Pathophysiology of beta thalassemia

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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. There are two copies of the alpha globin gene on chromosome 16, while a single beta globin gene resides on chromosome 11 adjacent to genes encoding the beta-like globin chains, delta and gamma globin (figure 1). In normal subjects, globin chain synthesis is very tightly controlled such that the ratio of production of alpha to non-alpha chains is 1.00 ± 0.05. (See "Structure and function of normal human hemoglobins".)

Thalassemia refers to a spectrum of diseases characterized by reduced or absent production of one or more globin chains:

- **Beta thalassemia** is due to impaired production of beta globin chains, which leads to a relative excess of alpha globin chains. These excess alpha globin chains are unstable, incapable of forming soluble tetramers on their own, and precipitate within the cell, leading to a variety of clinical manifestations. The degree of alpha globin chain excess determines the severity of subsequent clinical manifestations, which are profound in patients homozygous for impaired beta globin synthesis and much less pronounced in heterozygotes who generally have minimal or mild anemia and no symptoms. (See "Clinical manifestations and diagnosis of the thalassemias".)

- **Alpha thalassemia**, in comparison, is due to impaired production of alpha globin chains, which leads to a relative excess of beta globin chains. (See "Pathophysiology of alpha thalassemia".) The toxicity of the excess beta globin chains in alpha thalassemia on the red cell membrane skeleton appears to be less than that of the excess partially oxidized alpha globin chains in beta thalassemia [1]. This probably explains why the clinical manifestations are generally less severe in alpha compared with beta thalassemia of comparable genetic severity (except for homozygous alpha (0) thalassemia, which is incompatible with extrauterine life, leading to hydrops fetalis and/or death shortly after delivery).

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

DEFINITIONS — The thalassemias are profoundly heterogeneous from a genetic standpoint [2]; however, certain clinical terms are available to describe the phenotypic expression of this disorder:

Beta (0) thalassemia — Beta (0) thalassemia refers to the more than 40 different genetic mutations of the beta globin locus that result in the **absence** of production of beta globin. Patients homozygous or doubly heterozygous for beta (0) thalassemic genes cannot make normal beta chains and thus are unable to make any hemoglobin A.

Beta (+) thalassemia — Beta (+) thalassemia refers to the more than 30 different genetic mutations which result in **decreased** production of beta globin. Patients homozygous for beta (+) thalassemic genes are able to make some hemoglobin A, and are generally less severely affected than those homozygous for beta (0) genes.

Beta thalassemia major — Beta thalassemia major is the term applied to patients who have either no effective production (as in homozygous beta (0) thalassemia) or severely limited production of beta globin. These are the patients originally described by Cooley (Cooley's anemia). Starting during the first year of life, they have profound and life-long transfusion-dependent anemia, hepatosplenomegaly, and skeletal deformities due to bone marrow expansion; they are prone to infection and skeletal fractures and, unless appropriately treated, die during adolescence of iron overload syndromes.

Beta thalassemia minor — Beta thalassemia minor, also called beta thalassemia trait, is the term applied to heterozygotes who have inherited a single gene leading to reduced beta globin production. Such patients are asymptomatic, may be only mildly anemic, and are usually discovered when a blood count has been obtained for other reasons.

Beta thalassemia intermedia — Beta thalassemia intermedia is the term applied to patients with disease of intermediate severity, such as those who are compound heterozygotes of two thalassemic variants. Such patients may have the skeletal abnormalities and hepatosplenomegaly seen in thalassemia major. However, their hemoglobin concentrations usually range from 5 to 10 g/dL and they usually require transfusions only when they have an intercurrent event, such as an infection, which impairs erythropoiesis. Their clinical symptoms may not be apparent until well after the first year of life.

Anemia — Anemia in beta thalassemia is generally caused by the presence of two interrelated phenomena: death of red blood cell (RBC) precursors within the bone marrow (ineffective erythropoiesis, also called intramedullary hemolysis) and increased destruction of circulating RBCs (hemolytic anemia). Each of these phenomena will be described separately, although both are ultimately attributable to unbalanced globin chain synthesis.

Protection against severe malaria — Thalassemic RBCs offer innate protection against severe malaria due to Plasmodium falciparum [3,4]. This effect is associated with reduced parasite multiplication within the RBCs, while parasite invasion is intact [5]. How this occurs is not well understood. Among the contributing factors in beta thalassemia may be the variable degree of persistence of hemoglobin F [4,6], which is relatively resistant to hemoglobin digestion by malarial hemoglobinases [7]. (See "Protection against malaria in the hemoglobinopathies".)

The degree of parasite growth inhibition is less pronounced in beta thalassemia than in alpha thalassemia [5]. The combination of this selective malaria advantage and the less severe clinical disease may account for the relatively high prevalence of alpha thalassemia (up to 90 percent) compared with beta thalassemia in southeast Asia and Papua New Guinea [5]. (See "Pathophysiology of alpha thalassemia".)

Yersinia infection — Infection with Yersinia enterocolitica is a significant cause of morbidity in patients with thalassemia [8,9] and other iron overload syndromes, such as chronic liver disease and hereditary hemochromatosis. Yersinia enterocolitica is a siderophoric (iron-loving) organism; it contains several pathways to facilitate iron-uptake, which is essential for its growth [10]. (See "Microbiology and pathogenesis of Yersinia infections".)

Iron chelation with desferrioxamine may make host iron more bioavailable to Yersinia; transfused red cells may also be a particularly rich source of iron for these organisms. As an example, 57 percent of the clinically apparent Yersinial infections in one series of thalassemic patients occurred within 10 days of blood transfusion [9]. Recognition of these associations and unusual manifestations in these patients, such as an appendicitis-like syndrome, may direct clinicians to earlier anti-yersinial therapy, along with temporary cessation of chelation [8,11]. (See "Clinical manifestations and diagnosis of Yersinia infections".)

