Hemolytic anemias

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Introduction

Hemolysis is the accelerated destruction of red blood cells (RBCs), leading to decreased RBC survival. The bone marrow's response to hemolysis is increased erythropoiesis, reflected by reticulocytosis. If the rate of hemolysis is modest and the bone marrow is able to completely compensate for the decreased RBC life span, the hemoglobin concentration may be normal; this is called fully compensated hemolysis. If the bone marrow is unable to completely compensate for hemolysis, then anemia occurs. This is called incompletely compensated hemolysis.

Clinically, hemolytic anemia produces variable degrees of fatigue, pallor, and jaundice. Splenomegaly occurs in some conditions. The complete blood count shows anemia and reticulocytosis that depend on the acuity and severity of hemolysis, and the degree of bone marrow compensation. Secondary chemical changes include indirect hyperbilirubinemia, increased urobilinogen excretion, and elevated lactate dehydrogenase (LDH). Decreased serum haptoglobin levels and increased plasma-free hemoglobin may also be detected. Because free hemoglobin scavenges nitric oxide, e sophageal spasm or vascular sequelae such as nonhealing skin ulcers and pulmonary hypertension can occur in chronic hemolytic anemia. Chronic intravascular hemolysis produces hemosiderinuria, and chronic extravascular hemolysis increases the risk of pigmented (bilirubinate) gallstones.

The hemolytic anemias can be classified in different yet complementary ways (Table 7-1). They can be inherited

(eg, sickle cell disease or hereditary spherocytosis) or acquired (eg, autoimmune or microangiopathic). Alternatively, they can be characterized by the anatomic site of RBC destruction: extravascular or intravascular. Extravascular hemolysis, in which erythrocyte destruction occurs by macrophages in the liver and spleen, is more common. Intravascular hemolysis refers to RBC destruction occurring primarily within blood vessels. The distinction between intravascular and extravascular hemolysis is not absolute because both occur simultaneously, at least to some degree, in the same patient, and the manifestations of both can overlap. The site of RBC destruction in different conditions can be conceptualized to occur in a spectrum between pure intravascular and pure extravascular hemolysis. Some hemolytic anemias are predominantly intravascular (eg, paroxysmal nocturnal hemoglobinuria), and some are predominantly extravascular (eg, hereditary spherocytosis). Others have substantial components of both, such as sickle cell disease.

The hemolytic anemias can be classified according to whether the cause of hemolysis is intrinsic or extrinsic to the RBC. Intrinsic causes of hemolysis include abnormalities in hemoglobin structure or function, the RBC membrane, or RBC metabolism (cytosolic enzymes). Extrinsic causes may be due to a RBC-directed antibody, a disordered vasculature, or the presence of infecting organisms or toxins. In general, intrinsic causes of hemolysis are inherited and extrinsic causes are acquired, but there are notable exceptions. For example, paroxysmal nocturnal hemoglobinuria (PNH) is an acquired intrinsic RBC disorder, and congenital thrombotic thrombocytopenia purpura (TTP) is an inherited cause of extrinsic hemolysis. In this chapter, hemolytic anemias will be divided into those that are due to intrinsic or extrinsic abnormalities of the RBC.

Table 7-1 Methods of classification of hemolytic anemias

Classification	Example
Inheritance	
Inherited	Sickle cell anemia
Acquired	Autoimmune hemolytic anemia
Site of RBC destruction	
Intravascular	Paroxysmal nocturnal hemoglobinuria
Extravascular	Hereditary spherocytosis
Origin of RBC damage	
Intrinsic	Pyruvate kinase deficiency
Extrinsic	Thrombotic thrombocytopenic purpura

Hemolysis due to intrinsic abnormalities of the RBC

Intrinsic causes of hemolysis include abnormalities of hemoglobin structure or function, the RBC membrane, or RBC metabolism (cytosolic enzymes). Most intrinsic forms of hemolysis are inherited conditions.

Abnormalities of hemoglobin

Hemoglobin is the oxygen-carrying protein within RBCs. It is composed of four globular protein subunits, called globins, each with an oxygen-binding heme group. The two main types of globins are the α-globins and the β-globins, which are made in essentially equivalent amount in precursors of RBCs. Normal adult hemoglobin (Hb A) has two α-globins and two β-globins ($\alpha_2\beta_2$). Genes on chromosomes 16 and 11 encode the α-globins and β-globins, respectively. There are also distinct embryonic, fetal, and minor adult analogs of the α-globins and β-globins encoded by separate genes.

Hemoglobin production

The β-globin gene cluster is on chromosome 11 and includes an embryonic ε-globin gene, the two fetal γ-globin genes ($^A\gamma$ and $^G\gamma$), and the two adult δ- and β-globin genes. The α-globin gene cluster is on chromosome 16 and includes the embryonic ζ-globin gene and the duplicated α-globin genes (α_1 and α_2) which are expressed in both fetal and adult life. Both clusters also contain nonfunctional genes (pseudogenes) designated by the prefix ψ. The θ globin gene downstream of α_1 has unknown functional significance.

The expression of each globin gene cluster is under the regulatory influence of a distant upstream locus control region (LCR). The LCR for the β-cluster resides several kilobases upstream. A similar regulatory region, called HS-40, exists upstream of the α cluster. The LCRs contain DNA sequence elements that bind erythroid-specific and nonspecific DNA binding proteins and serve as a "master switch," inducing expression within the downstream gene cluster. In addition to binding specific transcriptional regulatory proteins, the LCRs also facilitate the binding and interaction of transcriptional regulatory proteins in proximity to the specific genes within the downstream cluster. These regulatory proteins influence the promoter function of the α-globin and β-globin genes to achieve a high level of erythroid- and development-specific gene expression.

Figure 7-1 details the organization of the α- and β-clusters with the associated upstream regulatory elements and the normal hemoglobin species produced during the developmental stages from intrauterine to adult life. Note that the genes are expressed developmentally in the same sequence in which they are organized physically in these clusters (left to right; 5′ to 3′). The process of developmental changes in the type and site of globin gene expression is known as hemoglobin switching. Switching within the cluster is influenced by differential enhancing and gene-silencing effects imparted by the combination of the LCR and local regulatory proteins,
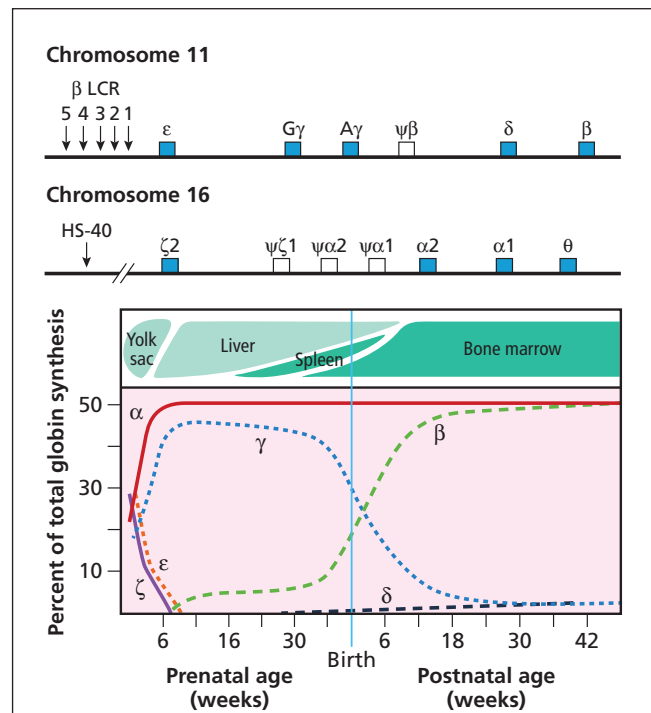

Figure 7-1 Hemoglobin gene clusters and developmental hematopoiesis. The organization of the α- and β-globin gene clusters are shown at the top of the figure. The bottom portion of the figure illustrates the developmental changes in Hb production, both by the site of production of blood and changes in the proportions of the different globins. Modified with permission from Stamatoyannopoulos G, Majerus PW, Perlmutter RM, et al, eds. *The Molecular Basis of Blood Diseases.* 3rd ed. Philadelphia, PA: W. B. Saunders; 2001.

but the entire process of regulatory determination remains incompletely defined. The ability to modulate the switch from the synthesis of γ- to β-globin chains has long been of interest because "reversing the switch" to enhance expression of fetal hemoglobin (Hb F) could successfully treat sickle cell disease.

Hemoglobin structure

Hemoglobin is a tetramer consisting of two pairs of globin chains. Heme, a complex of ferrous iron and protoporphyrin, is linked covalently to each globin monomer and can reversibly bind one oxygen molecule. The molecular mass of hemoglobin is approximately 64 kDa. The α-chains contain 141 amino acids, and the β-chains contain 146 amino acids, as do the β-like globins, δ and γ, which differ from β by 10 and 39 amino acids, respectively. The compositions of normal Hb species throughout development are depicted in Figure 7-1. The postembryonic hemoglbins are Hb A ($\alpha_2\beta_2$), Hb A$_2$ ($\alpha_2\delta_2$), and Hb F ($\alpha_2\gamma_2$).

When hemoglobin is deoxygenated, there are substantial changes in the structures of the individual globins and the hemoglobin tetramer. The iron molecule rises from the plane of its heme ring, and there is a significant rotation of each globin chain relative to the others. In the deoxy conformation, the distance between the heme moieties of the β-chains increases by 0.7 nm. This conformation is stabilized in a taut (T) conformation by salt bonds within and between globin chains and by the binding of allosteric modifiers such as 2,3-bisphosphoglycerate (2,3-BPG) and of protons. The binding of oxygen to hemoglobin leads to disruption of the salt bonds and transition to a relaxed (R) conformation.

Hemoglobin function

Hemoglobin enables RBCs to deliver oxygen to tissues by its reversible binding of oxygen. With the sequential binding of one oxygen molecule to each of the four heme groups, the salt bonds are progressively broken, which increases the oxygen affinity of the remaining heme moieties. Cooperation between the heme rings results in the characteristic sigmoid-shaped oxygen affinity curve. This phenomenon accounts for the release of relatively large amounts of oxygen with small decreases in oxygen tension.

Deoxygenation of hemoglobin is modulated by certain biochemical influences. For example, deoxyhemoglobin binds protons with greater avidity than oxyhemoglobin, which results in a direct dependence of oxygen affinity on pH over the physiologic pH range. This Bohr effect has two major physiologic benefits: (i) the lower pH of metabolically active tissue decreases oxygen affinity, which accommodates

oxygen delivery; and (ii) the higher pH level resulting from carbon dioxide elimination in the lungs increases oxygen affinity and oxygen loading of RBCs. An additional important influence on oxyhemoglobin dissociation is temperature. Hyperthermia decreases affinity, providing the opportunity to deliver more oxygen at the tissue level. 2,3-BPG, a metabolic intermediate of anaerobic glycolysis, is another physiologically important modulator of oxygen affinity. When 2,3-BPG binds in the pocket formed by the amino termini of the β-chains, it stabilizes the deoxy conformation of hemoglobin, thereby reducing its oxygen affinity. The intraerythrocytic molar concentrations of 2,3-BPG and hemoglobin are normally about equal (5 mM). When 2,3-BPG levels increase during periods of hypoxia, anemia, or tissue hypoperfusion, oxygen unloading to tissues is enhanced.

Carbon dioxide reacts with certain amino acid residues in the β-chain of hemoglobin; however, this does not play a significant role in carbon dioxide transport. It recently has been reported that hemoglobin binds nitric oxide in a reversible manner. The participation of hemoglobin in modifying regional vascular resistance through this mechanism has been proposed.

Disorders of hemoglobin

Disorders of hemoglobin can be classified as qualitative or quantitative disorders. Qualitative abnormalities of hemoglobin arise from mutations that change the amino acid sequence of the globin, thereby producing structural and functional changes in hemoglobin. There are four ways in which hemoglobin can be qualitatively abnormal: (i) decreased solubility, (ii) instability, (iii) altered oxygen affinity, and (iv) altered maintenance of the oxidation state of the heme-coordinated iron. Hemolytic anemia results from decreased solubility and instability of hemoglobin. Qualitative hemoglobin disorders often are referred to as hemoglobinopathies, even though the term technically can apply to both qualitative and quantitative disorders. Quantitative hemoglobin disorders result from the decreased and imbalanced production of generally structurally normal globins. For example, if β-globin production is diminished by a mutation, there will be a relative excess of α-globins. Such imbalanced production of α- and β-globins damages RBCs and their precursors in the bone marrow. These quantitative hemoglobin disorders are called thalassemias. Both qualitative and quantitative disorders of hemoglobin can be subdivided by the particular globin that is affected; for example, there are α-thalassemias and β-hemoglobinopathies, among others. We will begin this chapter with a review of the thalassemias and end the section with a discussion of several of the common qualitative hemoglobin disorders.

Thalassemia

Clinical case

A healthy 48-year-old female of African descent is referred to you for evaluation of refractory microcytic anemia. She has been treated with oral iron formulations many times throughout her life. Hemoglobin values have always ranged from 10-11 g/dL with a mean corpuscular volume (MCV) ranging from 69-74 fL. She has no other prior medical history. Her examination is entirely unremarkable. Peripheral blood smear is significant for microcytosis, mild anisopoikilocytosis, and a small number of target cells. The hemoglobin concentration is 10 g/dL with an MCV of 71 fL and mean corpuscular hemoglobin (MCH) of 23 pg. Additional laboratory studies include a transferrin saturation of 32% and a normal ferritin of 285 ng/mL. Hemoglobin electrophoresis reveals hemoglobin A 98% and hemoglobin A2 1.8%.

Thalassemia occurs when there is the quantitatively decreased synthesis of often structurally normal globin proteins. Mutations that decrease the synthesis of α-globins cause α thalassemia; mutations that decrease the synthesis of β-globins cause β-thalassemia.

Heterozygous thalassemia (thalassemia trait) appears to confer protection against severe *Plasmodium falciparum* malarial infection. As a result of this selective advantage, a wide variety of independent mutations leading to thalassemia have arisen over time and have been selected for in populations residing in areas where malaria is (or once was) endemic. In general, α-thalassemias are caused by deletions of DNA, whereas β-thalassemias are caused by point mutations. If a mutation decreases the synthesis of one globin, α or β, it produces a relative excess of the other and an imbalance between the two occurs. For example, if β-globin synthesis is diminished by a mutation, there will be a relative excess of α-globins. Such imbalanced production of α- and β-globins results in damage to precursors of RBCs in the bone marrow. This damage occurs largely because the excess unpaired globin is unstable, and it precipitates within early RBC precursors in the bone marrow and oxidatively damages the cellular membrane. If the α- and β-globin imbalance is severe, most of the RBC precursors in the bone marrow are destroyed before they can be released into the circulation. A severe microcytic anemia results. The body attempts to compensate for the anemia by increasing erythropoietic activity throughout the marrow and sometimes in extramedullary spaces, although this effort is inadequate and compensation is incomplete. This pathophysiologic process is called ineffective erythropoiesis.

The thalassemias can be described simply by two independent nomenclatures: genetic and clinical. The genetic nomenclature denotes the type of causative mutation, such as α-thalassemia or β-thalassemia. The clinical nomenclature divides the thalassemias into the asymptomatic trait state (thalassemia minor), severe transfusion-dependent anemia (thalassemia major), and everything in between (thalassemia intermedia). The two systems can be used together, giving α-thalassemia minor or β-thalassemia intermedia, for example.

β-Thalassemias

β-Thalassemia is prevalent in the populations of the Mediterranean region, the Middle East, India, Pakistan, and Southeast Asia, and is somewhat less common in Africa. It is rarely encountered in Northern European Caucasians.

Molecular basis

β-Thalassemia results from >200 different mutations of the β-globin gene complex (Figure 7-2). Abnormalities have been identified in the promoter region, messenger RNA (mRNA) cap site, 5′ untranslated region, splice sites, exons, introns, and polyadenylation signal region of the β-gene. Gene deletions are infrequent except in δβ and εγδβ thalassemias. A variety of single–base pair mutations or insertions or deletions of nucleotides represent the majority of described mutations. Thus, defects in transcription, RNA processing, and translation or stability of the β-globin gene product have been observed. Mutations within the coding region of the globin gene allele may result in nonsense or truncation mutations of the corresponding globin chain, leading to complete loss of globin synthesis from that allele (β^0 thalassemia allele). Alternatively, abnormalities of transcriptional regulation or mutations that alter splicing may
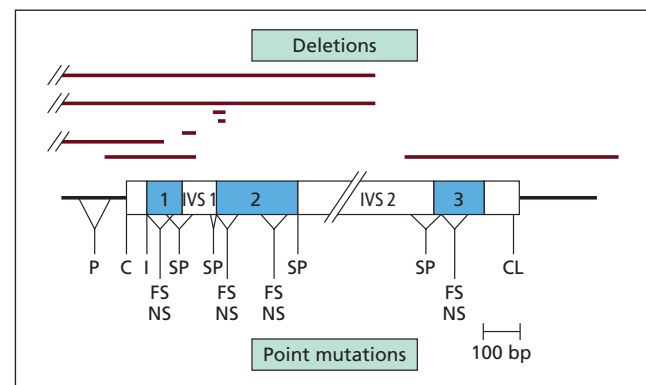

Figure 7-2 Common β-thalassemia mutations. The major classes and locations of mutations that cause β thalassemia are shown. Redrawn from Stamatoyannopoulos G et al., eds. *The Molecular Basis of Blood Diseases.* 3rd ed. Philadelphia, PA: WB Saunders; 2001. C = CAP site; CL = RNA cleavage [poly(A)] site; FS = frameshift; I = initiation codon; NS = nonsense; P = promoter boxes; SP = splice junction, consensus sequence, or cryptic splice site.

cause mild to markedly decreased, but not absent, globin gene synthesis (β^+ thalassemia allele). β-thalassemia major (Cooley anemia) and β-thalassemia intermedia can be due to various genotypes including homozygosity or compound heterozygosity for two β^0 alleles (β^0/β^0) or compound heterozygosity with a β^0 and β^+ allele (β^0/β^+). Patients with beta thalassemia trait are generally heterozygous, carrying a single β-thalassemia allele (β/β^0, β/β^+), but some patients who are homozygous or compound heterozygous for two very mild β^+ alleles may also have β-thalassemia minor phenotype. The clinical phenotype of patients with β-thalassemia is determined primarily by the globin chain imbalance due to the number and severity of the abnormal alleles inherited. Additional factors that contribute to the phenotype include the number of alpha globin genes, genetic determinants associated with increased gamma-chain production, and secondary genetic modifiers such as uridine diphosphate-glucuronosyltransferase (UDPG) gene polymorphisms.

