anemia in the adult" and "Overview of hemolytic anemias in children".)

The response to the anemia is evidenced by the presence of marrow erythroid hyperplasia and reticulocytosis (2.5 to 16 percent) [11,12,25]. In addition, serum erythropoietin (EPO) values are increased, as are levels of soluble transferrin receptor (sTfR), an indicator of increased erythroid activity [26,27]. (See "Causes and diagnosis of iron deficiency anemia in the adult".) The increases in EPO and sTfR are directly related to the HbH concentration which, in turn, reflects the globin chain imbalance [26].

The following section will review the multiple factors that contribute to the enhanced extravascular removal of alpha thalassemic RBCs. Although our understanding of these processes has increased, a number of questions have been raised concerning the pathogenesis of anemia in HbH disease that will be discussed in the following sections:

- How does decreased alpha globin chain synthesis produce abnormalities in the affected RBCs that are recognized in the microvasculature and by the macrophages of the reticuloendothelial organs, leading to removal of these RBCs?
- Why are patients with HbH disease anemic, since a rate of hemolysis three times normal falls easily within the normal marrow's five- to seven-fold compensatory activity? In fact, ferrokinetic studies show an increase in erythropoiesis up to six times normal in some but not all patients [25]. This discrepancy is thought to reflect ineffective erythropoiesis (see 'Ineffective erythropoiesis' below).

REMOVAL OF ALPHA THALASSEMIC RED BLOOD CELLS BY SPLEEN AND MACROPHAGES — In the final common pathway of extravascular destruction of circulating RBCs, abnormalities in RBC deformability slow the passage of affected RBC in the microvasculature, allowing the macrophages of the reticuloendothelial organs to carefully scrutinize these RBCs and detect any cell surface signals that could alert the macrophages to retard, bind and engulf the defective RBC.

RBC deformability — As mentioned above, the 7 to 8 micron diameter RBCs must undergo a remarkable elliptical elongation to allow passage through 3 micron diameter capillaries and even smaller slits in the sinusoids of the reticuloendothelial system. Any increase in rigidity will hold up passage through the microvascular bed and delay transit through the reticuloendothelial organs, such as the spleen. There are three major factors that control RBC deformability: the ratio of surface area to volume, internal viscosity as measured by the MCHC, and membrane features. (See "Red blood cell mechanics".)

Erythrocyte deformability measurements made via osmotic gradient ektacytometry have shown that HbH RBCs are very rigid [21]. The increase in RBC rigidity was more apparent when populations of defined MCHCs were compared in normal and HbH RBCs. The RBCs in HbH/CS are even more rigid than in HbH [22], and are most rigid in HbCS/CS [28]. This extreme rigidity could lead to the observed RBC fragmentation in parts of the vasculature in which there is elevated shear stress. These undeformable fragments are subject to rapid removal, while the rigidity of the intact RBC impairs passage through RE organ sinusoids.

The reduction in deformability (ie, increase in rigidity) was an unexpected finding, since two of the three factors (the third being membrane features) that determine RBC deformability would be expected to increase the deformability of RBCs in HbH disease:

- Measurements of osmotic fragility, which are an index of surface area to volume (SA/V) ratios, showed resistance to osmotic lysis [11], indicating a SA/V ratio higher than normal.
- The MCHC is very low in HbH RBCs, indicating that increased intracellular viscosity due to elevated hemoglobin concentrations would not be a factor contributing to reduced RBC deformability [21].

Membrane features — The preceding findings suggested that membrane features must play an important role

in the increased rigidity of HbH RBCs. Two features have been evaluated: deformability and stability.

- Deformability — Membranes from HbH RBCs are two to three times more rigid than normal [21,28]. Membranes from patients with HbH/CS are even more rigid, and the least deformable membranes are seen in HbCS/CS [28]. Ektacytometric studies suggested that an interaction of the excess beta globin chains in HbH RBCs with the cytosolic face of the membrane played an important role in the membrane rigidity (see 'Membrane bound beta globin chains' below) [21]. The further increase in rigidity in the HbCS variants appears to be caused by the combination membrane-bound beta globin chains and oxidized alpha CS globin chains [28].