HEMOLYTIC ANEMIA — By the classic hematologist's yardstick, hemolysis in beta thalassemia is intracorpuscular (ie, due to abnormalities primarily within the red cell) and extravascular (ie, occurring in the monocyte-macrophage system); as a result, there is little hemoglobinemia or hemoglobinuria. In addition to the presence of abnormal RBC size due to decreased overall hemoglobin production (ie, hypochromia and microcytosis), there are, depending upon the phenotype, grossly abnormal RBC shapes including target cells, tear-

drop cells (dacrocytes), fragmented forms, echinocytes, and the presence of RBC inclusions (picture 1).

A breakthrough in our understanding of the hemolysis in beta thalassemia occurred in 1963 when Fessas noted "large, single inclusions" in beta thalassemia major normoblasts and circulating RBCs [12]. The inclusions were fewer in number in mature RBCs and increased in number in RBCs from splenectomized patients.

Fessas proposed that these inclusions, which stained like Heinz bodies, were precipitated hemoglobin, and postulated that they were excess alpha globin chains [12]. Consistent with this hypothesis was the subsequent demonstration that the hemolysate of RBCs from these patients contained free alpha globin chains [13]. The importance of this imbalance in globin chain synthesis in the development of hemolysis in patients with beta thalassemia can be illustrated by the following observations:

- The shortening of 51Cr-labeled RBC survival (ie, the extent of hemolysis) is directly correlated with the degree of alpha globin chain excess in the RBCs [14].
- Subjects with beta thalassemia trait (ie, heterozygotes) are, as noted above, generally asymptomatic. However, patients with beta thalassemia trait who also have six (instead of the normal four) alpha globin genes, leading to a greater excess of alpha globin chains, develop a more severe phenotype (beta thalassemia intermedia, see above) [15].

The driving hypothesis is that excess alpha globin chains, either in soluble form or more likely when they aggregate, produce the RBC changes that lead to hemolysis. Such changes could then affect the material properties of RBCs that allow cells that are 7 to 8 microns in diameter to elongate, tank tread, and otherwise deform to pass through 3 micron diameter capillaries and 2 micron slits in the reticuloendothelial sinusoids. (See "Red blood cell mechanics".) Alternatively, but not exclusively, the surface of the RBCs could be altered to provide signal recognition by macrophages, leading to removal of the affected RBC.

Oxidant injury — Much of the pathophysiology of the thalassemias is thought to be due to oxidant injury. In normal RBC, hemoglobin is oxidized to methemoglobin at a rate of about 0.5 to 3 percent per day; the methemoglobin thus formed is subsequently reduced back to native hemoglobin via cytochrome b5 reductase (formerly called methemoglobin reductase) [16]. (See "Genetics and pathogenesis of methemoglobinemia".)

However, isolated alpha and beta chains are susceptible to oxidation to hemichromes, some of which are irreversibly modified [17-19]. Furthermore, while free beta chains can remain in soluble form and are capable of producing tetramers (Hemoglobin H), free alpha chains are unstable and aggregate and precipitate adjacent to the RBC membrane, where they are more likely to form irreversible hemichromes [20].

These iron containing hemichromes are capable of generating reactive oxygen species (ROS), perhaps by acting as Fenton reagents [21]. These ROS can, in turn, oxidize adjacent RBC membrane proteins and lipids [22-24]. In fact, in beta thalassemia intermedia, increased amounts of membrane iron have been found in association with denatured hemoglobin and hemichromes [25]. (See "Approach to the patient with suspected iron overload", section on 'Iron toxicity'.)

In one study, the majority of membrane-bound iron was in the "free" or non-heme form, which was especially prominent in splenectomized patients [26]. This latter observation suggests that splenic hemolysis in these patients may be mediated via "free" iron-driven oxidative mechanisms, and might be abated through use of membrane-permeable chelating agents [27]. However, the critical clinical studies to test this have not yet been done. (See "Iron overload syndromes other than hereditary hemochromatosis".)

Non-transferrin bound iron — As the body burden of iron increases, the saturation of circulating transferrin with iron increases and serum ferritin levels increase, as does the production of increased amounts of nontransferrin bound iron (NTBI, labile plasma iron, ie, iron complexed with citrate, acetate, albumin and other plasma components). This results in the off-loading of iron into tissues, especially to those cells with high levels of transferrin receptors (eg, heart, liver, thyroid, gonads, and pancreatic islets), with resulting tissue damage. (See "Approach to the patient with suspected iron overload", section on 'Iron toxicity'.)

NTBI is not a readily available assay and techniques for its determination have not been standardized. However, a transferrin saturation greater than 60 to 70 percent (the upper limit of normal for transferrin saturation is 45 percent in females and 50 percent in males) may be as useful in determining the potential for iron toxicity in patients with iron overload, including that due to multiple transfusions (eg, sickle cell disease), increased iron absorption (eg, hereditary hemochromatosis), ineffective erythropoiesis (eg, thalassemia and the sideroblastic anemias), and low utilization of transferrin-bound iron (eg, Diamond-Blackfan anemia) [28,29]. Serial measurement of NTBI, when available, might have some use as an indicator of iron chelator activity in these patients [30].

The former issue was explored in a study of 174 patients with thalassemia major or intermedia who were receiving regular transfusion and chelation therapy. NTBI was detected in 83 percent [31]. All patients with cardiac disease had transferrin saturations >70 percent and all were NTBI positive. Conversely, of the patients who were NTBI negative and/or had transferrin saturations <70 percent, none had preclinical or clinical cardiac disease.