Pathophysiology

The defect in β-thalassemia is a reduced or absent production of β-globin chains with a relative excess of α-chains. The decreased β-chain synthesis leads to impaired production of the $\alpha_2\beta_2$ tetramer of Hb A, decreased hemoglobin production, and an imbalance in globin chain synthesis. The reduction in Hb A in each of the circulating RBCs results in hypochromic, microcytic RBCs with target cells, a characteristic finding in all forms of β-thalassemia. Aggregates of excess α-chains precipitate and form inclusion bodies, leading to premature destruction of erythroid precursors in the bone marrow (ineffective erythropoiesis) (Figure 7-3). In more severe forms, circulating RBCs also may contain inclusions, leading to early clearance by the spleen. The precipitated α-globin chains and products of degradation may also induce changes in RBC metabolism and membrane structure, leading to shortened RBC survival. The response to anemia and ineffective erythropoiesis is increased production of erythropoietin leading to erythroid hyperplasia which can produce skeletal abnormalities, splenomegaly, extramedullary masses and osteoporosis. Ineffective erythropoiesis is associated with increased gastrointestinal iron absorption due to decreased hepcidin. RBC membrane damage with increased surface expression of anionic phospholipids, platelet activation, and changes in hemostatic regulatory proteins contribute to a hypercoagulable state in thalassemia.

Clinical features

The clinical manifestations of β-thalassemia are quite heterogeneous and depend on the extent of β-globin chain production as well as the coinheritance of any other abnormalities
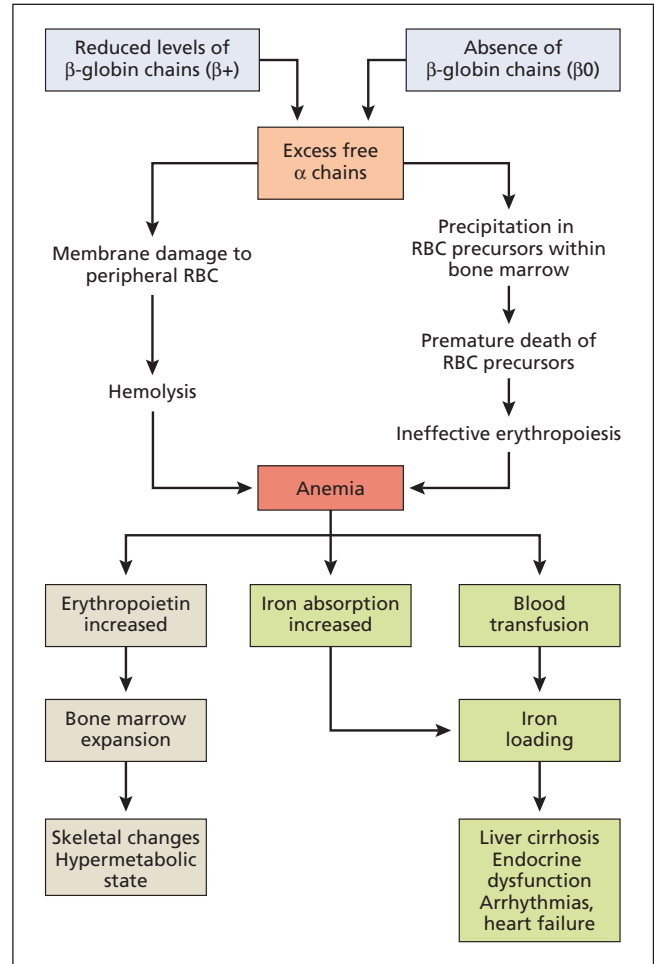

Figure 7-3 Pathophysiology of β-thalassemia. Effects of excess production of free α-globin chains in β-thalassemia. Adapted with permission from Viprakasit V and Origa R. In: *Guidelines for the Management of Transfusion Dependent Thalassaemia (TDT)*. 3rd ed. Nicosia, Cyprus: Thalassaemia International Federation; 2014.

affecting α- or γ-globin synthesis or structural abnormalities of Hb (eg, Hb S).

β-Thalassemia minor (trait) is asymptomatic and is characterized by mild microcytic anemia. Most commonly, it arises from heterozygous β-thalassemia (β-thalassemia trait). Neonates with β-thalassemia trait have no anemia or microcytosis; these develop with increasing age as the transition from Hb F to Hb A production progresses.

β-Thalassemia major (Cooley's anemia) is characterized by absence of or severe deficiency in β-chain synthesis. Symptoms are usually evident within the first 6-12 months of life as the Hb F level begins to decline. In the absence of adequate RBC transfusions, the infant will experience failure to thrive and a variety of clinical findings. Erythroid expansion leads to widening of the bone marrow space, thinning of the cortex, and osteopenia, predisposing to fractures. Growth retardation, progressive hepatosplenomegaly, gallstone formation, and cardiac disease are common. Most homozygotes do not

survive without transfusions beyond the age of 5 years. RBC transfusions ameliorate severe anemia and suppress ineffective erythropoiesis. A chronic transfusion program can allow for normal growth and development, prevent skeletal abnormalities, and suppress extramedullary hematopoiesis.

Iron overload is a major complication in β-thalassemia due to transfusions and increased gastrointestinal iron absorption. Each milliliter of transfused blood contains 1 mg of iron. Red cell transfusions are the major cause of iron loading in beta thalassemia major. Iron accumulates since the body does not have an active mechanism to excrete excess iron. Excess iron results in increased non-transferrin bound iron, which generates harmful reactive oxygen species leading to lipid peroxidation, and organelle and DNA damage causing apoptosis, fibrosis and organ damage. Uncontrolled transfusional iron loading leads to iron deposition in key organs leading to an increased risk of liver cirrhosis, hepatocellular carcinoma, heart failure, and endocrine complications including hypogonadotropic hypogonadism, diabetes, hypothyroidism, osteoporosis, and hypoparathyroidism. Over the last few years, patient survival has significantly improved due to improved iron chelation therapy, improved modalities to measure liver and cardiac iron load, and a comprehensive care approach. An increased frequency of *Yersinia enterocolitica* bacteremia is associated with iron overload and chelation therapy with deferoxamine.

In the modern era, β-thalassemia intermedia is often grouped under the term *non-transfusion dependent thalassemia* (NTDT), which is used to describe patients with moderate anemia who do not need life-long regular transfusions for survival, but need occasional or frequent transfusions in certain clinical settings for short periods of time. NTDT encompasses three clinically distinct forms of thalassemia including beta thalassemia intermedia, hemoglobin E/β-thalassemia, and hemoglobin H disease. These patients exhibit a wide spectrum of clinical findings including hepatosplenomegaly, extramedullary hematopoietic pseudotumors, bone deformities, leg ulcers, thrombotic events, pulmonary hypertension, silent infarcts, gallstones, and iron overload. These complications, except for iron overload are generally limited in the well-transfused thalassemia patient because transfusion interrupts the underlying pathophysiology. An increased incidence of cerebral thrombosis, venous thromboembolism, and pulmonary hypertension has been reported in β-thalassemia major and β-thalassemia intermedia following splenectomy, and these risks should be considered before splenectomy. Iron overload in NTDT occurs primarily due to increased gastrointestinal absorption in the setting of ineffective erythropoiesis. Thus, even in the absence of transfusion, some patients may have iron overload. The iron overload significantly increases with increasing number of transfusions.

$(\delta\beta)^0$ thalassemia is due to the deletion of both δ and β genes, while $(\gamma\delta\beta)^0$ is due to deletion of γ, δ and β genes. Heterozygotes have a phenotype similar to β-thalassemia trait and may have splenomegaly. Homozygotes or compound heterozygotes with a severe b+ or b^0 mutation may have thalassemia intermedia due to increased synthesis of hemoglobin F.

Heterozygotes for the rare εγδβ deletion present with moderately severe microcytic anemia in the neonatal period with hemolysis, erythroblastosis, hepatosplenomegaly, and may need transfusions at birth or during the first 6 months of life. In the adult, the hematologic findings are those of β-thalassemia trait, except that the Hb electrophoresis shows normal Hb F and A_2 levels. The homozygous state is incompatible with fetal life.

Laboratory findings

Patients with β-thalassemia trait may have a hemoglobin ranging from 9 g/dL to a normal value. Peripheral smear shows microcytic, hypochromic RBCs and target cells. Basophilic stippling is variable. The MCV is usually <70 fL, the MCH is reduced, and the reticulocyte count can be mildly elevated. Hb A_2 levels are elevated >3.5% (usually 4%-7%), and Hb F levels may be mildly increased. A variable degree of anemia with hypochromic, microcytic cells and target cells is observed in β-thalassemia intermedia. Laboratory abnormalities are similar to β-thalassemia trait, but more severe. A child with β-thalassemia major who is not receiving transfusions will have severe anemia. Peripheral blood smear findings include anisopoikilocytosis, target cells, severe hypochromia, nucleated red blood cells, and basophilic stippling. The reticulocyte count is slightly increased, and nucleated RBCs are abundant. These findings are exaggerated after splenectomy. Hemoglobin electrophoresis reveals persistent elevation of Hb F $(\alpha_2\gamma_2)$ and variable elevation of Hb A_2 $(\alpha_2\delta_2)$. Hb A is absent in homozygous β^0 thalassemia. In $(\delta\beta)^0$ or $(\gamma\delta\beta)^0$ hemoglobin ranges between 8-13 g/dL, and the MCV may be normal or low-normal. Hemoglobin A2 is however normal, and Hb F is increased between 5%-20%. Homozygotes for $(\delta\beta)^0$ or $(\gamma\delta\beta)^0$ thalassemia have 100% Hb F.

Management of the β-thalassemias

Individuals with β-thalassemia trait do not require therapy but should be identified to reduce the risk of inappropriate iron supplementation. Individuals of child-bearing age should be offered genetic counseling for informed reproductive choices.

Management of patients with β-thalassemia major and intermedia involves a comprehensive multidisciplinary care approach. RBC transfusion has been the mainstay in the management

of β-thalassemia major and its complications are described above. The goals of transfusions are to promote normal growth and development and to suppress ineffective erythropoiesis. A lifelong chronic blood transfusion program to maintain a pretransfusion Hb level between 9-10 g/dL sufficiently suppresses bone marrow expansion while minimizing transfusional iron loading.

Monitoring iron load is key to establishing an individualized, effective iron chelation regimen for each patient. Iron load is determined by serum ferritin, liver iron concentration, and cardiac iron load. Serum ferritin generally correlates with body iron stores and is an easy, convenient, and inexpensive measure to trend. However, it has several limitations since it is an indirect measure of true body iron burden, is an acute phase reactant, and it has a non-linear response to iron load at high levels. Liver iron concentration (LIC) can be determined by liver biopsy or by the new gold standard, liver MRI R2. Normal LIC is < 1.8 mg Fe/g dry weight. Cardiac MRI T2* correlates with cardiac iron load and the risk of developing heart failure increases with T2* values <20 ms. The risk for developing heart failure is highest when the cardiac T2* is <8 ms. A complete iron load evaluation thus includes at least serum ferritin every 3 months, yearly LIC by MRI R2 starting at age 5, and yearly cardiac iron T2* starting at 8-10 years of age. For young children, the risks of sedation should be weighed against the risks of severe liver iron overload. The main goals of iron chelation therapy are to maintain safe levels of body iron to prevent iron overload and its complications and to reduce accumulated iron. Iron chelation therapy is tailored to each individual based on transfusion rates and iron burden. In β-thalassemia major, iron chelation therapy with subcutaneous deferoxamine or oral deferasirox is initiated when serum ferritin levels reach approximately 1,000-1,500 ng/mL following approximately 12 months of scheduled transfusions or approximately 20 units of blood. Chelation is adjusted to maintain a ferritin <1,000-1,500 ng/mL, a LIC of 2-7 mg of iron/g dry weight, and a cardiac T2* >20 ms. Monitoring for chelator-specific complications should be performed.

In the modern era of improved transfusion support and chelation, and in light of recognized post-splenectomy complications of increased infection risk, venous thromboembolism, and pulmonary hypertension, splenectomy is generally not recommended. Often, increasing the transfusion targets is sufficient to reduce the degree of splenomegaly. Allogeneic bone marrow transplantation from a histocompatibility (human leukocyte antigen [HLA]-compatible) sibling has been performed in >1,000 patients and is now curative in most. The outcome has been influenced by the age of the patient, the presence of liver disease, and the extent of iron overload. Graft-versus-host disease represents the most common long-term complication. Recent studies exploring nonmyeloablative or unrelated donor transplantation are encouraging, even in patients with prior iron loading (for whom chelation therapy before transplantation is advised) or concomitant hepatitis C virus (HCV) infection. Many adults with thalassemia major have chronic HCV infection resulting from contaminated RBC products that they received before the early 1990s. Treatment with ribavirin-based regimens may be complicated by hemolysis resulting from ribavirin and have been limiting in thalassemia patients. New treatment regimens for HCV that do not include ribavirin and are not associated with hemolysis are encouraging. Recent proof of principle gene therapy studies in β-thalassemia major have achieved transfusion independence and are promising for the future.

Individuals with NTDT may require intermittent transfusions during acute episodes of worsening anemia, including infection or acute illness. Most indications for initiating a chronic transfusion program in NTDT are similar to those in β-thalassemia major. However, these are generally initiated later in childhood or in adulthood, depending on the severity of the phenotype. Iron overload in NTDT occurs primarily due to increased gastrointestinal absorption but significantly increases with increasing transfusions. Iron-associated complications are similar to those seen in beta thalassemia major, except cardiac siderosis is much less common. Serum ferritin and LIC measurements show a generally positive correlation and should be regularly evaluated in all patients over 10 years of age. In NTDT, the total body iron load may be higher than what the serum ferritin levels suggest. Thus a serum ferritin of >800 ng/mL warrants LIC evaluation. Chelation therapy should be initiated if the LIC is ≥5 mg Fe/g dry weight to reduce iron-associated morbidity. Deferasirox has been well studied in NTDT with a good efficacy and safety profile.

α-Thalassemias

There is a high prevalence of α-thalassemia in Africa, the Mediterranean region, Southeast Asia, and, to a lesser extent, the Middle East.

Molecular basis

Duplicated copies of the α-genes are normally present on each chromosome 16, making the defects in α-thalassemia more heterogeneous than in β-thalassemia. The α^+ thalassemias result from deletion of one of the linked genes, $-\alpha/\alpha\alpha$, or impairment due to a point mutation, designated $\alpha^T\alpha/\alpha\alpha$. The deletion type of α^+ thalassemia is due to unequal crossover of the linked genes, whereas the non-deletion type includes mutations resulting in abnormal transcription or translation or the production of unstable

α-globin. The $-\alpha/\alpha\alpha$ genotype (the "silent carrier" state) occurs in approximately one in three African Americans. Hemoglobin Constant Spring is a nondeletion α^+ thalassemia, common in Southeast Asia, resulting from a mutation that affects termination of translation and results in abnormally elongated α-chains. The $--/\alpha\alpha$ genotype of α-thalassemia trait due to loss of linked α-genes on the same chromosome (*cis* configuration), is more common in individuals of Asian descent, whereas the $-\alpha/-\alpha$ genotype (deletions in the *trans* position) is more common in individuals of African or Mediterranean descent.

Pathophysiology

As in the β-thalassemias, the imbalance of globin chain synthesis results in decreased hemoglobin synthesis and microcytic anemia. Excess γ- and β-chains form tetramers termed Hb Bart and Hb H, respectively. These tetramers are more soluble than unpaired α-globins (as in β-thalassemia) and form RBC inclusions slowly. Consequently, although α-thalassemia is associated with a hemolytic anemia, it does not lead to significant ineffective erythropoiesis. The homozygous inheritance of α^0 thalassemia $(--/--)$ results in the total absence of α-chains, death in utero, or hydrops fetalis. Unpaired γ-chains form Hb Bart (γ_4), and there may be persistence of embryonic hemoglobins. Hb Bart is soluble and does not precipitate; however, it has a very high oxygen affinity and is unable to deliver oxygen to the tissues. This leads to severe fetal tissue hypoxia, resulting in edema, congestive heart failure, and death. Hb H disease results from the coinheritance of α^0 thalassemia and α^+ thalassemia $(--/-\alpha)$ or α^0 thalassemia and a nondeletional form of α-thalassemia $(--/\alpha^T\alpha)$ such as Hb Constant Spring $(--/\alpha^{CS}\alpha)$. The excess β-chains form Hb H (β_4) that is unstable, causing precipitation within circulating cells and hemolysis. Patients have moderately severe hemolytic anemia.

Hb H also can be produced as an acquired phenomenon in the setting of myelodysplastic syndromes and some myeloid leukemias, in which somatic mutations of the *ATRX* gene downregulate α-globin production and cause globin chain imbalance. This condition is called the α-*thalassemia–myelodysplastic syndrome* (ATMDS). The X-linked *ATRX* gene encodes a chromatin-remodeling factor (X-linked helicase 2) that regulates α-globin production. Constitutional deletions of this gene produce the α-thalassemia–mental retardation syndrome.