- Stability — Membrane stability is a technical term used to describe the ability of isolated RBC membranes to resist fragmentation under an intense elliptically deforming shear stress [21]. Membrane stability is generally thought to be a function of the RBC membrane skeleton and the interaction of the skeleton with the major transmembrane protein, band 3. The same gradation noted above for membrane rigidity was noted for membrane stability: increased in HbH, more stable in HbH/CS, and maximally stable in HbCS/CS [28]. These changes are thought to be due to the membrane bound beta globin and alpha CS globin chains (see 'Role of oxidant injury' below). They are in marked contrast to the highly unstable RBC membranes in beta thalassemia, a change induced by partially oxidized alpha globin chains. (See "Pathophysiology of beta thalassemia", section on 'Membrane instability'.)

The combined consequences of increased RBC rigidity and increased membrane stability are likely to be slowed flow in the vasculature of the reticuloendothelial organs, giving macrophages ample opportunity to look for signals indicating RBC membrane senescence and/or damage, including bound IgG and complement components, or altered membrane phospholipid bilayer asymmetry (see below).

Red blood cell inclusions — The initial descriptions of HbH disease identified inclusions that occurred spontaneously in some RBCs, were much more common in splenectomized subjects, and could be induced by incubating RBCs with mild oxidants such as brilliant cresyl blue or new methylene blue [11,12,18,22]. At the time of splenectomy, inclusion rich RBCs can be found in the spleen, providing a reason for the increased number of inclusion-containing RBCs in HbH patients following splenectomy [18]. These inclusions were thought to be aggregates of beta chain tetramers (ie, beta-4 or HbH).

When RBCs from patients with HbH are separated by density into young, middle aged, and old cells, the level of soluble HbH is highest in young cells, where the number of inclusion containing cells is lowest. As the cells age (ie, became more dense), the amount of soluble HbH decreases while the proportion of inclusions increases. These observations led to the hypothesis that after about 45 days of in vivo aging, HbH began to precipitate within the RBC, forming inclusions which damage the RBC, leading to their removal at least partly in the spleen [18].

Inclusions have been described by electron microscopy in 67 and 79 percent of RBC in HbH and HbH/CS, respectively [22]. The cytosolic levels of HbH differ between these two variants, with HbH RBC having mean levels of 9.3 percent HbH while the value is 15.3 percent in patients with HbH/CS [29]. Thus, the cytosolic concentration of HbH is directly proportional to the number of inclusions.

Convincing proof that these inclusions consisted of beta globin chains was obtained by combining electron microscopy with monoclonal antibodies against beta globin [30]. The presence of inclusions, certainly those that are membrane bound, interferes with the ability of the RBC to undergo the requisite tank treading motion, thereby slowing its passage through the microvasculature.

The heme moiety attached to the beta-4 tetramers undergoes oxidative alteration, with conversion to hemichromes [31]. These iron containing hemichromes are capable of generating reactive oxygen species (ROS), perhaps by acting as Fenton reagents (see "Approach to the patient with suspected iron overload", section on 'Iron toxicity'). These ROS can, in turn, oxidize adjacent RBC membrane proteins and lipids [32,33]. Thus, further RBC membrane damage may occur as a result of local oxidative damage.

Phosphatidylserine signaling — An important question is how a globin chain synthetic defect within the RBC leads to changes in the outer RBC membrane. One hypothesis is that the denatured HbH inclusions lying adjacent to the membrane damage it via oxidant mechanisms (see above), resulting in an alteration in the normal pattern of phospholipid bilayer asymmetry.

One of the messages that macrophages identify on the surface of RBCs marked for removal is the presence of exposed phosphatidylserine (PS) which has "flopped" from the inner to the outer leaflet of the membrane phospholipid bilayer. In normal RBCs, PS is confined to the inner half of the membrane phospholipid bilayer and therefore does not provide a signal to macrophages [34]. (See "Red blood cell membrane dynamics and organization".)

Hemoglobin H, HbCS/CS, and HbH/CS have RBCs with PS on the outer leaflet of the membrane phospholipid bilayer, although the absolute number of such RBCs in the circulation is relatively small [35]. However, the low number may reflect rapid removal of such RBCs from the circulation by the macrophages in the spleen and other reticuloendothelial organs.

Membrane IgG and complement — Macrophages target RBCs for removal from the circulation by identifying the Fc domains of IgG on the RBC's outer membrane surface. Increases in membrane IgG and complement components are a likely contributor to the increased phagocytosis of alpha thalassemic RBCs [36]. Nonsplenectomized patients with HbH disease have RBC IgG levels that are normal. However, RBC IgG levels increase after splenectomy suggesting that such cells had been removed by the spleen. HbH/CS RBCs, even from nonsplenectomized subjects, have more membrane associated IgG than either normal or HbH RBCs [37]. Increased expression of antigens that may be similar to senescent antigens may provide the signal for IgG deposition.