In a separate study in 74 patients with thalassemia intermedia who had never been transfused or treated with iron chelating agents, mean levels of NTBI, serum ferritin, and liver iron concentration were above the reference range, indicating that many patients with thalassemia intermedia will be at risk of significant iron-related morbidity [32].

The use of transfusions of exogenous human transferrin normalized NTBI levels in an animal model of thalassemia intermedia and reversed or markedly improved their thalassemic phenotype [33].

Material properties of thalassemic RBC — Two observations provided the initial rationale for characterization of the material properties of beta thalassemic RBCs: the RBC shapes were grossly abnormal; and the RBCs demonstrated impaired filterability (a reflection of reduced deformability), which correlated with the observed shortening of in vivo RBC survival [34].

RBC deformability — There are three major determinants of RBC deformability (see "Red blood cell mechanics"):

- The ratio of RBC surface area to volume (SA/V), with a higher ratio facilitating deformation and a lower ratio leading to reduced deformability and splenic trapping.
- Cytoplasmic viscosity, which is primarily regulated by the mean corpuscular hemoglobin concentration (MCHC). Normal red cell hemoglobin concentrations of 27 to 35 g/dL have negligible effects on cytoplasmic viscosity; however, viscosity rises exponentially at values above 37 g/dL as would occur when there is significant RBC dehydration.
- Membrane deformability, which is regulated by the viscoelastic properties of the red cell's lipid bilayer and its underlying cytoskeleton. (See "Red blood cell membrane dynamics and organization".) Poorly deformable RBCs (ie, rigid RBCs) are unable to traverse the splenic cords, and are destroyed by components of the monocyte-macrophage system.

In beta thalassemia, the SA/V ratio is greater than normal but surprisingly, given the decreased amount of hemoglobin per red cell, there are also populations of dense dehydrated thalassemic RBCs with increased MCHC (see below) [35]. Furthermore, even at normal MCHC values, there is an as yet unexplained strong interaction of hemoglobin A (when present) with the membrane of the thalassemic RBC that also contributes to RBC rigidity [35]. This effect is more prominent in RBCs of splenectomized subjects, suggesting that populations of RBCs with these characteristics are selectively removed by the spleen.

There is increased rigidity (ie, reduced deformability) of RBCs from both nonsplenectomized and splenectomized patients with beta thalassemia intermedia [35,36].

- Red cells from nonsplenectomized patients show reduced deformability despite the fact that the SA/V is increased. Both an interaction of the excess alpha globin chains with the membrane and a component of membrane impairment appear to contribute to the reduction in deformability.
- Red cells from splenectomized subjects are even more rigid since the most abnormal cells, which are

ordinarily removed by the spleen, are still in the circulation [35]. The increase in RBC rigidity is due in part to a greater degree of hemoglobin interaction with the membrane in these RBCs, which occurs at a normal MCHC of about 33 g/dL. This is in striking contrast to the finding in sickle cell anemia, where increased hemoglobin viscosity and interaction between the hemoglobin molecule and the RBC membrane occur only at pathologically high MCHCs in the high 30s and low 40s [37].

Reduced membrane deformability — The preceding observations suggest a major role for a membrane defect in the impaired deformability of beta thalassemic red cells. In the above studies, isolated RBC membranes in nonsplenectomized patients were 2.5 to 3 times more rigid than normal [35]. Even in beta thalassemia trait, membrane rigidity is directly correlated with the amount of complex formation between hemoglobin and the major membrane protein spectrin, which in turn is dependent upon increased MCHC and oxidative injury (see below) [37].

Membrane skeletons prepared from beta thalassemic RBCs are grossly abnormal by scanning and transmission electron microscopy, and contain an increased content of globin [38,39]. This skeletal-bound globin is virtually all alpha globin in partially oxidized form [40].

The probable explanation for the increase in membrane rigidity in beta thalassemia rests on the changes produced by binding of partially oxidized alpha globin chains to the membrane cytoskeleton. These changes could be secondary to nonspecific mass effects or the consequences of oxidant attack (see below). Detailed analyses of RBC membrane cytoskeletons have shown that they retain about twice as much of the major integral membrane protein, band 3 protein, as controls [38]. This interaction between band 3 and the cytoskeleton lattice probably contributes to the increased membrane rigidity as is the case when another integral membrane protein, glycophorin A, is induced to bind to the membrane skeleton [41]. (See "Control of red blood cell hydration".)

In addition to the correlations between RBC abnormalities and the degree of alpha globin chain excess in beta thalassemia [14,15,42], there is direct in vitro support for the role of aggregated alpha globin chains in producing both RBC and membrane rigidity. When purified alpha globin chains are resealed within normal RBCs and then incubated for about 20 hours, there is a progressive rise in membrane-associated alpha globin chains accompanied by increased rigidity of the RBCs and their isolated membranes [43,44].

Membrane instability — Membranes from RBCs of splenectomized thalassemic subjects are very unstable and fragment readily, whereas membranes from nonsplenectomized thalassemic subjects may be only slightly unstable [35]. This difference can presumably be explained by splenic removal of RBCs with the most unstable membranes, which are also those most likely to undergo fragmentation. Thus, in the postsplenectomy state, many fragmented RBC with unstable membranes survive [35].

Membrane protein abnormalities — Protein 4.1, a key component of the skeleton, is defective in beta thalassemia intermedia with a 50 percent reduction in the ability of this protein to mediate the critical formation of the spectrin-protein 4.1-actin trimeric complex [45]. A clue to the abnormal function of beta thalassemic protein 4.1 came from studies showing that it had undergone partial oxidation [40].