Clinical features

In contrast to β-thalassemias, α-thalassemias can manifest in both fetal and postnatal life. The clinical manifestations of α-thalassemia are related to the number of functional α-globin genes (Figure 7-4). Heterozygotes for α^+

thalassemia $(-\alpha/\alpha\alpha)$, so-called silent carriers, are clinically normal. Thalassemia trait (two-gene deletion α-thalassemia) occurs in two forms: α^0 thalassemia trait $(--/\alpha\alpha)$ or homozygosity for α^+ thalassemia $(-\alpha/-\alpha)$. Individuals with thalassemia trait have a lifelong mild microcytic anemia. The clinical manifestations are variable in Hb H disease $(--/-\alpha)$, with severe forms demonstrating transfusion dependence and other individuals having a milder course. As in β-thalassemia, splenomegaly occurs commonly in the anemic patient. The homozygous state for Hb Constant Spring results in moderate anemia with splenomegaly. Hb H–Constant Spring $(--/\alpha^{CS}\alpha)$ is typically more severe than classical Hb H disease $(--/-\alpha)$. Homozygous α^0 thalassemia $(--/--)$ results in the Hb Bart hydrops fetalis syndrome.
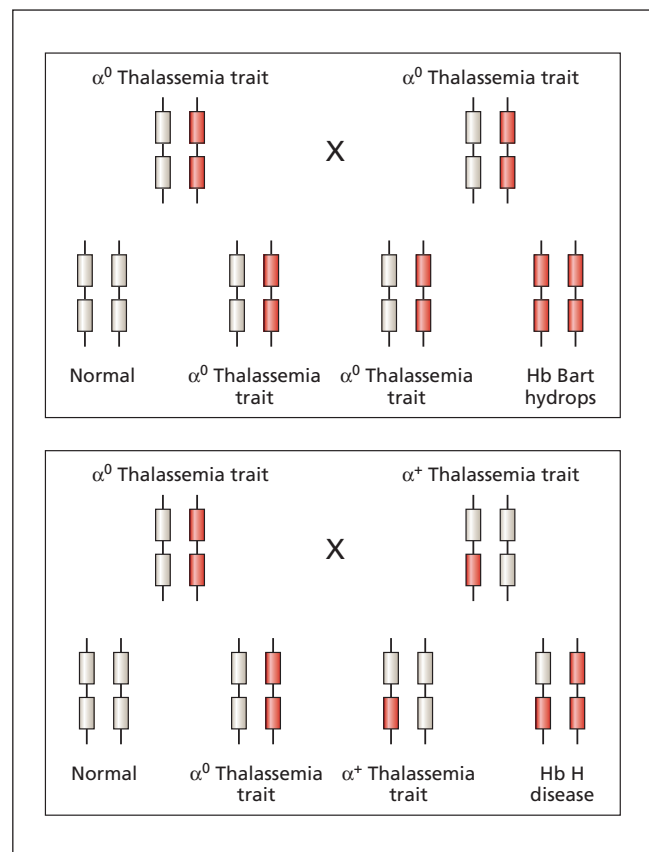

Figure 7-4 Genetics of α-thalassemia. The α-globin genes are represented as boxes. The red α-globin genes represent deletions or otherwise inactivated α-genes. The open boxes represent normal α-genes. The terms α^0 and α^+ thalassemia are defined in the text. The potential offspring of two parents with α^0 thalassemia trait is shown in the upper panel. The potential offspring of one parent with α^0 thalassemia trait and the other with α^+ thalassemia trait is shown in the lower panel (note the lack of hemoglobin Bart hydrops fetalis in these offspring). Redrawn from Stamatoyannopoulos G et al., eds. *The Molecular Basis of Blood Diseases.* 3rd ed. Philadelphia, PA: W. B. Saunders; 2001.

The lack of Hb F due to the absence of α chains produces intrauterine hypoxia, resulting in marked expansion of bone marrow and hepatosplenomegaly in the fetus and enlargement of the placenta. In utero death usually occurs between 30 and 40 weeks or soon after birth. Moreover, the mother often experiences morbidity from polyhydramnios.

Laboratory features

Minimal or no anemia or morphologic abnormalities of RBCs are observed in silent carriers (heterozygous α^+ thalassemia [$-\alpha/\alpha\alpha$]). The hemoglobin electrophoresis is normal. In newborns who are heterozygous for α^0 thalassemia ($--/\alpha\alpha$), the hemoglobin electrophoresis reveals 2%-5% Hb Bart and microcytosis (<95 fL). Children and adults heterozygous for α^0 thalassemia ($--/\alpha\alpha$) or homozygotes for α^+ thalassemia ($-\alpha/-\alpha$) have mild anemia with hypochromic, microcytic RBCs and target cells. The RBC indices are similar to those of β-thalassemia trait, but the hemoglobin electrophoresis is normal (or shows a reduction in Hb A_2). Molecular testing is required to confirm the diagnosis in alpha thalassemia due to one or two gene deletions. The high prevalence of the $-\alpha/-\alpha$ genotype in African Americans is noteworthy. About 2%-3% of all African Americans in the United States have asymptomatic microcytosis and borderline anemia (often mistaken for iron deficiency) as a result of this condition. Hb H disease ($--/-\alpha$) is characterized by anisopoikilocytosis and hypochromia with elevated reticulocyte counts. Hemoglobin electrophoresis reveals 5%-40% of the rapidly migrating Hb H. Supravital staining with brilliant cresyl blue will reveal inclusions representing in vitro precipitation of Hb H. The blood smear in Hb Bart hydrops fetalis syndrome ($--/--$) reveals markedly abnormal RBC morphology with anisopoikilocytosis, hypochromia, targets, basophilic stippling, and nucleated RBCs. The hemoglobin electrophoresis in a neonate reveals approximately 80% Hb Bart and the remainder Hb Portland ($\zeta_2\gamma_2$).

Management of the α-thalassemias

Individuals with 1 or 2 alpha gene deletions ($-\alpha/\alpha\alpha, --/\alpha\alpha, -\alpha/-\alpha$) do not generally require any specific treatment. Patients with Hb H disease are categorized as NTDT as described earlier and usually require no specific interventions. However, since the phenotype can be variable, close observation and follow up is important. Some patients, especially those with Hb H–Constant Spring, require intermittent or chronic RBC transfusions. Intermittent transfusions may be required in acutely worsening anemia due to infection or acute illness, or to allow for normal growth and development in childhood. For those patients with significant anemia and splenomegaly, a chronic transfusion program or splenectomy may prove useful. A fetus with homozygous α^0 thalassemia can be rescued with intrauterine transfusion, followed by postnatal chronic transfusions or stem cell transplantation. Because of the high prevalence of the α^0 genotype in Southeast Asian and certain Mediterranean populations, screening programs and genetic counseling can reduce the occurrence of births resulting in Hb Bart hydrops fetalis and Hb H disease.

Clinical case (continued)

The patient presented in this case likely has two copies of alpha deletions in the trans position ($-\alpha/-\alpha$). Patients with this condition usually have mild microcytic, hypochromic anemia. Targeted RBC forms suggest the presence of thalassemia in an otherwise healthy person. With single or double α-gene deletions, the hemoglobin electrophoresis is typically normal, unlike in β-thalassemia. α-Thalassemia is often a diagnosis of exclusion, and identification of similar findings in family members supports the diagnosis. Molecular testing for specific α gene deletions confirms the diagnosis. Iron deficiency should be ruled out. Exogenous iron should not be prescribed because it is unnecessary and potentially harmful. Patients are generally asymptomatic, require no treatment, and have a normal life expectancy.

Key points

• The thalassemias are characterized by a reduced rate of synthesis of one of the globin subunits of the hemoglobin molecule.
• The intracellular precipitation of the excess, unpaired globin chains in thalassemia damages red cell precursors and circulating red cells, resulting in ineffective erythropoiesis and hemolysis.
• The β-thalassemias are caused by >200 different mutations, usually point mutations, with a wide variety of genetic abnormalities documented.
• Patients with thalassemia major require transfusion support, and develop iron overload associated complications. A spectrum of clinical manifestations is observed in thalassemia intermedia, whereas the carrier state has no associated symptoms.
• The hemoglobin electrophoresis in β-thalassemia reveals increased levels of hemoglobin A_2 and variably increased hemoglobin F.
• The α-thalassemias are primarily due to DNA deletions. Four α-genes are normally present, so multiple phenotypes are possible when gene deletions occur.
• α-Thalassemia trait is characterized by mild anemia with microcytic indices and a normal hemoglobin electrophoresis.
• The clinical manifestations in hemoglobin H disease are variable, with some affected individuals requiring transfusions and others less symptomatic.
• Homozygous α^0 thalassemia manifests in fetal life with the formation of hemoglobin Bart (γ_4) and hydrops fetalis.

Sickle cell disease

Clinical case

A 17-year-old African American male with homozygous sickle cell anemia (HbSS) is admitted to the hospital with a 4-day history of a typical painful episode involving his arms and legs. There is no recent febrile illness. Past medical history is remarkable for few hospital admissions for pain crises and red cell transfusion once as a young child. He is in severe pain and appears ill, and vital signs are remarkable for a pulse of 129 and temperature of 38.5°C. Scleral icterus and moderate jaundice are noted. Laboratory data include hemoglobin 7.2 g/dL (baseline 9.1 g/dL), corrected reticulocyte count of 2%, and platelet count 72,000/μL. Liver function tests are elevated above baseline and include a direct bilirubin of 4.8 mg/dL, aspartate aminotransferase (AST) of 1,200 U/L, and alanine aminotransferase (ALT) 1,550 U/L. His creatinine is elevated at 4.3 mg/dL. Abdominal ultrasound is nondiagnostic. He is immediately started on intravenous fluids and opioid analgesics. Broad-spectrum antibiotics are empirically administered. Over the next 24 hours he becomes tachypneic and slightly confused. Hypoxemia develops despite oxygen supplementation, and anuria ensues. Serum creatinine has increased to 6.4 mg/dL, direct bilirubin to 7.8 mg/dL, AST to 2,725 U/L, and creatine phosphokinase (CPK) to 2,200 IU/L and hemoglobin has decreased to 5.8 g/dL. The patient undergoes simple transfusion and subsequently red cell exchange. Acute dialysis is required. He slowly improves during a prolonged 3-week hospitalization. No infectious etiology was identified.
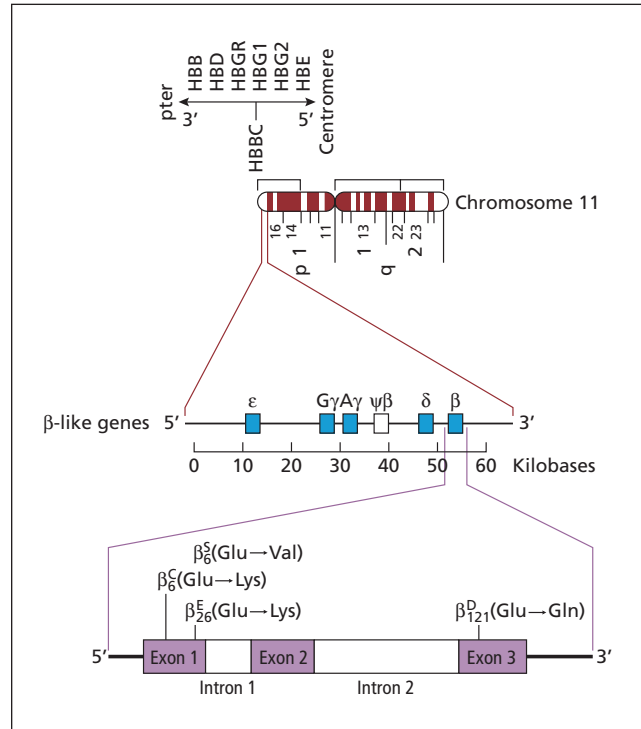

Figure 7-5 Common β-globin variants. The locations of the mutations within the chromosome (top), the β-globin cluster (middle), and the β-globin gene itself (bottom) are shown for four common β-globin variants.

Sickle hemoglobin (HbS) was the first hemoglobin variant discovered. It has been well characterized on a biochemical and molecular level. Heterozygosity for the sickle cell gene (β^S), called sickle cell trait, occurs in >20% of individuals in equatorial Africa; up to 20% of individuals in the eastern provinces of Saudi Arabia and central India; up to 6.3% in Hispanic populations; and approximately 5% of individuals in parts of the Mediterranean region, the Middle East, and North Africa. In HbS, a hydrophobic valine is substituted for the normal, more hydrophilic glutamic acid at the sixth residue of the β-globin chain (Figure 7-5). This substitution is due to a single nucleotide mutation (GAG/GTG) in the sixth codon of the β-globin gene. Heterozygous inheritance of HbS offers a degree of protection from severe malaria infection. This has been offered as an explanation for the evolutionary selection of the HbS gene despite the devastating effects of the homozygous state. The β^S gene is inherited in an autosomal codominant fashion. That is, heterozygous inheritance does not cause disease but is detectable (sickle cell trait); homozygous inheritance or compound heterozygous inheritance with another mutant β-globin gene results in disease. The *sickle cell syndromes* include all conditions in which β^S is inherited

(including sickle cell trait). In contrast, the term *sickle cell disease* includes only those genotypes associated with varying degrees of chronic hemolytic anemia and vaso-occlusive pain (not sickle trait): homozygous sickle cell anemia (HbSS), sickle-Hb C disease (HbSC), sickle-β^0 thalassemia (HbSβ^0), and sickle-β^+ thalassemia (HbSβ^+). Less common hemoglobin mutants, such as O^{Arab}, D^{Punjab}, or E, may be inherited in compound heterozygosity with β^S to result in sickle cell disease.

Sickle cell trait (Hb AS) occurs in 8%-9% of the African American population. It is associated with the rare complications of hematuria, renal papillary necrosis, pyelonephritis during pregnancy, and risk of splenic infarction at high altitude. Sickle trait also is associated with the extremely rare medullary carcinoma of the kidney and an increased risk of sudden death during extreme conditions of dehydration and hyperthermia. Recent publications have shown that individuals with sickle trait are at higher risk of chronic kidney disease and venous thromboembolism. This simple heterozygous state generally has an hemoglobin A:S ratio of approximately 60:40, because of the greater electrostatic attraction of α-chains to β^A rather than β^S chains. When the availability of α chains is limited by coinherited α-thalassemia, the A:S ratio is further increased.

Pathophysiology

The hallmark of sickle cell pathophysiology is the intraerythrocytic polymerization of deoxyhemoglobin S. When deoxygenation of Hb S occurs, the normal conformational change of the tetramer exposes on its external surface a hydrophobic β_6 valine (instead of the hydrophilic glutamate of Hb A), resulting in decreased solubility and a tendency of deoxyhemoglobin S tetramers to aggregate or polymerize. The rate and degree of this polymerization determines the rheologic impairment of sickle erythrocytes and the change in morphology for which the condition was named. Polymerization rate and extent are related to the intracellular concentration of Hb S, the type and fractional content of other hemoglobins present (particularly Hb F), and percent oxygen saturation. These variables correlate with the rate of hemolysis in sickle cell syndromes.

Multiple factors determine the clinical manifestations of sickle cell disease. In addition to physiologic changes such as tissue oxygenation and pH, multiple genetic polymorphisms and mutations may modify the presentation of the disease. This is best appreciated by examining the influence of the coinheritance of other hemoglobin abnormalities on the effects of Hb S. For example, the coexistence of α-thalassemia reduces the hemolytic severity as well as the risk of cerebrovascular accidents. High levels of fetal hemoglobin (Hb F) may substantially reduce symptoms as well as clinical consequences. Compound heterozygosity for a second abnormal hemoglobin (eg, Hb C, D, or E) or β-thalassemia also modifies some of the manifestations of disease (discussed later in this section) (Table 7-2).

Several restriction fragment-length polymorphisms (RFLPs) may be identified in the vicinity of a known gene and define the genetic background upon which a disease-causing mutation has arisen. For example, the coinheritance of a defined set of RFLPs around the β-globin gene can define a disease-associated "haplotype" that marks the sickle mutation within a specific population. These β-globin haplotypes also have been associated with variations in disease severity. This association is probably not related to the RFLPs themselves but rather is mediated through linked differences in γ-chain (Hb F) production. The β^S gene has been found to be associated with five distinct haplotypes, referred to as the Benin (Ben), Senegal (Sen), Central African Republic (CAR or Bantu), Cameroon (Cam), and Arab-Indian (Asian) haplotypes. This is evidence that the β^S gene arose by five separate mutational events. In general, the Asian and Sen haplotypes are associated with a milder clinical course, and CAR is associated with a more severe course.