Macrophage colony-stimulating factor — Serum concentrations of macrophage/monocyte colony-stimulating factor (M-CSF) are elevated in HbH disease and correlate inversely with the patient's hemoglobin concentration [38]. It has been proposed that M-CSF enhances the phagocytosis of HbH RBCs, thereby contributing to the severity of the anemia.

BIOCHEMICAL AND CELLULAR ALTERATIONS LEADING TO HEMOLYSIS — The challenge remains to discover how an impairment in synthesis of alpha globin chains produces the cellular alterations described above that in turn lead to hemolysis. Several biochemical studies provide a partial explanation.

Membrane bound beta globin chains — HbH disease is characterized by a prominent globin band seen on SDS polyacrylamide gel electrophoresis of the RBC membranes (figure 2) [39]. A variable amount of this globin consists of HbA, probably in the form of alpha-beta dimers. However, there is only beta globin when one isolates membrane skeletons. This probably represents excess unmatched beta globin that is now firmly attached to the membrane skeleton (figure 3) [30,39]. This membrane bound beta globin has also undergone partial oxidation [39].

These findings are a direct mirror image of the situation in severe beta thalassemia in which partially oxidized alpha globin chains are bound to the RBC membrane skeleton [39]. (See "Pathophysiology of beta thalassemia".) It has therefore been hypothesized that skeletal bound, partially oxidized beta globin chains account for much of the observed membrane pathophysiology in the alpha thalassemias. In both disorders, membrane damage produced by the accumulation of excess globin chains (ie, excess alpha globin chains in beta thalassemia and excess beta globin chains in alpha thalassemia) may be reduced by the action of proteases, which directly attack and destroy these excess globin chains [40].

Hemoglobin Constant Spring (CS) variants provide an opportunity for further exploring the pathophysiology of the alpha thalassemias. As noted above, HbH/CS usually produces a more severe anemia than seen in HbH disease. These RBCs are hyperhydrated and have more inclusions than HbH RBCs [23,41], are poorly deformable, and their membranes are very rigid and hyperstable [28]. The biochemical counterpart of these changes is the presence on the red cell membrane skeleton of partially oxidized alpha CS globin chains as well as partially oxidized beta globin chains; similar findings are seen in HbCS/CS [28].

Role of oxidant injury — There is a recurrent theme that membrane skeletal-associated globin chains with attached hemes, hemichromes [31], and iron causes local oxidant damage to the membrane. The earliest reports of HbH disease noted the presence of inclusions which could be much increased by incubation with mild oxidants, such as methylene blue and brilliant cresyl blue [11,12]. A clinical counterpart is the observation that the hemolysis may be exacerbated when patients with HbH disease are exposed to oxidant drugs such as the sulfonamides [18].

In experiments designed to model the oxidant damage, normal RBCs were incubated with methylhydrazine (MHZ) and phenylhydrazine (PHZ) [42]. MHZ induced the binding of oxidized beta globin chains to the membrane skeleton and the membranes became hyperstable, as is typical for severe alpha thalassemia. In contrast, PHZ induced the binding of oxidized alpha globin chains to the membrane skeleton. The resulting membranes were unstable as measured in the ektacytometer, exactly as seen in the RBCs in severe beta thalassemia. (See "Pathophysiology of beta thalassemia".) Similar findings can be induced by resealing isolated and purified alpha and beta globin chains within normal RBCs, providing further support for the hypothesis that specific oxidized excess globin chains are responsible for the cellular pathophysiology of thalassemic RBCs [43].

A variety of other observations are consistent with the hypothesis that the alpha thalassemic RBC is under considerable oxidant stress [44]:

- Hexosemonophosphate shunt activity is decreased in young HbH RBC [45]. This metabolic pathway controls the recycling of oxidized to reduced glutathione, as well as the generation of the reducing agent NADPH.
- Superoxide dismutase, GSH peroxidase, and catalase are increased in HbH RBCs and even further increased in HbH/CS RBCs [46].