Some studies [46] but not others [45] have noted a defect in the formation of spectrin tetramers from spectrin dimers in beta thalassemic RBCs, a step that is critical for the development of the skeletal lattice. The entire skeletal lattice may be somewhat reduced in density since small amounts of spectrin, actin, and protein 4.1 are found in the first lysate of a preparation of hypotonic RBC membranes [1]. This decrease in membrane spectrin is directly correlated with the amount of insoluble alpha globin chains and is associated with a decrease in membrane ankyrin (band 2.1), a protein that links the skeleton to band 3 [42]. These findings suggest that beta thalassemic membrane skeletal proteins are not assembled normally in severe beta thalassemia, being unstably attached due perhaps to the membrane association of partially oxidized alpha globin chains (see above) [1].

These changes are associated with reduced membrane thiol content, thus providing evidence of oxidation of membrane proteins (see below) [43,44,47], it has been proposed that the iron, heme, and hemichromes associated with the alpha globin chains produce oxidant damage (see above) [47]. The demonstration in a murine model of beta thalassemia intermedia that removal of membrane bound iron improves the abnormalities in RBC survival,

deformability, and hydration is compatible with a role for iron in the oxidant injury (see below) [48].

In addition, the active RBC and membrane proteases in beta thalassemic RBCs could contribute by degrading some membrane proteins. This subject is not written about explicitly, but investigators working with beta thalassemic RBC membranes quickly learn to add protease inhibitors to virtually all of the preparatory steps in separating thalassemic RBC membranes in order to avoid the multiple electrophoretic bands indicative of protease action [1]. The calcium content of beta thalassemia intermedia RBCs is markedly increased [49], a condition known to activate RBC proteases [50]. Although much of this calcium appears to be trapped in inside-out endocytic vesicles [51], small but sufficient amounts may be available to stimulate these proteases.

Red blood cell dehydration — The state of RBC hydration plays an important role in determining its deformability characteristics. As noted above, the intracellular hemoglobin concentration is one of the major determinants of cytoplasmic viscosity. Beta thalassemic RBCs uniformly have a low hemoglobin content, even in the asymptomatic heterozygous state [52]. Thus, it was unexpected to find in beta thalassemia heterozygotes that 10 percent of RBCs had an MCHC greater than 37 g/dL and 2 percent had an MCHC greater than 45 g/dL [37]. In addition, when specific cell density gradient profiles were performed on beta thalassemia intermedia RBCs, there were surprisingly dense populations in both nonsplenectomized and splenectomized subjects (figure 2) [35].

Red cell volume is controlled by a complex interplay between pores, channels, and pumps (figure 3) [53]. One important volume controller is the K-Cl cotransport system which normally functions in reticulocytes and appears to be involved in the remodeling process by which reticulocytes, with a mean corpuscular volume (MCV) of about 110 fL lose volume and attain the MCV characteristic of mature RBCs (85 to 95 fL). This cotransporter is appropriately activated by an increase in volume, resulting in potassium efflux with volume loss. However, it is inappropriately activated in RBCs containing Hb S or Hb C, leading to KCl efflux followed by osmotic water loss and cellular dehydration [54]. (See "Control of red blood cell hydration", section on 'K-Cl cotransport'.)

The K-Cl cotransport system is also activated beyond the reticulocyte stage in patients with beta thalassemia intermedia, which accounts in part for the cellular dehydration [35,54]. The onset of RBC dehydration could occur as a consequence of alterations developing during the circulation of RBCs or be induced during intramedullary erythropoiesis. When this question was studied, it became apparent that there were populations of reticulocytes that were already profoundly dehydrated in beta thalassemia intermedia. Thus, events within the marrow triggered by alpha globin chain accumulation produce lesions that lead to RBC dehydration.

Increased destruction by macrophages — The peripheral hemolysis in beta thalassemia is extravascular, occurring in macrophages of the reticuloendothelial (monocyte-macrophage) system. This observation provided the rationale for studies designed to determine the extent of macrophagic attack on beta thalassemic RBCs, the status of beta thalassemic macrophages, and the signals used by the macrophage to identify, retard, trap, and engulf thalassemic RBCs. As an example, an early study with mouse peritoneal macrophages found a 22-fold and 4-fold increase in phagocytosis of beta thalassemia intermedia RBCs from splenectomized and nonsplenectomized subjects, respectively, as compared with RBCs from normal controls [55].

It has been suggested that the 25 percent reduction in surface sialic acid levels in beta thalassemia intermedia RBCs [56] uncovers sites on the membrane that can be detected by mouse macrophages [55]. These uncovered sites also may promote antibody formation. Increased amounts of IgG are commonly seen on the red cell surface in patients with beta thalassemia [57]. Some of these IgG molecules are autoreactive and interact with terminal galactosyl residues which may have been newly exposed because of the loss of sialic acid.

Another change that may be important for macrophage attack is oxidative damage to the beta thalassemic RBC membrane produced by hemichromes, heme, and iron in the excess alpha globin chains. This type of injury can result in covalent crosslinking of band 3 to form dimers and tetramers which aggregate to form clusters [58]. These clusters of band 3 can form complexes with hemichromes and cell surface IgG and C3 [58], which are well known signals to macrophages [58,59]. It is presumed that the IgG and C3 recognize neoantigens produced by the hemichrome-mediated oxidative attack on band 3 [59].

An interesting observation is that the monocytes in patients with a variant of beta thalassemia, beta (0) thalassemia/Hb E, are more active than normal in attaching to (3-fold higher) and ingesting (30 percent higher) normal Rh-positive RBCs coated with anti-D IgG [59]. One possible explanation for this enhanced monocyte-macrophage activity in beta thalassemia is the presence of increased levels of cytokines, particularly tumor necrosis factor [60].