Although the deoxygenation-polymerization-sickling axiom provides a basic understanding of sickle cell disease, there is an increasing appreciation that interactions of sickle cells with other cells and proteins contribute to the hemolytic and vaso-occlusive processes. In vitro data show that sickle erythrocytes exhibit abnormally increased adherence to vascular endothelial cells as well as to subendothelial extracellular matrix proteins. Apparent endothelial damage is demonstrated by the increased number of circulating endothelial cells in sickle cell disease patients and by the increase in such cells during vaso-occlusive crises. The disruption of normal endothelium results in the exposure of extracellular matrix components, including thrombospondin, laminin, and fibronectin. Endothelial cell receptors include the vitronectin receptor $\alpha_V\beta_3$ integrin and the cytokine-induced vascular cell adherence molecule-1 (VCAM-1). RBC receptors include CD36 (glycoprotein IV), the $\alpha_{IV}\beta_1$ integrin, the Lutheran blood group glycoproteins, and sulfatides. Vaso-occlusion thus may be initiated by adherence of sickle erythrocytes to endothelial cells and extracellular matrix molecules exposed during the process of endothelial damage and completed by trapping of sickled, nondeformable cells behind this nidus of occlusion. Activation of blood coagulation resulting in enhanced thrombin generation and evidence for platelet hyperreactivity have been demonstrated in patients with sickle cell disease during steady-state and vaso-occlusive episodes. It has been suggested that the exposure of RBC membrane phosphatidylserine and circulating activated endothelial cells in sickle cell disease patients contribute to the hypercoagulability by providing procoagulant surfaces. The correlation of elevated

Table 7-2 Typical clinical and laboratory findings of the common forms of sickle cell disease after 5 years of age

Disease	Clinical severity	S (%)	F (%)	A$_2$ (%)*	A (%)	Hemoglobin (g/dL)	MCV (fL)
SS	Usually marked	>90	<10	<3.5	0	6-9	>80
Sβ^0	Marked to moderate	>80	<20	>3.5	0	6-9	<70
Sβ^+	Mild to moderate	>60	<20	>3.5	10-30	9-12	<75
SC	Mild to moderate	50	<5	0†	0	10-15	75-85
S⁻ HPFH	Asymptomatic	<70	>30	<2.5	0	12-14	<80

*Hb A$_2$ can be increased in the presence of Hb S, even in the absence of β-thalassemia. The classical findings are shown here.
†There will be 50% hemoglobin C that migrates near hemoglobin A$_2$ on alkaline gel electrophoresis or isoelectric focusing.
HPFH = hereditary persistence of fetal hemoglobin.

white blood cell counts to increased mortality and adverse outcomes identified by epidemiologic studies of sickle cell disease patients suggest that neutrophils also participate in vaso-occlusion. This concept has been further supported by the precipitation of vaso-occlusive episodes with markedly increased neutrophil counts associated with the administration of granulocyte colony-stimulating factor (G-CSF). These findings together support the concept that the products of multiple genes as well as inflammatory cytokines contribute to the pathology of sickle cell disease.

Laboratory features

The diagnosis of the sickle cell syndromes is made by the identification of Hb S in erythrocyte hemolysates. Historically, cellulose acetate electrophoresis at alkaline pH was used to separate Hb A, Hb A_2, and Hb S; and citrate agar electrophoresis at acidic pH was used to separate comigrating Hb D and Hb C from Hb S and Hb A_2, respectively. Currently, high-performance liquid chromatography (HPLC) and isoelectric focusing are used in most diagnostic laboratories to separate Hbs. In both Hb SS and $S\beta^0$ thalassemia, no Hb A is present. In Hb SS, however, the MCV is normal, whereas in Hb $S\beta^0$ thalassemia, the MCV is reduced. Hb A_2 is elevated in $S\beta^0$ thalassemia, but it also can be nonspecifically elevated in the presence of Hb S, so an elevation of A_2 alone cannot reliably distinguish Hb SS from $S\beta^0$ thalassemia. In sickle cell trait and $S\beta^+$ thalassemia, both Hb S and Hb A are identified. The A:S ratio is 60:40 in sickle trait (more A than S) and approximately 15:85 in $S\beta^+$ thalassemia (more S than A). Microcytosis, target cells, anemia, and clinical symptoms occur only in $S\beta^+$ thalassemia and not in sickle trait (Table 7-2). Review of the peripheral smear will reveal the presence of irreversibly sickled cells in Hb SS and Hb $S\beta^0$ thalassemia (Figure 7-6), but only rarely in $S\beta^+$ thalassemia and Hb SC. Turbidity tests (for Hb S) are positive in all sickle cell syndromes, including Hb AS (sickle trait). The classic sickle cell slide test or "sickle cell prep" (using sodium metabisulfite or dithionite) and the turbidity test detect only the presence of Hb S, so they do not differentiate sickle cell disease from sickle cell trait. Therefore, they have limited utility. Sickle cell disease can be diagnosed by DNA testing of the preimplanted zygote in the first trimester of pregnancy using chorionic villus sampling, in the second trimester using amniocentesis, or after birth using peripheral blood.

Clinical manifestations

Two major physiologic processes, shortened RBC survival (hemolysis) and vaso-occlusion, account for most of the clinical manifestations of sickle cell disease. The erythrocyte life span is shortened from the normal 120 days to
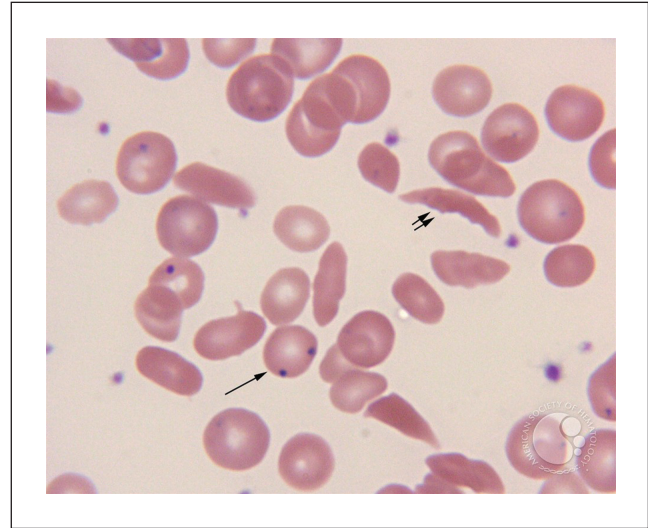

Figure 7-6 Irreversibly sickled cell. This peripheral blood film shows an irreversibly sickled cell (ISC) that occurs in sickle cell anemia (SS), $S\beta^0$ thalassemia (double arrow). ISCs are rare in hemoglobin SC and $S\beta^+$ thalassemia. Also note the Howell-Jolly bodies in this view (single arrow). Source: ASH Image Bank/John Lazarchick (image 00003961).

approximately 10-25 days, resulting in moderate to severe hemolytic anemia, with a steady-state mean hemoglobin concentration of 8 g/dL (ranging from 6 to 9 g/dL) in Hb SS disease. The anemia is generally well tolerated because of compensatory cardiovascular changes and increased levels of 2,3-BPG. Several conditions are associated with acute or chronic declines in the hemoglobin concentration, which may lead to symptomatic anemia (Table 7-3). The transient aplastic crisis resulting from erythroid aplasia is caused by human parvovirus infection, which may result in severe or life-threatening anemia. Lesser degrees of bone marrow

Table 7-3 Causes of acute exacerbations of anemia in sickle cell disease

Cause	Comment
Aplastic crisis	Caused by human parvovirus; does not recur
Acute splenic sequestration crisis	Often recurrent in childhood before splenic involution
Acute chest syndrome	Anemia may precede the onset of respiratory signs and symptoms
Vaso-occlusive crisis	Minimal decline only
Hypoplastic crisis	Mild decline; accompanies many infections
Accelerated hemolysis	Infrequent; accompanies infection of concomitant G6PD deficiency
Hepatic sequestration	Rare
Folate deficiency (megaloblastic crisis)	Rare, even in the absence of folate supplementation

"suppression" are associated with other infections. Sudden anemia may be caused by acute splenic sequestration in children with Hb SS or Sβ^0 (and in adults with Hb SC or Sβ^+ thalassemia) or, less frequently, hepatic sequestration, concomitant glucose-6-phosphate dehydrogenase (G6PD) deficiency, or superimposed autoimmune hemolysis. Chronic exacerbations of anemia may be the result of folate or iron deficiency or reduced erythropoietin levels due to chronic renal insufficiency. Because of the chronic erythrocyte destruction, patients with sickle cell disease have a high incidence of pigmented gallstones, which are often asymptomatic.

The acute painful "vaso-occlusive crisis" is the stereotypical and most common complication of sickle cell disease. These often unpredictable events are thought to be caused by obstruction of the microcirculation by erythrocytes and other blood cells, leading to painful tissue hypoxia and infarction. They most commonly affect the long bones, back, chest, and abdomen. Acute pain events may be precipitated by dehydration, cold temperatures, exercise (in particular swimming), pregnancy, infection, or stress. Often no precipitating factor can be identified. Painful episodes may or may not be accompanied by low-grade fever.

One of the first manifestations of sickle cell disease, acute dactylitis (hand-foot syndrome), results from bone marrow necrosis of the hands and feet. The first attack generally occurs between 6 and 18 months of life, when the Hb F level declines. Dactylitis is uncommon after age 3 years, as the site of hematopoiesis shifts from the peripheral to the axial skeleton. Long-bone infarcts with pain and swelling may mimic osteomyelitis. Other skeletal complications of sickle cell disease include osteomyelitis, particularly due to *Salmonella* and staphylococci, and avascular necrosis, especially of the femoral and humeral heads.

Sickle cell disease is a multisystem disorder. Organ systems subject to recurrent ischemia, infarction, and chronic dysfunction include the lungs (acute chest syndrome, pulmonary fibrosis, pulmonary hypertension, hypoxemia), central nervous system (overt and covert cerebral infarction, subarachnoid and intracerebral hemorrhage, seizures, cognitive impairment), cardiovascular system (cardiomegaly, congestive heart failure), genitourinary system (hyposthenuria, hematuria, proteinuria, papillary necrosis, glomerulonephritis, priapism), spleen (splenomegaly, splenic sequestration, splenic infarction and involution, hyposplenism), eyes (retinal artery occlusion, proliferative sickle retinopathy, vitreous hemorrhage, retinal detachment), and skin (leg ulcerations). The risk of life-threatening septicemia and meningitis because of encapsulated organisms, such as *Streptococcus pneumoniae*, is increased markedly in children with sickle cell disease. This susceptibility is related to functional and anatomic asplenia and decreased opsonization

because of deficient production of natural antibodies. The risk for such infections persists into adulthood.

There are many important clinical differences among the genotypes that cause sickle cell disease (Table 7-2). Hemoglobin SS is associated with the most severe anemia, most frequent pain, and shortest life expectancy (median age, 42 years for men and 48 years for women in one large, but old, study), although there is tremendous heterogeneity in these variables even within this genotype. Hemoglobin Sβ^0 thalassemia can closely mimic Hb SS, despite the smaller red blood cells, lower MCH concentrations, and higher levels of Hb F and Hb A$_2$ associated with this genotype. Patients with Hb SC generally live longer lives (median age, 60 years for men and 68 years for women) and have less severe anemia (~20% are not anemic at all), higher MCH concentrations and less frequent pain, but they have more frequent ocular and bone complications. Although Hb C does not enter into the deoxyhemoglobin S polymer, patients with Hb SC have symptoms, whereas those with sickle cell trait (AS) do not. This is thought to be caused by two important consequences of the presence of Hb C: the Hb S content in Hb SC is 10%-15% higher than that seen in sickle trait (Hb S of approximately 50% vs. 40%), and the absolute intraerythrocytic concentration of total Hb is increased. The latter phenomenon results from persistent loss of cellular K$^+$ and water from these cells induced by the toxic effect of Hb C on cell membranes. Another effect of this dramatic cellular dehydration is the generation of target cells, which are far more prevalent on the peripheral smear than sickled forms (Figure 7-7). Finally, in Hb SC disease, the increased hematocrit combined with the higher MCH concentration (MCHC) and cellular dehydration results in higher whole blood viscosity, which
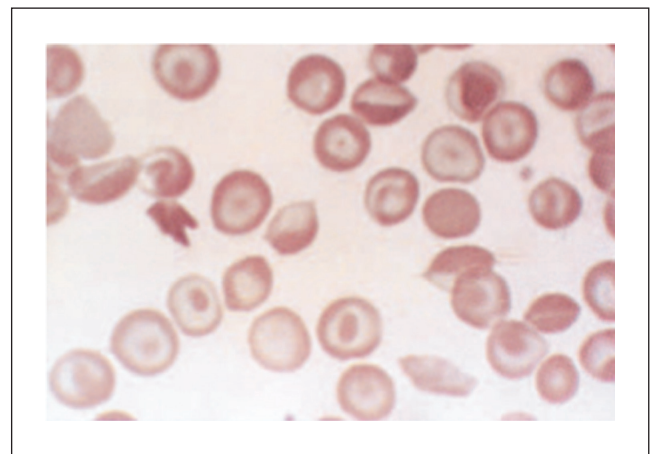

Figure 7-7 Sickle-hemoglobin C disease. This peripheral blood film shows no irreversibly sickled cells, as expected for hemoglobin SC, but shows instead a large number of target cells and several dense, contracted, and folded cells containing aggregated and polymerized hemoglobin.

Table 7-4 Important completed randomized clinical trials in sickle cell disease

Clinical trial	Year	Outcome
Penicillin Prophylaxis in Sickle Cell Disease (PROPS)	1986	Oral penicillin greatly reduces the incidence of invasive pneumococcal infections in children.
Penicillin Prophylaxis in Sickle Cell Disease II (PROPS II)	1995	Penicillin prophylaxis can be discontinued at 5 years of age.
Multicenter Study of Hydroxyurea in Patients With Sickle Cell Anemia (MSH)	1995	Hydroxyurea reduces the frequency of painful episodes and appears to reduce the frequency of acute chest syndrome, transfusions, and hospitalizations.
National Preoperative Transfusion Study	1995	Simple transfusion to increase the Hb concentration to 10 g/dL is as effective as exchange transfusion to reduce Hb S to <30%.
Stroke Prevention Trial in Sickle Cell Anemia (STOP)	1998	First overt stroke can be prevented with red blood cell transfusions in high-risk children identified by transcranial Doppler (TCD) ultrasonography.
Optimizing Primary Stroke Prevention in Sickle Cell Anemia (STOP 2)	2005	Discontinuation of prophylactic red blood cell transfusions after 30 months results in a high rate of reversion to abnormal TCD velocities and stroke.
Hydroxyurea to Prevent Organ Damage in Very Young Children With Sickle Cell Anemia (BABY HUG)	2011	Hydroxyurea starting at 9-18 months of age did not prevent splenic and renal damage (the trial's primary endpoints), but it did decrease the frequency of dactylitis and painful episodes (secondary outcomes).
Stroke with Transfusions Changing to Hydroxyurea (SWiTCH)	2012	Terminated early due to futility for the primary composite endpoint of recurrent stroke and resolution of iron overload. There was an excess of recurrent strokes in the hydroxyurea arm ($N = 7$) compared with continued transfusions ($N = 0$).

may increase the likelihood of vaso-occlusion. Patients with Hb Sβ^+ thalassemia have less severe anemia and pain than patients with Hb Sβ^0 thalassemia. This is the result of smaller cells, lower MCHC, increased content of Hb F and Hb A$_2$, and, most important, the presence of significant amounts (10%-30%) of Hb A that interferes with polymerization.

Treatment

Treatment of sickle cell disease includes general preventative and supportive measures, as well as treatment of specific complications. The National Institutes of Health recently published "Evidence-based management of sickle cell disease expert panel report, 2014: guide to recommendations" which is an excellent resource for addressing the spectrum of treatment issues. Table 7-4 summarizes the results of major clinical trials influencing current clinical practice.

Preventive interventions

Children should receive the 13-valent pneumococcal conjugate vaccine (PCV-13), the 4-valent meningococcal conjugate vaccine (MCV-4), the 23-valent pneumococcal polysaccharide vaccine (PPV-23), and vaccines against *Haemophilus influenzae* and hepatitis B virus, in addition to twice-daily penicillin prophylaxis at least until the age of 5 years. Vaccinations against influenza on an annual basis and the pneumococcal vaccine at 5-year intervals (after the childhood PCV-13 and PPV-23 vaccinations) should be administered to all patients. Folic acid supplements are used

by some to prevent depletion of folate stores and megaloblastic anemia related to chronic hemolysis, but this is probably unnecessary in industrial countries where diets are better and flour is fortified with folate. Screening transcranial Doppler (TCD) ultrasonography to determine risk of overt stroke should be performed at least yearly for children of age 2-16 years with Hb SS or Sβ^0 thalassemia (see further discussion of TCD in the sections "Central nervous system disease" and "RBC transfusion" in this chapter). Ophthalmologic examinations should be performed periodically beginning around age 10 years. Genetic counseling services by trained individuals should be available for families with members having sickle cell syndromes.

Painful episodes

Acute pain unresponsive to rest, hydration, and oral analgesics at home requires prompt attention and is the leading cause for hospitalization. Painful episodes can be associated with serious complications, and a high frequency of pain is a poor prognostic factor for survival. It is essential to consider infectious and other etiologies of pain in the febrile patient. A complete blood count should be obtained. Because some degree of negative fluid balance often is present, oral or intravenous hydration is important. Caution must be used with intravenous hydration of adults, especially, who may have decreased cardiac reserve. Prompt administration of analgesics is a priority, and the selection of agents should be individualized based on previous experience. Parenteral opioids, preferably morphine or hydromorphone, are often necessary

for both children and adults. The addition of nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen or ketorolac, may decrease the requirement for opioid analgesics but should be used with appropriate vigilance in sickle cell disease because of potential nephrotoxicity. Maintenance analgesia can be achieved with patient-controlled analgesia (PCA) pumps or with administration at fixed intervals. Constant infusion of opioids requires close monitoring because the hypoxia or acidosis resulting from respiratory suppression is particularly dangerous. Meperidine is discouraged because of its short half-life and the accumulation of the toxic metabolite normeperidine, which lowers the seizure threshold. Use of pain assessment instruments and attention to the level of sedation at regular intervals are necessary. Oxygen supplementation is not required unless hypoxemia is present. The use of incentive spirometry has been shown to reduce pulmonary complications in patients presenting with chest or back pain. It has been demonstrated that the number of hospitalizations for painful events can be reduced by prompt intervention in an outpatient setting dedicated to sickle cell disease management. Nonpharmacologic management techniques should be considered as well as evaluation for depression for the patient with frequent episodes or chronic pain. Blood transfusion is not indicated in the treatment of uncomplicated painful episodes.