Other evidence of RBC membrane damage — Several studies have provided additional evidence of RBC membrane damage in alpha thalassemia. As an example, one can prepare an integral protein-rich fraction of RBC membranes and measure the binding of the major skeletal protein, spectrin. This preparation is called inside-out vesicles (IOVs). Vesicles from HbH RBCs bind only one-half the amount of spectrin as vesicles from normal RBCs [47]. This defect can be reproduced by adding heme-containing alpha globin chains to normal RBC membranes [48].

These findings suggest that assembly of the skeleton in alpha thalassemia RBCs is defective. Consistent with this hypothesis is the presence of spectrin, actin, and band-4.1 free in the supernatant wash medium during the preparation of RBC membranes by stepwise hypotonic lysis of alpha thalassemic but not normal RBCs [1]. However, the significance of these abnormalities is uncertain since they suggest that the membrane would be unstable, while, as noted above, the alpha thalassemic membrane is hyperstable [21].

INEFFECTIVE ERYTHROPOIESIS — A number of observations suggest that it is the combination of ineffective erythropoiesis, a suboptimal increase in erythropoiesis, and peripheral hemolysis that leads to the anemia in HbH disease.

- Some older studies showed that the erythropoietic rate could increase up to six times normal in HbH disease [24,25]. However, ferrokinetic studies have shown that overall erythropoiesis was increased up to three times normal, while the red cell radioiron utilization rate, a measure of ineffective erythropoiesis, was slightly lower than normal at 75 percent (normal >80 percent) [49]. Since the rate of hemolysis in HbH is only increased three-fold, it is not clear why the marrow, which normally has a five-fold or greater capacity to increase erythropoiesis, does not do so.
- Other signs of increased erythroid activity include increases in serum soluble transferrin receptor (sTfR) and the reticulocyte production index [26,27]. The magnitude of these findings is proportional to the HbH level.

In HbH/CS, peripheral red cell destruction is increased, with a 51Cr red cell survival of 8.3 days (normal: 26 days); erythropoiesis is increased up to five times normal, and there is a modest component of ineffective erythropoiesis, with an iron reutilization level of 59 percent (normal >80 percent) [49].

In HbCS/CS, which should have the phenotype of an alpha thalassemia trait, subjects are anemic with hemoglobin values of 10.9 g/dL, a ^{51}Cr survival of 13.7 days, an erythropoietic rate three times normal, and a modest degree of ineffective erythropoiesis (iron reutilization rate 58 percent).

Several factors are thought to contribute to the ineffective erythropoiesis in HbH disease including excess beta globin chains, apoptosis (programmed cell death), and perhaps IgG deposition on the surface of erythroid precursors. Although the beta globin inclusions in HbH occur primarily in circulating red cells as they age [18], excess beta globin chains also may accumulate and precipitate in marrow erythroid precursors [50,51]. This deposition of insoluble beta globin could provide the basis for oxidant or other cellular injury leading to the intramedullary death of RBC precursors. The increase in ineffective erythropoiesis in HbH/CS and HbCS/CS is probably due to the binding of alphaCS, in addition to beta chains, to the membranes of erythroid precursors.

Ineffective erythropoiesis has been correlated with apoptosis of marrow erythroblasts in severe beta thalassemia, at rates which are about five times normal. (See "Pathophysiology of beta thalassemia", section on 'Apoptosis of red cell precursors'.) In fact, the extent of erythroid apoptosis correlates very closely with the extent of ineffective erythropoiesis in both alpha and beta thalassemia variants (figure 4) [49].

One of the consequences and hallmarks of apoptosis is the movement of phosphatidylserine (PS) from the inner to the outer leaflet of the membrane phospholipid bilayer. As mentioned above, the movement of PS to the outer leaflet may be one of the signals that promotes removal of thalassemic cells by the reticuloendothelial system. The deposition of IgG on the cell surface may provide another such signal. Erythroblasts from patients with HbH have excessive amounts of IgG on their surfaces, suggesting that this may contribute to the ineffective erythropoiesis [37].

HYDROPS FETALIS AND HEMOGLOBIN BART'S — Hydrops fetalis syndrome with hemoglobin Bart's is the most severe form of alpha thalassemia as there is no alpha globin chain production. Because alpha chains are produced after the fifth or sixth week of fetal life, affected fetuses have great difficulty in synthesizing a functional hemoglobin. Gamma chains accumulate and form gamma-4 tetramers (Hb Bart's); HbBart's binds oxygen but cannot release it to tissues because its affinity for oxygen is at least 10 times greater than HbA. Unlike HbH, HbBart's has little propensity to precipitate and form inclusions [52]. Similar abnormalities are seen with homozygous alpha globin gene deletion in mice; induction of a human alpha globin transgene can rescue these animals from perinatal death [53].