Membrane phospholipid phosphatidylserine (PS) is also recognized by macrophages as a signal for attachment and phagocytosis [61]. PS is confined to the inner half of the membrane phospholipid bilayer of normal RBCs and therefore does not provide a signal to macrophages. (See "Red blood cell membrane dynamics and organization".) However in some beta thalassemic RBCs, the membrane phospholipid bilayer is scrambled in such a way that some PS moves to the outer leaflet [62,63]. The number of such cells varies from less than 0.2 percent (similar to normals) to as much as 20 percent [63]. Some of this loss of membrane organization appears to be caused by oxidative damage induced by alpha globin chain aggregates [63].

HYPERCOAGULABLE STATE — Several experienced clinicians believe that beta thalassemia intermedia and beta thalassemia major may be thrombophilic disorders, with an increased frequency of deep vein thrombosis and pulmonary embolism [64-66]. Blood levels of procoagulant activation peptides are somewhat increased in these patients; the underlying pathophysiology may relate to the affected RBCs expressing PS on their outer leaflet. Platelet PS forms a nidus for thrombosis via tenase activation, and RBC PS may have a similar role [67]. (See "Overview of hemostasis", section on 'Multicomponent complexes'.)

INEFFECTIVE ERYTHROPOIESIS — The second process leading to anemia in beta thalassemia is the increased destruction of erythroid precursors within the sites of RBC production, a process called ineffective erythropoiesis or intramedullary hemolysis. It is presumed that the accumulation of excess alpha globin chains, either soluble or more likely in aggregated precipitated forms, is the proximate cause for the intramedullary death of RBC precursors [68].

Abnormal erythrokinetics — For much of the 1900s, up until the use of kinetic studies, beta thalassemia was defined as a hereditary hemolytic disease. What remained unexplained in patients with homozygous beta thalassemia was the discrepancy between the extreme severity of the anemia, the extraordinary increase in extramedullary erythropoiesis, and the relatively modest reticulocytosis.

These discrepancies were resolved when isotopic studies demonstrated enormous degrees of ineffective erythropoiesis in severe beta thalassemia. Ferrokinetic analysis revealed that as little as 15 percent (compared with 75 to 90 percent in normal subjects) of the radiolabeled iron entering the bone marrow compartment appeared in circulating RBCs 7 to 10 days later [69]. These findings indicated that ineffective erythropoiesis could account for as much as 60 to 75 percent of total erythropoiesis in beta thalassemia major.

As mentioned above, the presence of subpopulations of profoundly dehydrated reticulocytes indicates that the defect in volume control occurred during erythropoiesis. Studies described below focus on observations identifying events occurring during erythropoiesis that play a major role in the pathophysiology of this disease.

Morphologic changes in RBC precursors — Ultrastructural studies have revealed a number of abnormalities in orthochromic normoblasts in patients with Cooley's anemia. These include glycogen accumulation, plasma membrane enfolding, vacuole formation, hyaline figures, and Heinz bodies [70]. Polychromatophilic and orthochromic erythroblasts also contain cytoplasmic aggregates of electron dense material [71] which, on the basis of biochemical analysis, are thought to be aggregates of alpha globin chains [72]. Final proof that the aggregates are alpha globin chains comes from studies using anti-alpha globin monoclonal antibodies combined with laser confocal fluorescence microscopy [73-75].

These studies also showed the heterogeneous presence of large aggregates of alpha globin chains, occurring as early as the proerythroblast stage [70,71,76]. The causes for the heterogeneity of this alpha globin deposition are largely unresolved. An initial hypothesis suggested that the erythroid precursors that were capable of turning on fetal programs, thereby synthesizing gamma chains, would be protected from the deposition of excess alpha globin

chains. However, this hypothesis was disproved by studies using monoclonal anti-gamma chain antibodies in which erythroid precursors with large amounts of alpha globin deposits also had large amounts of gamma chains [77].

Beta thalassemic erythroid precursors containing apparent alpha globin chain precipitates also have reduced protein synthetic capacity [78]. Iron loaded mitochondria are also seen [76], paralleling the ringed sideroblasts that occur in the myelodysplastic syndromes in which ineffective erythropoiesis is also present. The relationship between these observations and the presence of ineffective erythropoiesis is not well understood.

Altered assembly of membrane proteins — In normal erythroid precursors, the membrane skeleton proteins spectrin and band 4.1 and the major transmembrane protein band 3 are generally incorporated smoothly and in an orderly fashion into the cell membrane. (See "Red blood cell membrane dynamics and organization".) In contrast, in the erythroid precursors of patients with Cooley's anemia, the incorporation of spectrin and particularly band 4.1 is frequently disorderly, and discontinuous [73]. When double immunofluorescence was performed using antibodies to alpha globin chains, spectrin, and band 4.1, there was clear evidence of colocalization of alpha globin chain aggregates with abnormal areas of incorporation of spectrin and, to a greater degree, band 4.1 into the membrane skeleton. These findings suggest that alpha globin chain aggregates damage adjacent skeletal proteins, either by mass action or perhaps by the oxidant injury induced by the associated iron, heme, or hemichromes.

This study also provided evidence for a decrease in band 3 incorporation into beta thalassemic erythroid precursor membranes at the proerythroblast and basophilic erythroblast stages [73]. In comparison, band 3 incorporation was essentially normal in polychromatophilic and orthochromic erythroblasts. Band 3 incorporation, when present, always occurred in a smooth, regular pattern and never colocalized with alpha globin chain aggregates. Thus, the deficit in band 3 incorporation that occurs early in erythropoiesis is either surmounted with further maturation or the erythroid precursors that are deficient in band 3 die [73].