Acute chest syndrome

The diagnosis of acute chest syndrome is based on a new radiographic pulmonary infiltrate associated with symptoms such as fever, cough, and chest pain. As the nonspecific term implies, various insults or triggers can lead to acute chest syndrome. Young age, low Hb F, high steady-state hemoglobin, and elevated white blood cell count in steady state have been identified as risk factors. In a multicenter prospective study, bacterial (often atypical) or viral infections accounted for approximately 30% of episodes, whereas fat emboli from the bone marrow were responsible for approximately 10% of events, with pulmonary infarction as another common suspected cause. In children, fever is a common presenting symptom, whereas chest pain is more common in adults. Acute chest syndrome often develops in patients who initially present only with an acute painful event. Early recognition of the condition is of utmost importance because acute chest syndrome has become the leading cause of death for both adults and children with sickle cell disease. Management includes maintaining adequate oxygenation and administration of antibiotics to address the major pulmonary pathogens and community-acquired atypical organisms. Fluid management needs particular attention to prevent pulmonary edema by limiting oral and intravenous hydration to 1.0-1.5 times maintenance (after

correction of any dehydration). Pain control to avoid excessive chest splinting and the use of incentive spirometry are key adjunctive measures. Bronchodilator therapy is effective if there is associated reactive airway disease, which is particularly common in children. Transfusion of RBCs should be considered if there is hypoxemia or acute symptomatic, exacerbation of anemia. Exchange transfusion should be performed for hypoxemia despite oxygen supplementation, widespread (bilateral, multilobar) infiltrates, and rapid clinical deterioration. Patients with acute chest syndrome are at risk for recurrences as well as subsequent chronic lung disease. Preventive measures include hydroxyurea therapy and chronic RBC transfusions.

Central nervous system disease

Without primary prevention, overt stroke may occur in 11% of young sickle cell anemia patients (but is much less common in SC disease and $S\beta^+$ thalassemia), accounting for significant morbidity and mortality. The more frequent use of neuroimaging has identified a substantial incidence of subclinical cerebrovascular disease, with 25%-40% of children having covert or silent strokes. The majority of overt strokes result from ischemic events involving large arteries with associated vascular endothelial damage, including intimal and medial proliferation. Hemorrhagic events are more common in adults and may result from rupture of collateral vessels (moya moya) near the site of previous infarction. Suspicion of a neurologic event requires emergent imaging with computed tomography (CT) to assess for hemorrhage followed by MRI. The acute management of overt stroke includes transfusion, usually by an exchange technique, to reduce the Hb S percentage to <30%. Chronic transfusion therapy to maintain the Hb S <30% decreases the chance of recurrent overt stroke but does not eliminate it. After 3-5 years of such transfusions and no recurrent neurologic events, some physicians "liberalize" the transfusion regimen to maintain the Hb S <50%. The optimal duration of transfusions is not known, and they often are continued indefinitely. A randomized controlled trial (the SWiTCH study) of continued chronic transfusions versus hydroxyurea for long-term secondary stroke prevention was stopped early due to futility, and there was an excess of recurrent strokes in the hydroxyurea arm ($N = 7$) compared with continued transfusions ($N = 0$).

An abnormally increased TCD blood flow velocity can identify children with Hb SS at high risk of primary overt stroke. A randomized controlled trial of prophylactic transfusions versus observation for children with abnormal TCD velocities showed a reduced risk of the first stroke in patients receiving transfusions (the STOP study). The use of hydroxyurea currently is being explored as means of primary stroke

prevention in a phase 3 multicenter randomized controlled trial for children with abnormal TCD velocities (the TWiTCH study).

Silent cerebral infarcts are the most common neurologic complications in children with sickle cell anemia. A randomized clinical trial assigned children ages 5-15 with sickle cell anemia to receive regular blood transfusions or observations. Regular blood-transfusion therapy significantly reduced the incidence of the recurrence of cerebral infarct (6% in treatment arm versus 14% in observation arm).

Pregnancy

Pregnancy poses some risk to the mother as well as to the fetus. Spontaneous abortions occur in approximately 5% of pregnancies in sickle cell anemia, and preeclampsia occurs at an increased frequency in sickle cell disease. Preterm labor and premature delivery are common. All patients should be followed in a high-risk prenatal clinic, ideally at 2-week intervals with close consultation with a hematologist. Patients should receive folic acid 1 mg/d, in addition to the usual prenatal vitamins, and should be counseled regarding the additional risks imposed by poor diet, smoking, alcohol, and substance abuse. Data do not support the routine use of prophylactic transfusions. Simple or exchange transfusions, however, should be instituted for the indications outlined previously, as well as for pregnancy-related complications (eg, preeclampsia). Close follow-up is indicated postpartum when the patient is still at high risk for complications. The option of contraception with an intrauterine device, subcutaneous implant, progesterone-only contraceptives, or condoms should be discussed with all women of childbearing age.

RBC transfusion

Patients with sickle cell disease often receive transfusions unnecessarily. RBC transfusions, however, may be effective for certain complications of the disease. Transfusion is indicated as treatment of specific acute events, including moderate to severe acute splenic sequestration, symptomatic aplastic crisis, cerebrovascular accident (occlusive or hemorrhagic), acute ocular vaso-occlusive events, and acute chest syndrome with hypoxemia. Although the first two events only require correction of anemia and thus are treated with simple transfusion, stroke, ocular events, and severe acute chest syndrome are best treated with exchange transfusion aimed at decreasing the percentage of Hb S to <30% and increasing the Hb level to 9-10 g/dL. In addition, transfusions are indicated for the prevention of recurrent strokes as well as for the treatment of high-output cardiac failure. As mentioned, an abnormal TCD velocity can identify children with Hb SS at high risk of primary overt stroke, which can be prevented by chronic transfusion therapy. Transfusion has also been advocated for patients with severe pulmonary hypertension and chronic nonhealing leg ulcers and to prevent recurrences of priapism, but clinical trial data are lacking. When chronic transfusion is indicated, RBCs may be administered as a partial exchange transfusion, which may offer a long-term advantage of delaying iron accumulation. The goal of chronic transfusion is usually to achieve a nadir total hemoglobin level of 9-10 g/dL with the Hb S under 30%-50%. It is important to avoid the hyperviscosity associated with hemoglobin levels >11-12 g/dL in the presence of 30% or more Hb S. Patients with Hb SC requiring transfusion pose special challenges, with the need to avoid hyperviscosity usually necessitating exchange transfusion (goal Hb A >70%) to ensure the hemoglobin concentration does not exceed 11-12 g/dL.

Preoperative transfusion in preparation for surgery under general anesthesia may afford protection against perioperative complications and death but is probably not indicated in all cases, particularly minor procedures in children. In a multicenter study, simple transfusion to a total hemoglobin level of 10 g/dL afforded protection equal to partial exchange and was associated with less red cell alloimmunization. Another recently completed randomized trial (TAPS) included patients with sickle cell anemia undergoing low- or medium-risk surgery. Subjects were randomized to either preoperative transfusion or no transfusion. Thirty-nine percent of 33 patients in the no-preoperative-transfusion group had clinically important complications, compared with 15% in the preopeative-transfusion group ($P = 0.023$). Patients undergoing prolonged surgery or with regional compromise of blood supply (eg, during orthopedic surgery), hypothermia, or a history of pulmonary or cardiac disease may do better with preoperative exchange transfusion. Transfusions also may be useful for some patients preparing for intravenous ionic contrast studies, dealing with chronic intractable pain, or facing complicated pregnancy. Transfusions are not indicated for the treatment of steady-state anemia, uncomplicated pain events, uncomplicated pregnancy, most leg ulcers, or minor surgery not requiring general anesthesia.

Up to 30% of patients with sickle cell disease who repeatedly undergo transfusion will become alloimmunized to RBC antigens (especially E, C, and Kell), and this risk increases with increasing exposure. Alloimmunization predisposes patients to delayed transfusion reactions. Severe painful crises with a decrease in the hemoglobin level within days to weeks of a transfusion should alert the clinician to consider this diagnosis. Identification of a new alloantibody may not be made acutely, and reticulocytopenia can be an associated finding. In this situation, additional transfusions are hazardous and should be avoided if at all possible. Universal RBC phenotyping and matching for the antigens of

greatest concern (eg, C, D, E, and Kell) can minimize alloimmunization.

Modifying the disease course

In addition to chronic transfusions, two other disease-modifying treatments currently are available: (i) hydroxyurea, which is ameliorative; and (ii) hematopoietic stem cell transplantation, which is curative. On the basis of knowledge that patients with high hemoglobin F levels have less severe disease, many investigators tested a variety of experimental strategies for pharmacologic induction of hemoglobin F production and identified hydroxyurea as efficacious and practical. A multicenter, randomized, placebo-controlled trial then found that daily oral administration of hydroxyurea significantly reduced the frequency of pain episodes, acute chest syndrome, and transfusions in adult Hb SS patients (MSH study). No serious short-term adverse effects were observed, although monitoring of blood counts was required to avoid potentially significant cytopenias. Interestingly, the therapeutic response to hydroxyurea sometimes precedes or occurs in the absence of a change in Hb F levels, suggesting that a reduction in white blood cell count and other mechanisms may be beneficial. Laboratory studies revealed that hydroxyurea reduced adherence of RBCs to vascular endothelium, improved RBC hydration, and increased the time to polymerization. Follow-up at 9 years indicates that patients taking hydroxyurea seem to have reduced mortality without evidence for an increased incidence of malignancy. Classical indications for hydroxyurea include frequent painful episodes, recurrent acute chest syndrome, severe symptomatic anemia, and other severe vaso-occlusive events. Given the safety of hydroxyurea and that Hb SS is a morbid condition, many clinicians use hydroxyurea more liberally even when the classical indications for hydroxyurea therapy are not present. There are now guideline recommendations to strongly consider the use of hydroxyurea in patients with sickle cell anemia who have daily chronic pain that interferes with quality of life. Clinical trials of hydroxyurea in children also show a reduction in the frequency of painful episodes, but no convincing evidence yet indicates that early hydroxyurea therapy prevents or delays the onset of organ damage. Pregnancy should be avoided while taking hydroxyurea. Hematopoietic stem cell transplantation has been used primarily for children with stroke or other severe disease manifestations, with an event-free survival rate of >80%. In most centers, few patients meet the usual eligibility criteria, which includes an HLA-matched sibling donor. Alternative donor sources such as umbilical cord blood are now being used. Novel approaches such as nonmyeloablative conditioning regimens and haplotype transplants have shown early success with up to 60%

engraftment and minimal graft-versus-host disease in adults. These efforts are undergoing further development and, as the procedure becomes more refined, stem cell transplantation for patients with sickle cell disease could be greatly expanded.

Clinical case (continued)

The case in this section describes a patient with sickle cell anemia who has experienced pain episodes but no other major complications related to his disease. He is admitted for a pain crisis, and multiorgan failure ensues. Acute multiorgan failure is a well-described complication of sickle cell disease. High baseline hemoglobin levels may represent a key risk factor. Acute multiorgan failure often is precipitated by a severe acute pain crisis and is thought to be secondary to widespread intravascular sickling, fat embolization, and subsequent ischemia within affected organs. Aggressive transfusion therapy can be lifesaving and result in complete recovery.

Key points

• The clinical manifestations of sickle cell disease are primarily due to hemolysis and vaso-occlusion.
• Multiple cellular and genetic factors contribute to the phenotypic heterogeneity of sickle cell disease.
• The hemoglobin F level is a major determinant of clinical manifestations and outcomes.
• Pneumococcal sepsis is now uncommon, but it remains a potential cause of death in infants and young children, so universal newborn screening, compliance with penicillin prophylaxis, and vaccination remain a priority.
• Human parvovirus infection is the cause of aplastic crisis.
• Splenic sequestration is a consideration in the differential diagnosis of a sudden marked decrease in the hemoglobin concentration.
• There are differences in frequency of clinical events and survival among the various genotypes of sickle cell disease.
• Sickle cell disease is a leading cause of stroke in young individuals, and a substantial incidence of covert or silent infarctions recently has been appreciated.
• A randomized clinical trial has demonstrated efficacy of red cell transfusion in preventing first stroke in children with abnormal TCD velocity.
• A randomized clinical trial demonstrated that preoperative simple transfusion was as effective as exchange transfusion. The preoperative management of the older patient, particularly with cardiac or pulmonary dysfunction, has not been defined.
• A randomized, placebo-controlled clinical trial has established the efficacy of hydroxyurea in reducing the frequency of painful episodes and acute chest syndrome. A follow-up study suggests a reduction in mortality for patients taking hydroxyurea.
• The causes of acute chest syndrome include infection, fat embolism, and pulmonary infarction.

Hemoglobin E

Hb E is a β chain variant with highest frequency in Southeast Asia. The highest prevalence occurs in Myanmar and Thailand, where the gene frequency may approach 70% in certain regions. The gene frequency is also high in Laos, Cambodia, and Vietnam. It is also found in Sri Lanka, North East India, Nepal, Bangladesh, Malaysia, Indonesia, and the Philippines. It has become more common in the United States during the past 20-30 years as a result of immigration. The structural change is a substitution of glutamic acid by lysine at the 26th position of the β-globin chain (Figure 7-4). The mutation is also thalassemic because the single-base GAG/AAG substitution creates a cryptic splicing site, which results in abnormal mRNA processing and reduction of mRNA that can be translated. Hb E is also slightly unstable in the face of oxidant stress. Hb E is sometimes referred to as a "thalassemic hemoglobinopathy."

Individuals with hemoglobin E trait are asymptomatic with or without mild anemia (hemoglobin >12 g/dL), and mild microcytosis. Peripheral smear may be normal or may show hypochromia, microcytosis, target cells, irregularly contracted cells, and basophilic stippling. HbE usually makes up 30% or less of total hemoglobin. The HbE concentration will be lower with the coinheritance of α-thalassemia. Homozygotes (Hb E disease) are usually asymptomatic with no overt hemolysis or splenomegaly. Individuals may have mild anemia, microcytosis (MCV approximately 65-69 fL in adults and 55-65 fL in children), and reduced MCH. Peripheral smear will show hypochromia, microcytosis, and a variable number of target cells and irregularly contracted cells. HbE plus HbA2 will make up 85%-99% of the total hemoglobin. The compound heterozygous state, HbE-β thalassemia, results in a very variable phenotype ranging from thalassemia trait, thalassemia intermedia to thalassemia major depending on the β mutation. It is now one of the more common forms of thalassemia in the United States. It is characterized by microcytic anemia, with mildly increased reticulocytosis. The peripheral smear will include anisocytosis, poikilocytosis, hypochromia, microcytosis, target cells, nucleated red blood cells, and irregularly contracted cells. HbE-β^0 thalassemia is associated with a mostly HbE electrophoretic pattern, with increased amounts of HbF and HbA$_2$. The electrophoretic pattern in HbE-β^+ thalassemia is similar except for the presence of approximately 15% HbA. HbE comigrates with HbC and HbA$_2$ on cellulose acetate electrophoresis and isoelectric focusing.

Patients with HbE disease are usually asymptomatic and do not require specific therapy. However, patients who coinherited HbE and β-thalassemia, especially those with HbE-β^0, may have significant anemia. Some need intermittent or chronic RBC transfusions, and some may benefit from splenectomy.

Hemoglobin C

Hb C is the third most common mutant hemoglobin, after Hb S and Hb E. The Hb C mutation arose in West Africa. The prevalence in African Americans is 2%-3%. The hemoglobin mutant results from the substitution of lysine for glutamic acid as the sixth amino acid of β-globin, the consequence of a single nucleotide substitution (GAG/AAG) in the sixth codon (Figure 7-5). The resultant positive-to-negative charge difference on the surface of the Hb C tetramer results in a molecule with decreased solubility of both the oxy and deoxy forms that may undergo intraerythrocytic aggregation and crystal formation. Hb C stimulates the K:Cl cotransport system, promoting water loss and resulting in dehydration and poorly deformable RBCs that have a predilection for entrapment within the spleen. Consequently, patients with Hb CC and patients with Hb C-β thalassemia have mild chronic hemolytic anemia and splenomegaly. Patients may develop cholelithiasis, and the anemia may be more exaggerated in association with infections. Heterozygous individuals (Hb C trait) are clinically normal, however, identifying the diagnosis is important for genetic counseling. The coinheritance of Hb S and Hb C results in a form of sickle cell disease, Hb SC (see the section "Sickle cell disease" in this chapter).

Laboratory studies in Hb CC show a mild hemolytic anemia, microcytosis, and slightly elevated reticulocyte counts. The MCHC is elevated because of the effect of Hb C on cellular hydration. The peripheral blood smear shows prominent target cells, microcytosis, and irregularly contracted red cells. RBCs containing hemoglobin crystals also may be seen on the blood smear, particularly in patients who have had splenectomy. Individuals with Hb C trait have normal hemoglobin levels, and microcytosis is common. The peripheral smear may be normal or may show microcytosis and target cells. Confirmation of the diagnosis requires identification of Hb C; which comigrates with Hb A$_2$, Hb E, and Hb O^{Arab} on cellulose acetate electrophoresis and isoelectric focusing. Thus, Hb C must be distinguished by citrate gel electrophoresis or HPLC.

Specific treatment for patients with Hb CC is not generally necessary.