If the molecular defect allows the fetus to synthesize zeta chains, an embryonic variant of the alpha chain, then the fetus can make HbPortland (zeta 2 gamma 2) and survive in utero until the third trimester [54]. These fetuses are functionally severely anemic, despite the fact that the measured hemoglobin concentration may be as high as 10 g/dL due to the presence of HbBart's and HbPortland. The RBC are hypochromic and microcytic, and erythropoiesis is vastly expanded to compensate for the resulting profound tissue hypoxia, with compensatory extramedullary erythropoiesis in the liver and spleen. A small component of hemoglobin H (beta-4 tetramers) may also be present if the fetus can synthesize beta chains.

The severe functional anemia causes heart failure with anasarca and capillary leak ("hydrops"). The capillary leak may be caused by increased secretion of vascular endothelial growth factor (VEGF) by the stressed fetal heart [55]. Other defects include a massively enlarged placenta and severe maternal complications, such as hypertension and polyhydramnios.

Hydrops fetalis is incompatible with extrauterine life. However, antenatal diagnosis with transfusional and/or parental stem cell infusions followed by allogeneic bone marrow transplantation has resulted in some surviving patients [56,57].

SUMMARY — Thalassemia refers to a spectrum of diseases characterized by reduced or absent production of one (or rarely more) of the globin chains of hemoglobin. Alpha thalassemia is due to impaired production of alpha globin chains, which leads to a relative excess of gamma globin chains in the fetus and newborn, and beta globin chains in children and adults.

- In alpha thalassemia, excess beta globin chains are unstable and some precipitate within the cell, leading to a variety of laboratory findings (eg, hypochromic, microcytic red cells) and clinical manifestations (eg, anemia, ineffective erythropoiesis, decreased red cell deformability, hemolysis, oxidant sensitivity), depending upon how many of the four normally present alpha globin chain genes are deleted or mutated. (See 'Definitions' above and "Clinical manifestations and diagnosis of the thalassemias", section on 'The alpha thalassemia syndromes'.)

- Since all normal hemoglobins of postnatal life contain alpha chains, homozygous alpha (0) thalassemia (ie, loss of all four alpha globin genes) is incompatible with extrauterine life, leading to hydrops fetalis and/or death shortly after delivery, unless blood transfusion and/or hematopoietic cell transplantation can be performed in time. (See 'Hydrops fetalis and hemoglobin Bart's' above.)

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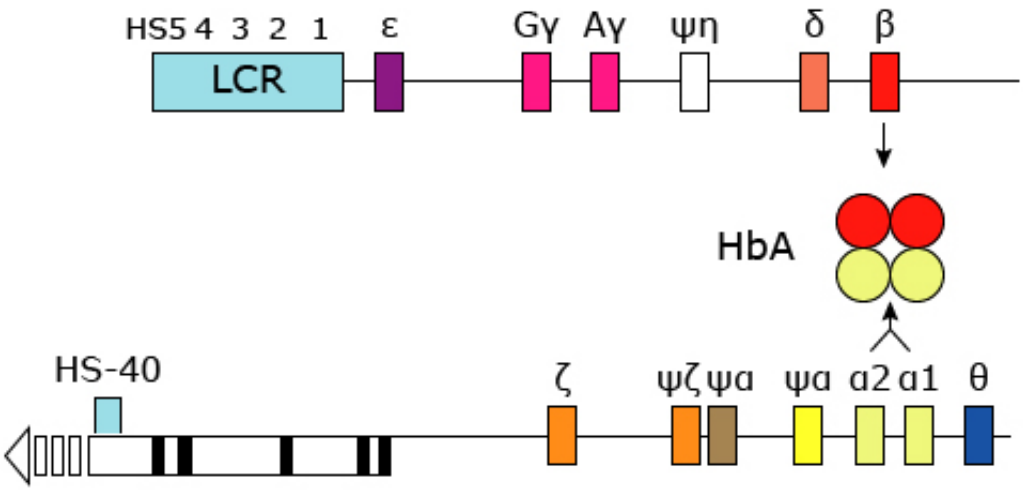
Topic 7103 Version 10.0