Apoptosis of red cell precursors — The intramedullary cell death of up to 60 to 75 percent of erythroid precursors in thalassemia as detected by ferrokinetic analysis led to a conceptual problem. Such a large number of cells dying by necrosis should leave a large morphologically detectable graveyard of erythroid precursors. The absence of such a finding led to the consideration that apoptosis or programmed cell death could be playing a major role in the ineffective erythropoiesis.

Subsequent studies confirmed this hypothesis in patients with thalassemia by demonstrating accelerated or enhanced apoptosis in erythroid precursors as detected by DNA ladder formation [75], alterations in DNA [79,80], increased Hoechst dye 33342 staining [81], and the movement of phosphatidylserine (PS) to the membrane outer leaflet [79-81]. The degree of apoptosis was found to be strongly correlated with the extent of erythroid expansion, as measured by the absolute number of marrow erythroid precursors and also by serum concentrations of soluble transferrin receptor [81]. (See "Pathophysiology of alpha thalassemia", section on 'Ineffective erythropoiesis'.)

The role of apoptosis in patients with HBE/beta thalassemia was substantiated by a study that analyzed phosphoproteins in stem cells (HSC) from affected patients. A significant change in 229 phosphoproteins was identified, including an abundance of proteins involved in apoptosis, such as cytochrome C and caspase-6 [82].

Cells undergoing apoptosis seem to transmit this fact to neighboring macrophages and are expeditiously removed [83]. One of the signals is thought to be the apoptosis-associated movement of PS to the outer leaflet. As mentioned above, PS on the outer leaflet is also thought to contribute to hemolysis by the same mechanism of providing a signal for macrophagic attack.

DISEASE HETEROGENEITY IN HEMOGLOBIN E/BETA THALASSEMIA — It has been estimated that, over the next 30 years, there will be 100,000 new cases of Hb E/beta thalassemia [84]. This double heterozygosity for two relatively mild traits produces remarkable clinical heterogeneity, with hemoglobin concentrations ranging from 2.6 to 13.5 g/dL (average 7.7 g/dL); it occurs at a frequency of 3 to 9 percent in Thailand [85,86]. A clinical severity score for Hb E/beta thalassemia (Mahidol score) has been developed based upon steady-state hemoglobin, age of onset, requirement for and age at first red cell transfusion, splenic size, and presence of growth retardation [86].

Hb E/beta thalassemia is especially severe in Sri Lanka, where individuals in one study of 69 subjects (mean age 26.6 years) had either beta (0) or severe beta (+) thalassemia mutations in addition to heterozygosity for Hb E [87]. Mean hemoglobin levels were 6.2 g/dL (range: 4.4 to 8.3) and subjects had received a mean of 48 transfusions (range: none to 143). Iron overload was present, with mean ferritin levels of 947 ng/mL (controls: 48 ng/mL), mean liver iron concentrations (LIC) of 7.2 mg Fe/g dry weight (normal <2), and extremely low hepcidin levels (mean 2.1 ng/mL; controls: 28). Even the 23 subjects who had received less than 20 units of red cells had evidence for severe iron overload, with a mean LIC of 8.7 mg Fe/g dry liver weight.

The pathophysiology of this heterogeneity and its severity remain a puzzle [88]. If, as suggested by the evidence cited above, the major problem in beta thalassemia is accumulation of excess unmatched alpha globin chains, then patients with beta (0) thalassemia should be clinically worse than patients with beta (+) thalassemia, and there is a general trend in this direction [89,90]. Similarly, coinheritance of alpha thalassemia and homozygosity for a beta thalassemic chromosome with Xmn1 cleavage site at position -158 of the G gamma globin gene, which is associated with somewhat higher levels of Hb F, should (and does) lessen the severity of the disease [89].

Hemoglobin E is mildly oxidatively unstable [91,92]. This observation has led to the suggestion that susceptibility to oxidative attack may play a role in the disease variability and its unexpected severity. Red cells from these patients show evidence of oxidative stress (eg, elevated values of superoxide dismutase, glutathione peroxidase, and methemoglobin), reduced serum concentrations of vitamins E and C, and also show loss of phospholipid membrane asymmetry [92-94]. However, none of these findings seems to provide a sufficient explanation for the variability and severity, although a high incidence of infection with Plasmodium vivax has been noted in a pilot study of patients with this disorder from Sri Lanka [95]. (See "Molecular pathology of the thalassemic syndromes", section on 'Hb E: A special case'.)

A group of Thai investigators have proposed that the severity of this variant might be correlated with the splicing process of beta E pre-MRNA, with a high ratio of correctly to aberrantly spliced MRNA being seen in 30 percent of the milder cases [96].

LESSONS FROM A MURINE MODEL OF BETA THALASSEMIA — The preceding observations are testaments to our understanding, or lack of understanding, of the pathophysiology of beta thalassemia and its variants. It has been difficult to pinpoint some of the critical pathophysiologic events in human beta thalassemia because of the complex genetic and environmental background of these patients. As an example, many patients inherit complex combinations of beta thalassemia along with hemoglobinopathies (such as Hb E) and alpha thalassemia. In addition, patients in countries where beta thalassemia is prevalent may be exposed to malaria and other infectious agents that can contribute to the anemia [89].

For these reasons, there are advantages to the study of inbred murine strains of beta thalassemia. One strain — the Hbbth-1 mouse — has a deletion of the beta major globin gene and, in the homozygous state, produces only beta minor globin and has a phenotype similar to that of human beta thalassemia intermedia. It shares the major features of the human disease [97]:

- Increased RBC and RBC membrane rigidity
- Reduced membrane stability
- Evidence of RBC dehydration
- Binding of alpha globin chains to the RBC membrane skeleton
- A strong correlation between the amount of membrane-bound alpha globin and both membrane rigidity and severity of the anemia
- Abnormal assembly of the membrane proteins 4.1 and spectrin in erythroid precursors [98].