Hemoglobin D

Hb D is usually diagnosed incidentally. Hb D^{Punjab} (also called HbD$^{Los Angeles}$) results from the substitution of glutamine for glutamic acid at the 121st position of the β-chain (Figure 7-4). This mutant has a prevalence of approximately 3% in the Northwest Punjab region of India but also is encountered in other parts of the world. Patients who are homozygous (Hb DD) may have a mild hemolytic anemia. Individuals who are heterozygous (Hb AD) are clinically

normal with normal blood counts and a peripheral smear with the occasional target cell. The major clinical relevance of Hb D is with compound heterozygous inheritance with HbS, resulting in a form of sickle cell disease, perhaps as a result of the low-affinity of Hb D promoting hemoglobin deoxygenation. The diagnosis of Hb AD (D trait) or Hb DD is made by hemoglobin electrophoresis. Hb S and Hb D have similar electrophoretic mobility on alkaline cellulose acetate electrophoresis and isoelectric focusing. They can be differentiated by acid citrate agar electrophoresis, HPLC, or solubility studies. This distinction is important for genetic and prognostic counseling.

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Key points

• Hemoglobins C, D, and E are common hemoglobin variants that can have significant consequences when coinherited with hemoglobin S.
• Homozygosity for hemoglobin E (EE) is a mild condition, but compound heterozygosity for HbE and β-thalassemia can be a clinically significant thalassemia syndrome.

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Unstable hemoglobin

Unstable hemoglobin variants are inherited in an autosomal dominant pattern, and affected individuals are usually heterozygotes. Unstable hemoglobins constitute one of the largest groups of hemoglobin variants, although individually, each is rare. In both Hb Köln (β_{98} Val/Met substitution) and In Hb Zurich (β_{63} His/Arg), the amino acid substitution destabilizes the heme pocket. Other mechanisms that destabilize hemoglobin include (i) alteration of the $\alpha_1\beta_1$ interface region (eg, Hb Tacoma, β_{30} Arg/Ser); (ii) distortion of the helical configuration of structurally important regions (eg, Hb Bibba, α_{136} Leu/Pro); and (iii) introduction of the interior polar amino acid (eg, Hb Bristol, β_{67} Val/Asp). Unstable γ-chain variants (eg, Hb Poole, γ_{130} Trp/Gly) can cause transient hemolytic anemia in the neonate that will spontaneously resolve.

These abnormal hemoglobins precipitate spontaneously or with oxidative stress. Precipitated hemoglobin inclusions (Heinz bodies seen using a supravital stain) impair erythrocyte deformability, resulting in premature erythrocyte destruction by macrophages of the liver and spleen. The severity of the hemolysis varies with the nature of the mutation but may be accelerated by fever or ingestion of oxidant drugs.

An unstable hemoglobinopathy should be suspected in a patient with hereditary nonspherocytic hemolytic anemia. The hemoglobin level may be normal or decreased. Hypochromia of the RBCs (resulting from loss of hemoglobin

due to denaturation and subsequent pitting), "bite cells," and basophilic stippling may occur. The evaluation includes hemoglobin electrophoresis (which is often normal), crystal violet Heinz body staining, and the isopropanol stability test. The isopropanol test may be falsely positive in the neonate due to high fetal hemoglobin levels, so the heat-stability test should be used during the first months of life. Management includes avoidance of oxidant agents, and some recommend supplementation with folic acid. Splenectomy may be useful for patients with severe hemolysis and splenomegaly. The risk of thrombosis is high after splenectomy in individuals with a severely unstable hemoglobin, and thus, patients should be educated and closely evaluated in this regard.

Hemoglobin M includes various α, β, and γ chain variants that are associated with an increased tendency to oxidation to methemoglobin resulting in cyanosis and hemolysis. Some unstable hemoglobins also may have altered oxygen affinity, which could exacerbate (decreased oxygen affinity) or ameliorate (increased oxygen affinity) the degree of anemia.

Abnormalities of the RBC membrane

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Clinical case

A 36-year-old woman is referred for evaluation of moderate anemia. She has been told she was anemic as long as she can remember, and she has intermittently been prescribed iron. She occasionally has mild fatigue but is otherwise asymptomatic. Her past history is significant only for intermittent jaundice and a cholecystectomy for gallstones at age 22 years. She takes no medications. A cousin and an aunt have also had anemia and jaundice. Her examination is significant for mild splenomegaly. Prior laboratory data reveal hematocrit values between 29% and 33%. Today's hematocrit is 27%, MCV 98 fL, MCHC 38 g/dL. Corrected reticulocyte count is 7%. Review of the peripheral blood smear reveals numerous spherocytes.

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Hereditary spherocytosis (HS), hereditary elliptocytosis (HE), and hereditary pyropoikilocytosis (HPP) are a heterogeneous group of disorders with a wide spectrum of clinical manifestations. This group of disorders is characterized by abnormal shape and flexibility of RBCs because of a deficiency or dysfunction of one or more of the membrane proteins, which leads to shortened RBC survival (hemolysis). Multiple genetic abnormalities, including deletions, point mutations, and defective mRNA processing, have been identified as underlying causes. The HS syndromes generally are due to private mutations unique to each kindred. In contrast, some HE syndromes are due to specific mutations in individuals from similar locales (eg, Melanesian elliptocytosis), suggesting a founder effect.

RBC membrane protein composition and assembly

The RBC membrane consists of a phospholipid-cholesterol lipid bilayer intercalated by integral membrane proteins such as band 3 (the anion transport channel) and the glycophorins (Figure 7-8). This relatively fluid layer is stabilized by attachment to a membrane skeleton. Spectrin is the major protein of the skeleton, accounting for approximately 75% of its mass. The skeleton is organized into a hexagonal lattice. The hexagon arms are formed by fiber-like spectrin tetramers, whereas the hexagon corners are composed of small oligomers of actin that, with the aid of other proteins (4.1 and adducin), connect the spectrin tetramers into a two-dimensional lattice. The membrane cytoskeleton and its fixation to the lipid-protein bilayer are the major determinants of the shape, strength, flexibility, and survival of RBCs. When any of these constituents are altered, RBC survival may be shortened.

A useful model to understand the basis for RBC membrane disorders divides membrane protein–protein and protein–lipid associations into two categories. Vertical interactions are perpendicular to the plane of the membrane and involve a spectrin–ankyrin–band 3 association facilitated by protein 4.2 and attachment of spectrin–actin–protein 4.1 junctional complexes to glycophorin C. Horizontal interactions, which are parallel to and underlying the plane of the membrane, involve the assembly of α- and β-spectrin chains into heterodimers, which self-associate to form tetramers. Because the distal ends of spectrin bind to actin, with the aid of protein 4.1 and other minor proteins, a contractile function of the cytoskeleton may be important for normal RBC survival. Conceptually, HS is caused by defects in vertical protein–protein interactions in the RBC membrane, whereas HE is caused by defects in horizontal interactions (Figure 7-8).

Hereditary spherocytosis

HS is common in individuals of Northern European descent with an occurrence of approximately 1 in 2,000. Penetrance is variable, and the prevalence of a clinically recognized disorder is much lower. In 75% of cases, the inheritance pattern is autosomal dominant with sporadic cases representing the remaining 25%, half of which represent an autosomal recessive inheritance pattern and the other half de novo mutations. HS is characterized by spherocytic, osmotically fragile RBCs and is both clinically and genetically heterogeneous.

Pathophysiology

The pathophysiology of HS generally involves aberrant interactions between the skeleton and the overlying lipid
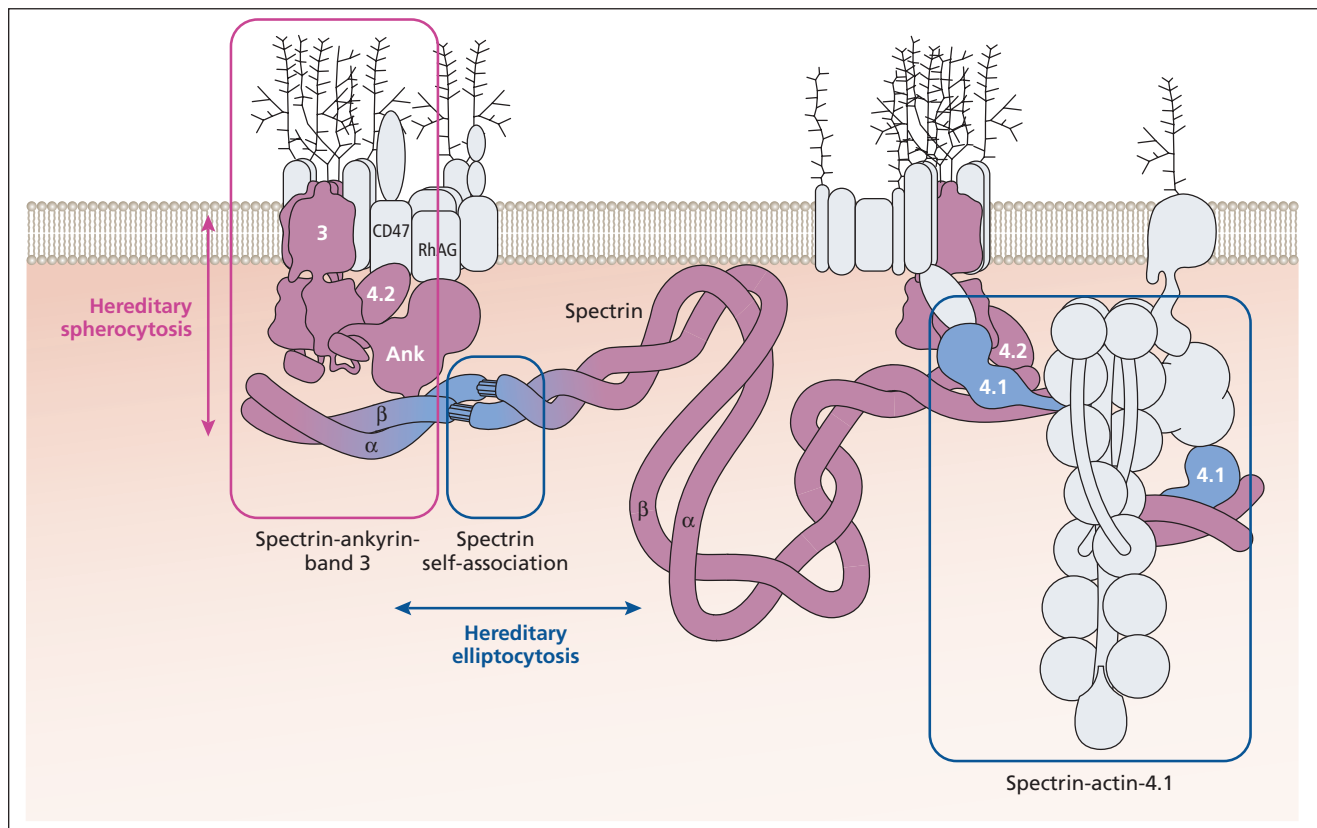

Figure 7-8 Membrane defects in hereditary spherocytosis and elliptocytosis. Redrawn with permission of Elsevier from Lux S. In: Orkin S et al. eds. *Nathan and Oski's Hematology and Oncology of Infancy and Childhood*, 8th ed., Philadelphia, PA: W.B. Saunders; 2015:515-579.

Table 7-5 Defects of red blood cell membrane proteins in hereditary spherocytosis, elliptocytosis, and pyropoikilocytosis

Class of defect	Hereditary spherocytosis	Hereditary elliptocytosis and pyropoikilocytosis
Protein deficiency	Spectrin	Spectrin[†]
	Ankyrin*	Protein 4.1
	Band 3	Glycophorin C
	Protein 4.2	
Protein dysfunction	β-spectrin abnormality affects	Defective spectrin dimer self-association due to spectrin mutations
	β-spectrin-protein 4.1 interaction*	Protein 4.1 abnormality affects β-spectrin-protein 4.1 interaction

*Red cells of the patients are also partially deficient in spectrin.

[†]Seen in patients with hereditary pyropoikilocytosis in cases in which it coexists with a spectrin mutation that affects spectrin self-association.

bilayer ("vertical interactions"). A common epiphenomenon in HS RBCs is a varying degree of spectrin loss, which is usually due to a defect in one of the membrane proteins involved in the attachment of spectrin to the membrane rather than a primary defect in the spectrin molecule itself. Spectrin as the major protein of the skeleton forms a nearly monomolecular submembrane layer that covers most of the inner-membrane surface; therefore, the density of this skeletal layer in HS erythrocytes is reduced. Consequently, the lipid bilayer is destabilized, leading to loss of membrane lipid and, thus, surface area through microvesiculation. The result of these changes is a progressively spheroidal RBC. The inherent reduced deformability of spherocytes makes it difficult for them to traverse the unique constraining apertures that characterize splenic vascular walls. The spleen "conditions" RBCs, enhancing membrane loss. Retained and further damaged by the hypoxic and acidic environment in the spleen, they ultimately are destroyed prematurely.

The molecular basis of HS is heterogeneous (Table 7-5). A deficiency or defect of the ankyrin molecule represents the most common cause of dominant HS. In 30%-45% of cases, the defect includes both ankyrin and spectrin deficiency, in 30% spectrin only, and in 20% band 3 mutations. Various mutations of the ankyrin gene have been identified. Multiple band 3 mutations have been described. Although less frequent, mutations of the β-spectrin gene have been found in autosomal dominant HS, whereas α spectrin gene abnormalities have been identified only in recessively inherited HS. Mutations in the protein 4.2 gene have been found primarily in Japanese patients with autosomal recessive HS.

Clinical manifestations

The clinical expression ranges from an asymptomatic and often undiagnosed condition with nearly normal hemoglobin levels (compensated hemolysis) to severe hemolysis and anemia. Patients with mild HS have a relatively uneventful course, although some may develop pigmented (bilirubinate) gallstones in childhood or adult life. Mildly anemic patients may be diagnosed later in life as adults during evaluation for unrelated conditions. Patients with moderately severe disease may present with several additional complications. Aplastic crisis, which may be the initial presentation for some patients, may require urgent attention. The cause of aplastic crisis is human parvovirus infection, which produces selective suppression of erythropoiesis, resulting in reticulocytopenia and inability to compensate for ongoing RBC destruction. In contrast, the "hyperhemolytic crisis" is characterized by accelerated hemolysis, leading to increased jaundice and splenic enlargement, which is a common problem in children. Other complications include the rare megaloblastic crisis secondary to acquired folic acid deficiency usually associated with high-demand situations, such as pregnancy. Leg ulcerations have been rarely reported. Patients with severe hemolysis and resulting expansion of the erythroid compartment in the bone marrow can develop maxillary hyperplasia interfering with dentition or extramedullary hematopoietic masses that may mimic malignancy. Patients may manifest a variety of issues attributable to splenomegaly, including early satiety, left upper-quadrant fullness, and hypersplenism. HS may be diagnosed in the neonatal period based on a positive family history or marked jaundice. The diagnosis also should be considered in patients of all ages with intermittent jaundice, mild "refractory" anemia, or splenomegaly. Rare associated syndromes suggest that mutant RBC membrane proteins may reside in other tissues. For example, distal renal tubular acidosis may occur in HS patients harboring mutant band 3 (the anion channel protein).

Laboratory evaluation

In addition to the usual laboratory abnormalities indicating hemolysis, the principal diagnostic feature is the identification of spherocytes on the peripheral blood smear (Figure 7-9a). The extent of spherocytosis is variable and, in mild cases, it may be missed even by the experienced clinician. Additional morphologic abnormalities, including cells with membrane extrusions and elliptocytes, may be observed. The RBC indices may provide a clue, with an increase in the
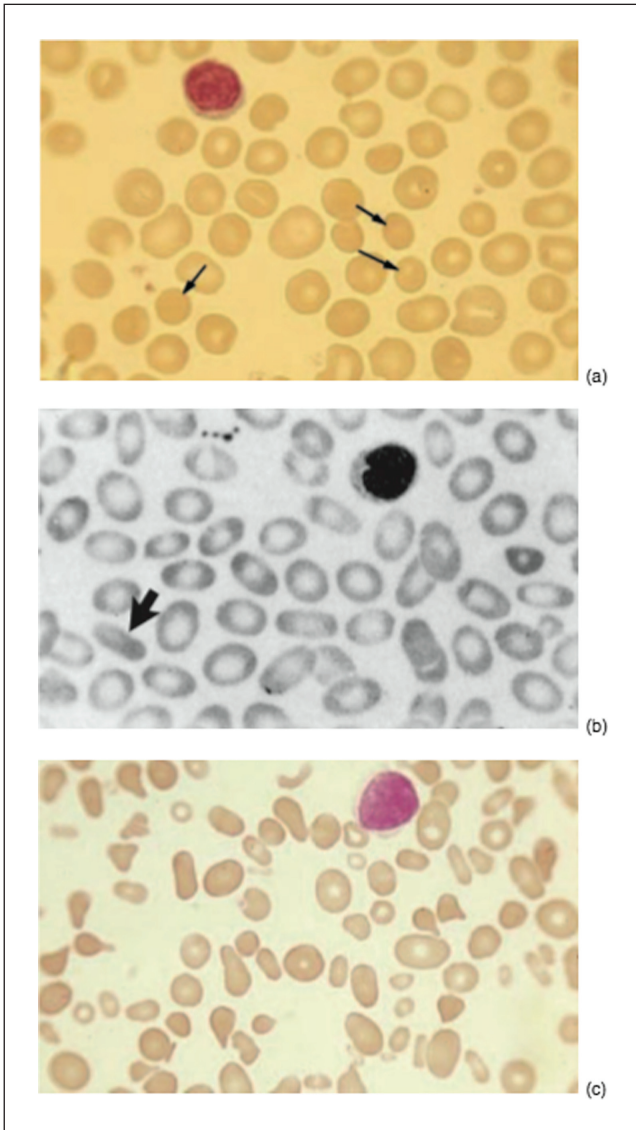
Figure 7-9 Peripheral blood findings in inherited disorders of the red cell membrane: (a) numerous spherocytes (arrows), (b) numerous elliptocytes and a rod-shaped cell (arrow), and (c) marked poikilocytosis.