GRAPHICS

Alpha and beta globin gene clusters in man

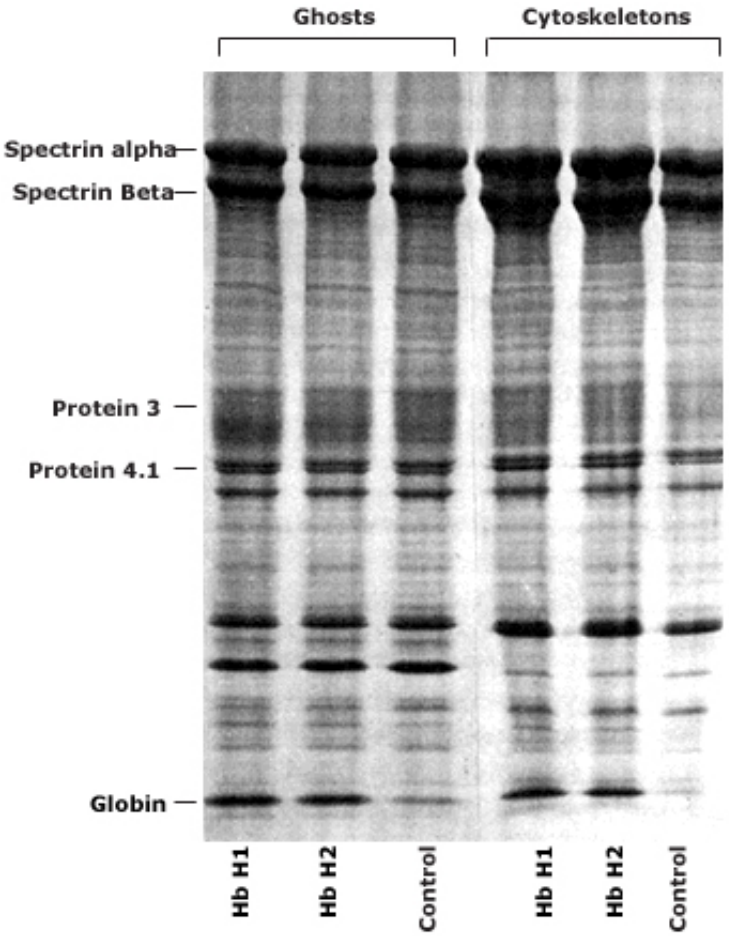


Schematic representation of the alpha (lower) and beta (upper) globin gene clusters in man, showing the position of the various genes, as well as the locus control region (LCR) for the beta globin gene cluster and the various hypersensitive sites for the beta globin gene cluster (HS 1 to 5) and the alpha gene cluster (HS-40). The only genes active after birth in the beta globin gene cluster are those for G(gamma), A(gamma), delta, and beta globin, while those active after birth in the alpha globin gene cluster are the two alpha genes (alpha 2 and alpha 1). The hemoglobin A tetramer (HbA) shown between the two clusters is formed from the products of the beta globin gene (on chromosome 11) and the two alpha globin genes (on chromosome 16).

Figure drawn by Dr. Ross Hardison and reproduced with permission from the Globin Gene Server, which can be found at: http://globin.cse.psu.edu.

Graphic 65642 Version 3.0

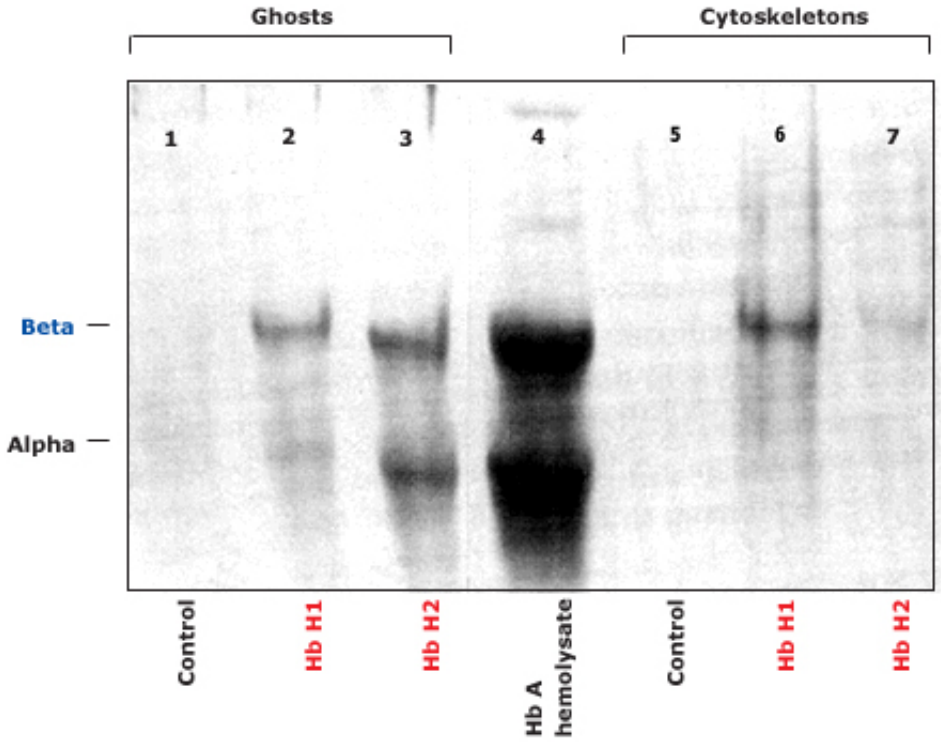
Globin bands attached to the red cell cytoskeleton in hemoglobin H disease