Binding of oxidized alpha globin chains to the RBC membrane and a contribution of membrane bound iron to oxidative injury of the membrane also appear to be important in the murine model as illustrated by the following observations:

- The extent of oxidation of membrane bound alpha globin correlates directly with membrane instability [99].
- Removal of RBC membrane iron with the chelator deferiprone improves RBC survival, deformability, and hydration and reduces the extent of oxidation of membrane thiols [48].

SUMMARY — Beta thalassemia is an inherited disorder due to impaired production of beta globin chains. This leads to a profound excess of highly unstable alpha globin chains in patients homozygous for impaired beta globin chain production. The following abnormalities in red cell precursors and circulating red cells follow from such accumulations of excess alpha chains:

- Death of developing red cell precursors within the bone marrow (ie, ineffective erythropoiesis). (See 'Ineffective erythropoiesis' above.)
- Increased destruction of circulating red cells (ie, increased hemolysis). (See 'Hemolytic anemia' above.)
- The net result of these two processes is a severe degree of anemia, associated with markedly increased erythropoiesis, with resulting bone marrow expansion, extramedullary hematopoiesis, hepatomegaly and splenomegaly, and a host of adverse clinical sequelae. (See "Clinical manifestations and diagnosis of the thalassemias", section on 'Clinical manifestations'.)

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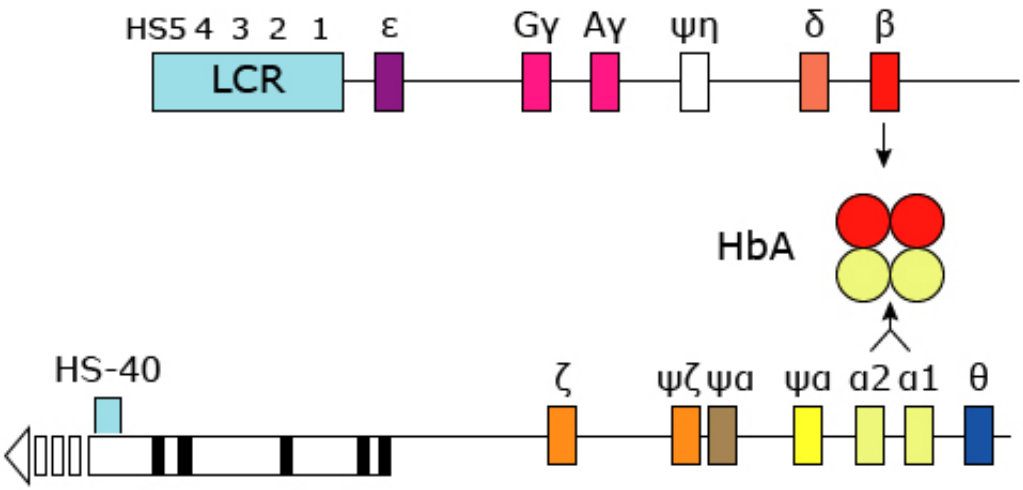
Topic 7090 Version 14.0

GRAPHICS

Alpha and beta globin gene clusters in man

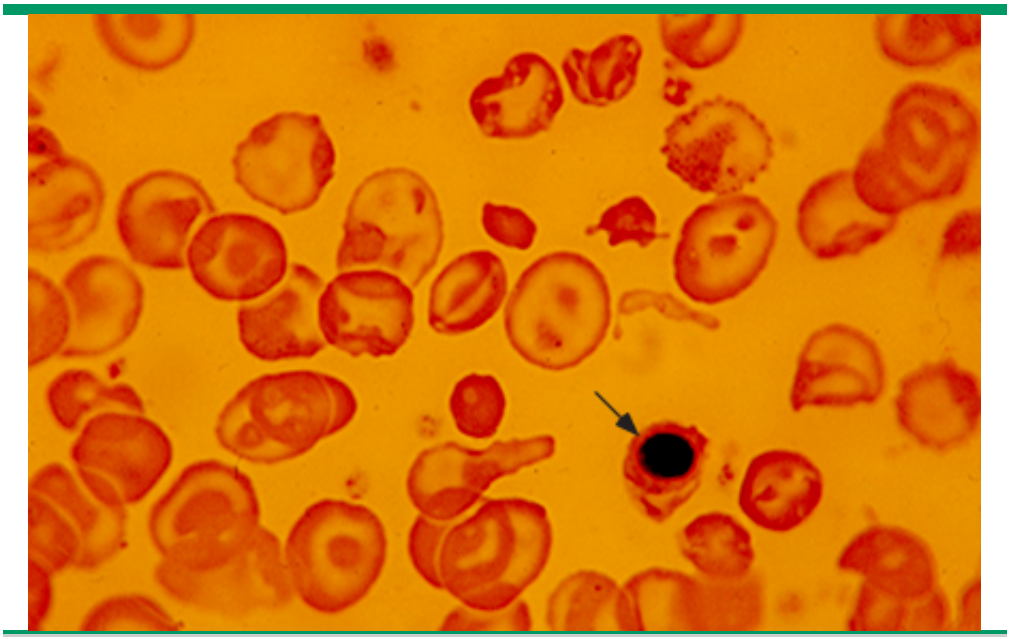


Schematic representation of the alpha (lower) and beta (upper) globin gene clusters in man, showing the position of the various genes, as well as the locus control region (LCR) for the beta globin gene cluster and the various hypersensitive sites for the beta globin gene cluster (HS 1 to 5) and the alpha 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