MCHC (due to cellular dehydration) even in the context of minimal anemia. The osmotic fragility test using increasingly hypotonic saline solutions will support the diagnosis with the finding of increased RBC lysis compared with normal RBCs. Sensitivity of the test is enhanced by 24-hour incubation at 37°C, but mild cases still can be missed by the test. Osmotic fragility testing is a test for spherocytes of any cause, not a specific test for HS. Related to standard osmotic fragility testing is the newer osmotic gradient ektacytometry, which measures deformability of whole RBCs measured as a function of osmolality using a laser-diffraction viscometer. Ektacytometry appears to be more sensitive than standard osmotic fragility testing, and it can help differentiate HS

from HE, HPP, and stomatocytosis. A different test for HS and related cytoskeleton-associated hemolytic anemias is the eosin-5-maleimide (EMA) binding assay. EMA binds to band 3 on RBCs, and a reduction in binding, measured by fluorescence intensity, corresponds to a quantitative reduction in erythrocyte band 3, consistent with HS. The EMA-binding test has a higher predictive value for HS than standard osmotic fragility testing. Review of the complete blood count, reticulocyte count, and peripheral smear from family members may prove helpful. The differential diagnosis for spherocytes and increased osmotic fragility include autoimmune hemolytic anemia, so a direct antiglobulin test (DAT) should be performed as part of the evaluation when the family history is negative. Likewise, HS should be considered in the differential diagnosis of DAT-negative hemolytic anemia.

Treatment

As with other hemolytic anemias, folic acid supplementation should be considered for patients with severe anemia, even though overt folic acid deficiency rarely is encountered in the industrial nations due to supplementation in grain products. Patients need to be aware of the signs and symptoms of aplastic and hyperhemolytic crises to seek prompt medical attention. The definitive treatment of HS is splenectomy, which ameliorates the hemolytic anemia in almost all patients, although the underlying intrinsic defect of the circulating RBCs is not altered. In rare patients with HS and severe hemolysis, the procedure markedly diminishes the hemolytic rate but may not fully correct the anemia. Controlled clinical trial data are not available to provide guidelines in making the decision to recommend splenectomy. Thus, the indications for splenectomy are somewhat controversial, but the prevailing view advocates surgery for patients with symptomatic hemolytic anemia or its complications, particularly gallstones. Additional considerations for splenectomy in the pediatric population include failure to thrive, recurrent hyperhemolytic episodes, or complications of chronic anemia, including a hypermetabolic state. The laparoscopic technique often is preferred to open splenectomy. Accessory spleens are common, so a thorough search should be performed at the time of splenectomy. The patient should receive pneumococcal, *H. influenzae* type b, and meningococcal vaccines before the procedure, and pediatric patients usually receive prophylactic penicillin for at least several years thereafter to reduce the risk of bacterial sepsis. Thromboembolic events may occur following splenectomy, although data are limited. Because of the increased frequency of postsplenectomy infections in young children, splenectomy should not be performed before the age of 5 years except in patients with particularly severe disease.

Partial splenectomy has been advocated to resolve the anemia of HS yet maintain some residual splenic phagocytic function. Long-term results of partial splenectomy (4-6 years) in small observational studies are promising, but the spleen may increase in size and the hemoglobin concentration may fall after splenectomy. Markers of splenic function indicate variable degrees of residual activity, but postoperative penicillin is recommended.

Clinical case (continued)

The patient presented in this section should be suspected of having HS. It is not uncommon for the diagnosis to be made in adulthood, as patients with mild or moderate disease are often well compensated. An elevated reticulocyte count, elevated MCHC, intermittent jaundice, history of gallstones, and spherocytes on peripheral smear all support the diagnosis. The diagnosis should be confirmed by demonstrating increased osmotic fragility of RBCs, especially if the spherocytosis is not obvious on the peripheral blood film, and by a negative DAT. Family members should be evaluated for anemia.

Key points

• HS is the most common inherited hemolytic anemia of individuals from Northern Europe.
• Abnormalities in ankyrin, spectrin, band 3, and protein 4.2 ("vertical interactions") that result in a reduction in the quantity of spectrin account for the red cell membrane loss characteristic of HS.
• HS should be suspected in cases of direct antiglobulin test-negative hemolytic anemia when spherocytes are identified on the peripheral blood smear. A positive family history is supportive of the diagnosis.
• Clinical manifestations of HS vary from a lack of symptoms to severe hemolysis.
• The diagnosis of HS can be supported by the osmotic fragility test, the sensitivity of which is increased with incubation at 37°C. Osmotic gradient ektacytometry and the EMA-binding assay are newer, often more sensitive, tests for membranopathies like HS and HE.
• Splenectomy decreases hemolysis and reduces gallstone formation, but it should be reserved for symptomatic or severe patients.

Hereditary elliptocytosis and hereditary pyropoikilocytosis

The clinical presentation, inheritance, and alteration in RBC shape and physical properties and the underlying molecular defects are considerably more heterogeneous in HE than in HS. Three distinct subtypes are distinguished: (i) common HE, characterized by biconcave elliptocytes and, in more severe forms, rod-shaped cells, poikilocytes, and fragments (Figure 7-9b); (ii) spherocytic HE, a phenotypic hybrid between HE and HS; and (iii) Southeast Asian ovalocytosis with unique spoon-shaped erythrocyte morphology. Clinical manifestations range from asymptomatic carrier state to severe transfusion-dependent hemolytic anemia with poikilocytosis and erythrocyte fragmentation. In most cases, the inheritance of HE is autosomal dominant. The exception is HPP, a rare and severe variant of common HE that is recessively inherited (Figure 7-9c).

Pathophysiology

The underlying defects involve horizontal interactions between proteins of the membrane skeleton, especially spectrin–spectrin and spectrin–protein 4.1 interactions. These defects weaken the skeleton. Under the influence of shear stress in the microcirculation, the cells progressively lose the ability to regain the normal disc shape and are stabilized in the elliptocytic or poikilocytic shape. In severely affected patients, the weakening of the skeleton grossly diminishes membrane stability, leading to RBC fragmentation.

Different underlying molecular defects have been identified in common HE, consistent with the heterogeneous nature of the disorder (Table 7-5). In the majority of cases, patients have mutant α- or β-spectrin, resulting in defective self-association and an increased percentage of spectrin heterodimer in the membrane. A partial or complete absence or dysfunction of protein 4.1 occurs in some patients with missense and deletion mutations. Patients with HPP appear to be compound heterozygotes. Coinheritance of a mutation leading to spectrin deficiency and a mutation of spectrin resulting in a qualitatively defective molecule has been identified in some patients with the condition. Southeast Asian ovalocytosis is prevalent among certain ethnic groups in Malaysia, the Philippines, Papua New Guinea, and probably other Pacific countries as well. It is an asymptomatic condition characterized by rigid RBCs of a unique spoon-shaped morphology. Affected individuals are heterozygous for a mutation of band 3.

Clinical manifestations, laboratory evaluation, and treatment

HE must be differentiated from a variety of other conditions in which elliptocytes and poikilocytes commonly are found on the peripheral blood smear, including iron deficiency, thalassemia, megaloblastic anemia, myelofibrosis, and myelodysplasia. As opposed to HE, however, the percentage of elliptocytes in these other conditions usually does not exceed 60%. The presence of elliptocytes and evidence of dominant inheritance of elliptocytosis in other family

members differentiate HE from the previous conditions. Whereas most patients with common HE are asymptomatic, occasional patients who are homozygotes or compound heterozygotes for 1 or 2 molecular defects have more severe hemolytic disease. African American neonates with common HE may have severe hemolysis, with striking RBC abnormalities similar to HPP, which abates during the initial months of life. Approximately 10% of HE patients and all HPP patients have mild-to-moderate anemia with clinical features of pallor, jaundice, anemia, and gallstones. The most severe form of elliptocytosis, HPP, typically is inherited recessively and is characterized by a striking micropoikilospherocytosis and fragmentation with some elliptocytes. A markedly low MCV, typically in the range of 50 to 60 fL, may be observed. In HPP, RBCs are thermally unstable and fragment at temperatures of 46°C-48°C, reflecting the presence of mutant spectrin in the cells. Additional specialized laboratory investigation includes separation of solubilized membrane proteins by polyacrylamide gel electrophoresis, which may reveal either an abnormally migrating spectrin or a deficiency or abnormal migration of protein 4.1. An increased fraction of unassembled dimeric spectrin in the extract can be detected by electrophoresis of extracts under nondenaturing conditions.

Treatment is not necessary for most individuals with common HE. Splenectomy may be of benefit for patients with symptomatic hemolytic anemia or its complications (see earlier discussion of splenectomy for hereditary spherocytosis).

Key points

- HE is due to defects in the interactions of red cell cytoskeleton proteins ("horizontal interactions"), with spectrin abnormalities accounting for most of the cases.
- The majority of patients with HE are not symptomatic and require no therapy.
- HPP is a rare condition with apparent coinheritance of spectrin defects leading to markedly abnormal red cells characterized by increased thermal instability.

Other RBC membrane disorders

Stomatocytosis

Stomatocytes have a wide transverse slit or stoma toward the center of the RBC (Figure 7-11). A few stomatocytes (between 3% and 5%) are found on blood smears of healthy individuals. Several inherited and acquired disorders are associated with stomatocytosis. The inherited forms are associated with abnormalities in erythrocyte cation permeability and volume, which is either increased (hence, the designation hydrocytosis), decreased (xerocytosis), or near normal.
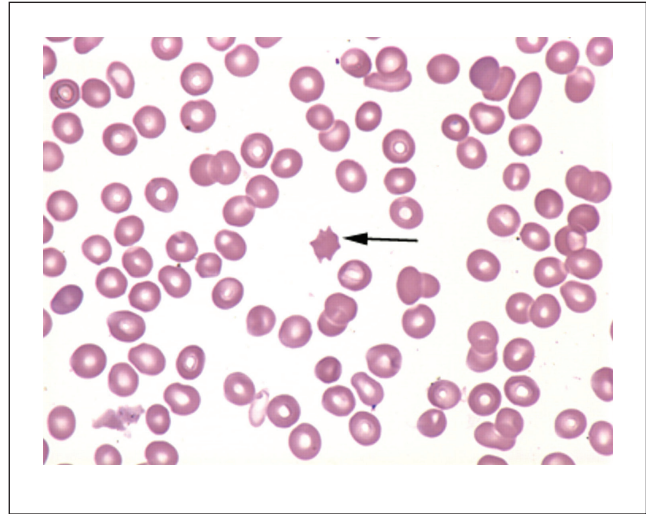

Figure 7-10 Acanthocytes.

Of significant clinical importance is the recognition that patients with hereditary stomatocytosis have an increased risk of developing thrombotic events after splenectomy. Acquired stomatocytosis can be seen in acute alcoholism and hepatobiliary disease (although target cells are more common) and occasionally in malignant neoplasms and cardiovascular disorders. Stomatocytes also may occur as an artifact.

Acanthocytosis

Spur cells, or acanthocytes (from the Greek *acantha*, or thorn; Figure 7-10), are erythrocytes with multiple irregular projections that vary in width, length, and surface distribution. Several conditions are associated with this morphology. In severe liver disease, acanthocyte formation is a two-step process involving the transfer of free nonesterified cholesterol from abnormal plasma lipoproteins into the
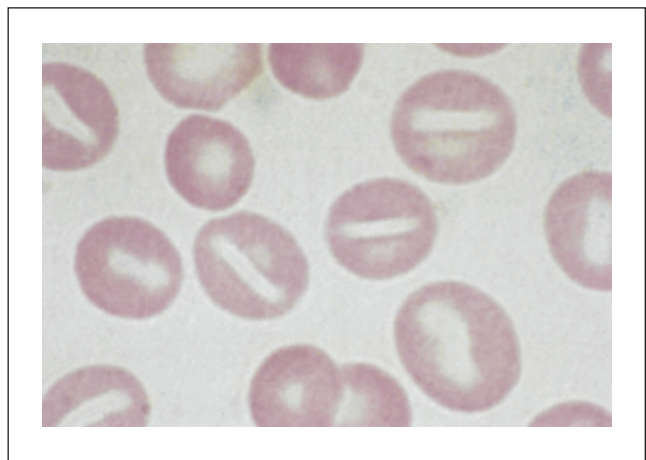

Figure 7-11 Stomatocytes.

erythrocyte membrane and then the subsequent remodeling of abnormally shaped erythrocytes by the spleen. Rapidly progressive hemolytic anemia is seen in association with advanced and often end-stage alcoholic cirrhosis, sometimes referred to as Zieve syndrome, or other conditions such as metastatic liver disease, cardiac cirrhosis, Wilson disease, and fulminant hepatitis.

In abetalipoproteinemia, the primary molecular defect involves a congenital absence of apolipoprotein B in plasma. Consequently, all plasma lipoproteins containing this apoprotein as well as plasma triglycerides are nearly absent. Plasma cholesterol and phospholipid levels also are markedly reduced. The role of these lipid abnormalities in producing acanthocytes is not well understood. The most striking abnormality of the acanthocyte membrane in abetalipoproteinemia is an increase in membrane sphingomyelin. Abetalipoproteinemia is an autosomal recessive disorder that manifests in the first month of life with steatorrhea. Retinitis pigmentosa and progressive neurologic abnormalities, such as ataxia and intention tremors, develop between 5 and 10 years of age and progress to death by the second or third decade of life.

Acanthocytes have also been described in patients with the McLeod phenotype, a condition in which the erythrocytes have reduced surface Kell antigen. The affected red cells lack the Kx protein which is a membrane precursor of the Kell antigen and needed for its expression. The Kx antigenic protein is encoded by the X chromosome, so males are affected with mild compensated hemolysis with variable acanthocytosis (8%-85%). Due to lyonization, female carriers are asymptomatic with occasional acanthocytes and may be identified by flow cytometric analysis of Kell blood group antigen expression. In some ethnicities, the frequency of the Kx antigen is >99%, and thus, individuals with the McLeod phenotype can develop major problems with alloimmunization after immunizing events such as a transfusion. As such, autologous donation should be considered where possible. The McLeod phenotype is a key feature of McLeod syndrome, a rare multisystem disease characterized by neuropsychiatric, neuromuscular, cardiac, and hematological abnormalities. The subtle hematological abnormalities may precede the neurological complications for decades until patients develop premature dementia, cognitive impairment, social retraction, personality changes, a choreatic movement disorder, or dystonia. McLeod phenotype has also been associated with X-linked granulomatous disease.

Rh deficiency (null) syndrome

This term is used to designate rare cases of either absent (Rh_{null}) or markedly reduced (Rh_{mod}) expression of the Rh antigen in association with mild to moderate hemolytic anemia. Three proteins (RhCE, RhD, and Rh50) comprise the Rh protein family. This disorder arises through autosomal recessive inheritance of either a suppressor gene unrelated to the Rh locus or a silent allele at the locus itself. The normal, complexed structure forms an integral membrane protein; its loss disrupts membrane architecture. Rh_{null} cells have increased rates of cation transport and sodium–potassium membrane adenosine triphosphate (ATP)-ase activity that results in dehydrated RBCs. This dehydration results in stomatocytes and occasional spherocytes on the peripheral blood smear. Laboratory evaluation shows increased RBC osmotic fragility, reflecting a marked reduction of the membrane surface area. The relationship between the absence of the Rh antigen proteins and RBC alterations leading to hemolysis presumably involves membrane microvesiculation, leading to diminished erythrocyte flexibility. Splenectomy results in improvement of the hemolytic anemia.

Abnormalities of RBC enzymes

Clinical case

A 23-year-old African American male who recently underwent cadaveric renal transplant for end-stage renal disease is referred for evaluation of anemia. His past history is significant for an episode of hemolytic uremic syndrome (HUS) that led to renal failure 2 years prior to referral. He had no further relapses of HUS or thrombotic thrombocytopenic purpura (TTP). His posttransplant course has been unremarkable with good graft function and no rejection. When he left the hospital, his hematocrit was 31%. His discharge medications included prednisone, cyclosporine, trimethoprim/sulfamethoxazole, and acyclovir. He complains of increasing fatigue and dyspnea over the 10 days since discharge. Friends have noted yellowing of his eyes. He denies any fever or infectious symptoms. On physical examination, he has a heart rate of 112, blood pressure (BP) of 89/45, and scleral icterus. Otherwise, the examination is unremarkable. Current hematocrit is 20%, corrected reticulocyte count 10%, LDH 1,543 U/L. Serum creatinine is 1.8 mg/dL and the platelet count is 302,000 /mm^3, similar to hospital discharge. On review of the peripheral blood smear, polychromatophilia is noted. A moderate number of bite and blister cells are identified.

Normal metabolism of the mature RBC involves two principal pathways of glucose catabolism: the glycolytic pathway and the hexose-monophosphate shunt. The three major functions of the products of glucose catabolism in the erythrocyte are (i) maintenance of protein integrity, cellular deformability, and RBC shape; (ii) preservation of hemoglobin iron in the ferrous form; and (iii) modulation of the oxygen affinity of hemoglobin. These functions are served by the regulation of appropriate production of five specific molecules: ATP, reduced glutathione, reduced NADH, reduced NADPH,

and 2,3-BPG. Maintenance of the biochemical and structural integrity of the RBC depends on the normal function of >20 enzymes involved in these pathways as well as the availability of five essential RBC substrates: glucose, glutathione, NAD, NAD phosphate (NADP), and adenosine diphosphate (ADP).