SDS-PAGE analysis of ghosts and cytoskeletons of red cells from two patients with hemoglobin H (HbH) disease and a normal control. A prominent globin band is seen in both the ghosts and cytoskeletons of the cells from patients with HbH disease.

Graphic 53915 Version 2.0

Beta globin attached to the red cell cytoskeleton in hemoglobin H disease



Red blood cell ghosts and cytoskeletons from patients with hemoglobin H (Hb H) disease analyzed by Triton acid urea gel electrophoresis are shown here; the reference standard is normal (Hb A) hemolysate (lane 4). Alpha and beta globin bands are not found on normal red cell ghosts (lane 1) or their cytoskeletons (lane 5). Although both beta and alpha globin bands are attached to the Hb H ghosts (lanes 2 and 3), only beta globin bands are attached to the cytoskeleton (lanes 6 and 7).

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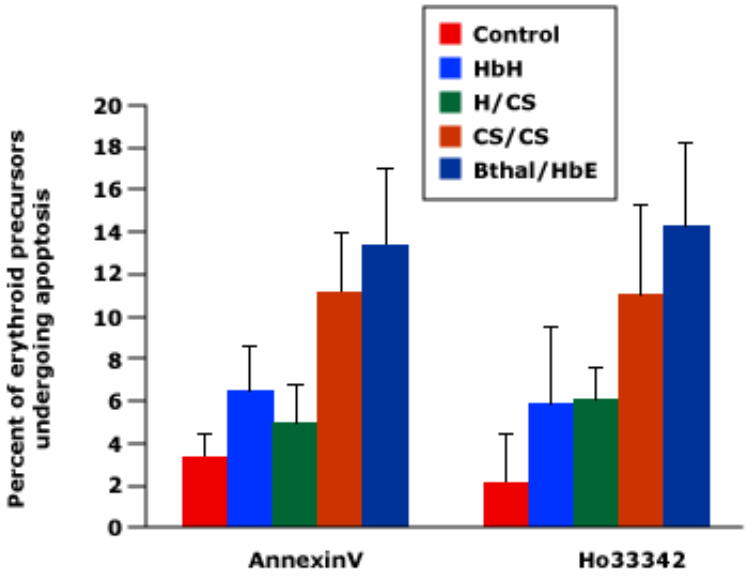
Graphic 63845 Version 2.0

Apoptosis in thalassemia variants



These graphs show the percentage of erythroid precursors undergoing apoptosis (mean ± 1 SD) in normal controls and in patients from Thailand with clinical diagnoses of α and β thalassemia, using both the annexin V and the Hoechst 33342 methodologies.

hbH: hemoglobin H disease; H/CS: hemoglobin H/hemoglobin constant spring; CS/CS: homozygous hemoglobin constant spring; bthal/hbE: beta thalassemia/hemoglobin E disease.

Graphic 65431 Version 1.0

Disclosures

Disclosures: **Stanley L Schrier, MD** Nothing to disclose. **William C Mentzer, MD** Equity Ownership/Stock Options: Johnson & Johnson [Anemia (Erythropoietin)]. **Donald H Mahoney, Jr, MD** Nothing to disclose. **Stephen A Landaw, MD, PhD** Employment (Spouse): Mass Medical Society (New England Journal of Medicine).

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