Peripheral blood smear in beta thalassemia intermedia

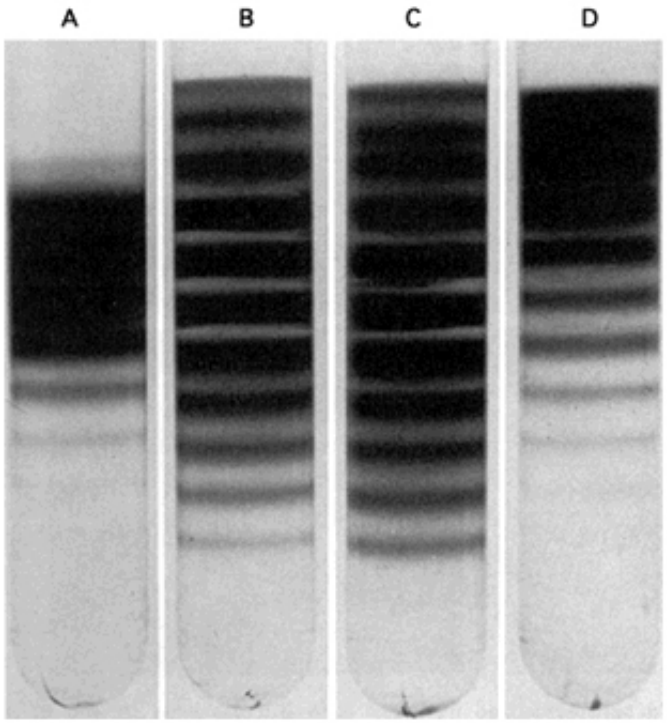


Peripheral smear from a patient with beta thalassemia intermedia post-splenectomy. This field shows target cells, hypochromic cells, microcytic cells, red cell fragments, red cells with bizarre shapes, and a single nucleated red cell (arrow).

Courtesy of Stanley Schrier, MD.

Graphic 76666 Version 2.0

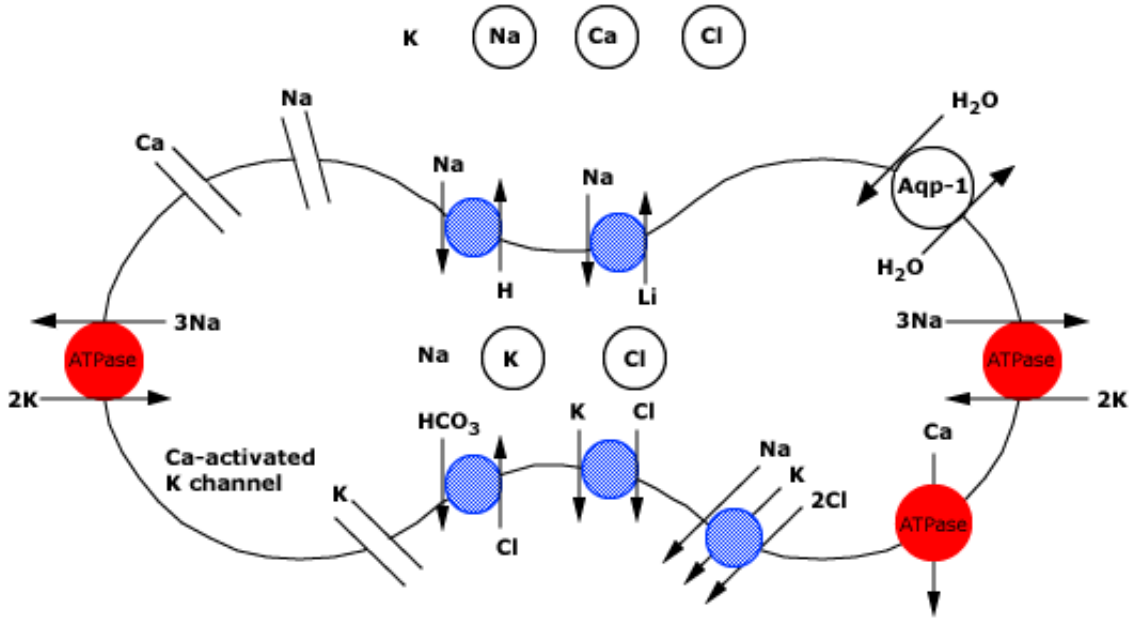
Dense red cells in beta thalassemia



Analysis of the density distribution of normal erythrocytes (A), erythrocytes from a nonsplenectomized individual with beta-thalassemia intermedia (B), erythrocytes from a splenectomized individual with beta thalassemia intermedia (C), and HbH erythrocytes (D) on discontinuous Stractan density gradients. The more dense cells are at the bottom of the gradient; dense populations are present only in the nonsplenectomized and splenectomized subjects with beta thalassemia.

Graphic 60097 Version 1.0

Red blood cell ion transport mechanisms



Schematic representation of the transport mechanisms regulating red cell hydration. The extracellular concentrations of sodium and calcium are higher than those within the cell, creating favorable gradients for entry, while the intracellular concentration of potassium is higher than that in the extracellular fluid, creating a favorable gradient for potassium exit by the K-Cl cotransporter or the calcium-activated (Gardos) potassium channel. The red transporters are active, the blue transporters are passive. Band 3 protein primarily functions as a Cl-HCO3 exchanger. Its primary physiological function is to facilitate CO2 transport from tissues to alveoli; it also plays an important role in defining red cell shape and membrane stability. Water movement passively follows that of cations and anions, or changes in tonicity of the red cell's environment. Transport of water can occur at a much faster rate via water channels (aquaporin-1, Aqp-1).

Graphic 62852 Version 3.0

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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