The primary function of the glycolytic pathway is the generation of ATP, which is necessary for the ATPase-linked sodium–potassium and calcium membrane pumps essential for cation homeostasis and the maintenance of erythrocyte deformability. The production of 2,3-BPG is regulated by the Rapoport–Luebering shunt, which is controlled by bisphosphoglyceromutase, the enzyme that converts 1,3-BPG to 2,3-BPG. Concentration of 2,3-BPG in the RBC in turn regulates hemoglobin oxygen affinity, thus facilitating the transfer of oxygen from hemoglobin to tissue-binding sites. The major function of the hexose-monophosphate shunt is preservation and regeneration of reduced glutathione, which protects hemoglobin and other intracellular and membrane proteins from oxidant injury.

Abnormalities of the glycolytic pathway

Defects in the glycolytic pathway lead to a decrease in the production of ATP or a change in the concentration of 2,3-BPG. Deficiencies of erythrocyte hexokinase, glucose phosphate isomerase, phosphofructokinase, and pyruvate kinase (PK) all lead to a decrease in ATP concentration. Although genetic disorders involving nearly all of the enzymes of the glycolytic pathway have been described, PK accounts for >80% of the clinically significant hemolytic anemias from defects in this pathway. With the exception of phosphoglycerate kinase deficiency, which is X-linked, all other glycolytic enzyme defects are autosomal recessive.

PK deficiency is the most common congenital nonspherocytic hemolytic anemia caused by a defect in glycolytic RBC metabolism. The syndrome is both genetically and clinically heterogeneous. PK deficiency has a worldwide distribution but is more common among those of northern and eastern European heritage. Severe cases can present either with neonatal jaundice or in early childhood with jaundice, splenomegaly, and failure to thrive. Alternatively, a mild presentation with fully compensated hemolytic anemia has been described. Osmotic fragility of the patient's RBCs is typically normal and may be helpful in differentiating this condition from HS. Several screening tests have been developed to diagnose PK deficiency, but often they lack sensitivity to diagnose specific PK variants. Reference laboratories can perform quantitative measurement of the erythrocyte enzyme level necessary to diagnose this condition accurately.

Both glucose phosphate isomerase and hexokinase deficiencies produce nonspherocytic hemolytic anemia associated with decreased erythrocyte ATP and 2,3-BPG content.
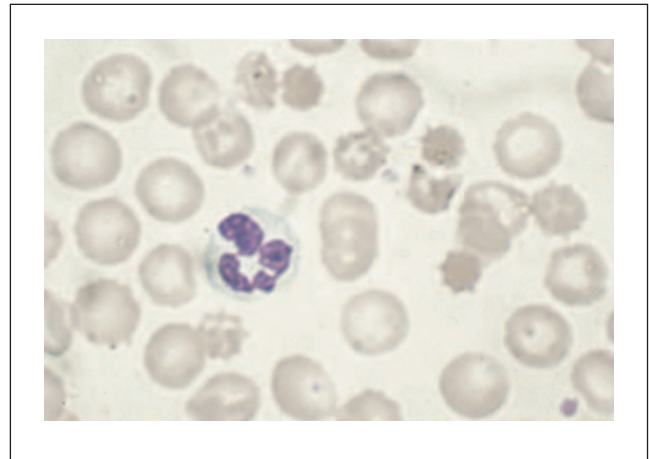

Figure 7-12 Pyruvate kinase deficiency. The peripheral blood film shows many small dense crenated cells (echinocytes).

These disorders are rare; patients often present in childhood with mild to moderate anemia and reduced exercise tolerance. A form of acquired hexokinase deficiency occurs in Wilson disease, in which elevated copper levels in the blood inhibit hexokinase in a fluctuating fashion that may lead to intermittent brisk intravascular hemolysis. Phosphofructokinase deficiency was first described as a muscle glycogen storage disease; some patients with this deficiency have a chronic hemolytic anemia. In phosphofructokinase deficiency, low levels of erythrocyte ATP lead to low-grade hemolysis, but the limiting symptoms are usually weakness and muscle pain on exertion. Children with phosphoglycerate kinase have associated neuromuscular manifestations, including seizures, spasticity, and mental retardation.

These enzymopathies are associated with anemia of variable severity. Peripheral blood smears from patients with PK deficiency show small dense crenated cells (echinocytes or "prickle cells") (Figure 7-12). In the most severe cases, marked reticulocytosis, nucleated RBCs, and substantial anisopoikilocytosis can be seen. The MCV is usually normal or increased, reflecting the contribution of reticulocytes. A marked increase in the reticulocyte count (up to 70%) occurs after splenectomy in PK deficiency.

Patients with severe hemolysis should receive folate supplementation. Splenectomy generally is reserved for patients with poor quality of life, chronic transfusion requirements, need for cholecystectomy, and persistent severe anemia. The response is variable, but most patients with PK deficiency benefit with an increase in the hemoglobin level. Splenectomy may be complicated by postoperative thromboembolic phenomena.

Abnormalities of the hexose-monophosphate shunt

G6PD deficiency is the most frequently encountered abnormality of RBC metabolism, affecting >200 million people worldwide.

A survival advantage has been noted in G6PD-deficient patients infected with *P. falciparum* malaria, possibly accounting for its high gene frequency, especially in endemic regions. The gene for G6PD is carried on the X chromosome and exhibits extensive polymorphism. Enzyme deficiency is observed in males carrying a variant gene. Females with a variant gene have two clonal RBC populations, one normal and one deficient; the clinical presentation depends on the extent of inactivation ("lyonization") of the affected X chromosome bearing the abnormal gene. Worldwide, >300 genetic variants of G6PD have been described and are categorized according to whether the defect leads to normal activity, moderately deficient activity, or severely deficient activity, and whether it is associated with hemolytic anemia. G6PD enzyme variants are distinguished based on electrophoretic mobility. G6PD B, the wild-type enzyme, and G6PD A^+, a common variant in the African American population, demonstrate normal enzyme activity and are not associated with hemolysis. G6PD A^- is present in approximately 10%-15% of African American males. This variant is an unstable enzyme, which results in a decrease in enzyme activity in aged RBCs. In contrast, other G6PD variants have reduced catalytic activity and marked instability or are produced at a decreased rate, rendering both reticulocytes and older cells susceptible to hemolysis. Enzymatic deficiency of this type is seen in up to 5% of persons of Mediterranean or Asian ancestry, as well as Ashkenazi Jews. The common example of this deficiency is the G6PD B variant, G6PD-Mediterranean.

Hemolysis in G6PD-deficient RBCs is due to a failure to generate adequate NADPH, leading to insufficient levels of reduced glutathione. This renders erythrocytes susceptible to oxidation of hemoglobin by oxidant radicals, such as hydrogen peroxide. The resulting denatured hemoglobin aggregates and forms intraerythrocytic Heinz bodies, which bind to membrane cytoskeletal proteins. Membrane proteins are also subject to oxidation, leading to decreased cellular deformability. Cells containing Heinz bodies are entrapped or partially destroyed in the spleen, resulting in loss of cell membranes through pitting of Heinz bodies and leading to hemolysis.

The severity of hemolytic anemia in patients with G6PD deficiency depends on the type of defect, the level of enzyme activity in the erythrocytes, and the severity of the oxidant challenge. Ingestion of an oxidant drug is sometimes the precipitating cause (Table 7-6). Hemolytic anemia in patients with G6PD deficiency may first be recognized during an acute clinical event that induces oxidant stress, such as infection, diabetic ketoacidosis, or severe liver injury. In children, infection is a common precipitating event. Hemolysis triggered by exposure to naphthalene (moth balls) is now much less common in children. Individuals with G6PD A^- do not

Table 7-6 Agents that cause clinically significant hemolysis in patients with G6PD deficiency

Acetanilide	Pentaquine
Dapsone	Phenylhydrazine
Dimercaptosuccinic acid	Phenazopyridine
Furazolidone	Primaquine
Glibenclamide	Sulfacetamide
Isobutyl nitrite	Sulfamethoxazole
Methylene blue	Sulfanilamide
Nalidixic acid	Sulfapyridine
Naphthalene	Thiazolesulfone
Niridazole	Toluidine blue
Nitrofurantoin	Trinitrotoluene (TNT)
Pamaquine	Urate oxidase

Data from Beutler E. In: Starbury JB et al. eds. *The Metabolic Basis of Inherited Disease.* 5th ed. New York: McGraw-Hill, 1983. Updated from Beutler E. In: Beutler E et al. eds. *Williams Hematology.* 6th ed. New York: McGraw-Hill, 2001.

manifest anemia until they are exposed to an oxidant drug or other oxidant challenge. Such an exposure may provoke an acute hemolytic episode with intravascular hemolysis. In the G6PD A^- variant, an adequate reticulocyte response can result in restoration of the hemoglobin concentration even if the offending drug is continued because the newly formed reticulocytes are relatively resistant to oxidant stress given their higher G6PD levels. Women heterozygous for G6PD A^- usually experience only mild anemia upon exposure to oxidant stress because a population of G6PD sufficient (normal) cells coexists. The G6PD-Mediterranean variant is more severe than the African G6PD A– variant and is thus prone to more severe hemolytic episodes. Men and heterozygous women with the G6PD-Mediterranean variant can experience severe hemolysis in the face of oxidant stress, and the offending agent must be removed because the reticulocytes have low enzyme levels and are prone to hemolysis.

Certain G6PD variants may result in a congenital nonspherocytic hemolytic anemia with persistent splenomegaly. Affected individuals are extremely susceptible to the oxidant stress associated with the drugs and disorders mentioned previously and also may exhibit severe hemolysis ("favism") after ingestion of fava beans. Hemolytic anemia due to favism may be severe or even fatal, particularly in children. G6PD deficiency predisposes to neonatal jaundice, and it may be the result of impairment of hepatic function, hemolysis, or both.

When hemolytic anemia occurs after the ingestion of an oxidant drug or in association with the clinical states leading to oxidant stress, G6PD deficiency should be considered. Significant anemia, hyperbilirubinemia, elevated plasma Hb, and hemoglobinuria may be due to brisk intravascular

hemolysis. G6PD deficiency should be considered in an individual with evidence of chronic DAT-negative hemolysis. The peripheral blood smear reveals RBCs with the Hb confined to one side of the cells, with the remainder appearing as an Hb-free ghost (eccentrocytes) (Figure 7-13). The morphology previously has been described as bite or blister cells, interpreted as the result of removal of denatured Hb by the spleen; however, it appears that the accumulated oxidized Hb actually remains and is adherent to the RBC membrane. Brilliant cresyl blue staining may reveal Heinz bodies. Screening or quantitative biochemical assays can be used to make the diagnosis. In the G6PD A⁻ variant, during an acute hemolytic episode, an elevated reticulocyte count will raise the mean level of erythrocyte G6PD and render a false-negative result. G6PD levels, therefore, should be checked several months after the acute event when there will be RBCs of varying ages. Although defects of other hexose-monophosphate shunt enzymes (eg, phosphogluconate dehydrogenase, glutathione reductase) are rare, they should be considered in cases of oxidant-induced hemolysis in which G6PD levels are normal.

Abnormalities of nucleotide metabolism

Pyrimidine-5′-nucleotidase deficiency is an enzymatic abnormality of pyrimidine metabolism associated with hemolytic anemia. The peripheral blood smear in patients with this defect often shows RBCs containing coarse basophilic stippling. Lead intoxication also inactivates the enzyme, leading to an acquired variant of pyrimidine-5′-nucleotidase deficiency.

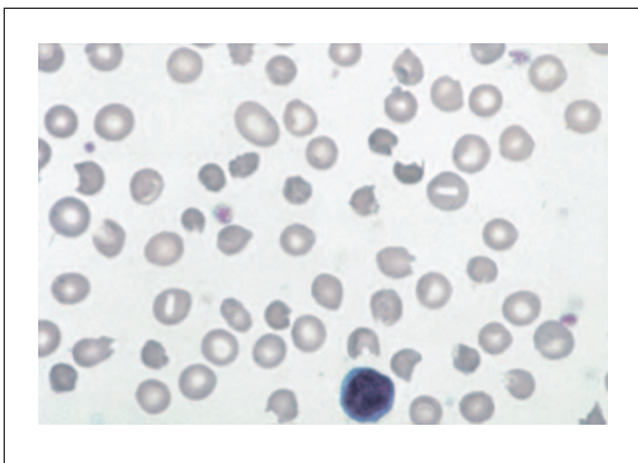Adenosine deaminase (ADA) excess is an unusual abnormality. It is caused by a genetically determined increase in the activity of a normal erythrocyte enzyme. The excessive deaminase activity prevents normal salvage of adenosine and causes subsequent depletion of ATP and hemolysis.

Clinical case (continued)

The patient presented in this section should be suspected of having G6PD deficiency. Patients with the African American variant (G6PD A–) are often asymptomatic until they ingest medication or experience an infection, which leads to oxidant stress of the RBCs. Trimethoprim/sulfamethoxazole may be an offending agent. During the early phases of hemolysis, eccentrocytes can be seen on review of the peripheral blood smear. A Heinz body preparation will show the typical inclusions, which consist of denatured hemoglobin. G6PD levels may be misleading in the acute setting, as values may be normal due to reticulocytosis. Treatment is primarily supportive. Offending drugs should be discontinued and alternative agents chosen. If the prescribed agent is necessary and cannot be substituted, however, a trial of continuation is reasonable, as hemolysis often is compensated in the G6PD A– variant even if drug administration is continued.

Key points

• The glycolytic pathway provides the erythrocyte with the ATP necessary for maintenance of membrane integrity, preservation of ferrous hemoglobin, and oxygen affinity. Additional products of the pathway are NADH for methemoglobin reduction and 2,3-BPG for regulating the oxygen affinity of hemoglobin.
• Glucose metabolism through the hexose monophosphate shunt produces NADPH to maintain the antioxidative activity of the red cell.
• Enzymopathies represent a major consideration in the differential diagnosis of inherited DAT-negative nonspherocytic hemolytic anemias.
• PK deficiency is the most common defect of the glycolytic pathway. The echinocyte is the characteristic abnormality observed on the peripheral blood smear.
• The most common enzyme deficiency is G6PD with >300 genetic variants. Oxidant stress in the presence of deficient G6PD activity results in hemolysis with the generation of blister cells and bite cells (eccentrocytes).
• In the G6PD A– deficiency, a quantitative measurement of the enzyme levels should be delayed until after the acute hemolytic episode. In the G6PD B variant (eg, G6PD-Mediterranean), levels are low in red cells of all ages.
• Defects of purine and pyrimidine metabolism are infrequent. The peripheral blood smear in pyrimidine-5′-nucleotidase deficiency shows red cells with coarse basophilic stippling.
• Iron overload can occur in non-transfused inherited chronic hemolytic anemias due to increased gastrointestinal iron absorption.

Figure 7-13 G6PD deficiency. The peripheral blood smear shows several red cells with the hemoglobin confined to one side of the cells with the remainder appearing as a hemoglobin-free ghost (eccentrocytes).

Hemolysis due to extrinsic abnormalities of the RBC

Clinical case

A 68-year-old male is admitted to the hospital with complaints of weakness, shortness of breath, and chest pain. Over the prior year, he has experienced weight loss and intermittent night sweats, and has generally felt poorly. His prior history is significant for diet-controlled diabetes and elevated cholesterol. He is taking no medications. On examination, he appears chronically ill and pale. Scleral icterus is noted. Axillary adenopathy and splenomegaly are appreciated. His fingertips are mildly cyanotic appearing. Laboratory data are significant for a spun hematocrit of 24% and an MCV of 143 fL. LDH is elevated at 2,321 U/L, indirect bilirubin at 2.1 mg/dL, and reticulocyte count at 13%. The peripheral blood smear shows agglutinated RBCs. The blood bank reports a direct Coombs test positive for complement (3+) but negative for immunoglobulin G (IgG). Serum protein electrophoresis reveals a monoclonal IgM. Abdominal CT scan reveals splenomegaly and diffuse adenopathy.

Hemolytic anemia due to immune injury to RBCs

In autoimmune hemolytic anemia (AHA), shortened RBC survival is mediated by autoantibodies. AHA is classified by the temperature at which autoantibodies bind optimally to the patient RBCs. In adults, the majority of cases (80%-90%) are mediated by antibodies that bind to RBCs at 37°C (warm autoantibodies). In the cryopathic hemolytic anemias, the autoantibodies bind most avidly to RBCs at temperatures <37°C (cold autoantibodies). Some patients exhibit both warm and cold reactive autoantibodies. These cases are classified as mixed AHA.

The warm- and cold-antibody classifications are further divided by the presence or absence of an underlying related disease. When no underlying disease is recognized, the AHA is termed *primary* or *idiopathic*. *Secondary* cases are those in which the AHA is a manifestation or complication of an underlying disorder. In general, the secondary classification should be used in preference to idiopathic only when the AHA and the underlying disease occur together more often

Table 7-7 Classification of immune injury to red blood cells

I. Warm-autoantibody type: autoantibody maximally active at 37°C
  A. Primary or idiopathic warm AHA
  B. Secondary warm AHA
      1. Associated with lymphoproliferative disorders (eg, Hodgkin disease, lymphoma)
      2. Associated with the rheumatic disorders (eg, SLE)
      3. Associated with certain nonlymphoid neoplasms (eg, ovarian tumors)
      4. Associated with certain chronic inflammatory diseases (eg, ulcerative colitis)
      5. Associated with ingestion of certain drugs (eg, α-methyldopa)

II. Cold-autoantibody type: autoantibody optimally active at temperatures <37°C
  A. Mediated by cold agglutinins
      1. Idiopathic (primary) chronic cold agglutinin disease (usually associated with clonal B-lymphocyte proliferation)
      2. Secondary cold agglutinin hemolytic anemia
          a. Postinfectious (eg, *Mycoplasma pneumoniae* or infectious mononucleosis)
          b. Associated with malignant B-cell lymphoproliferative disorder
  B. Mediated by cold hemolysins
      1. Idiopathic (primary) paroxysmal cold hemoglobinuria
      2. Secondary
          a. Donath-Landsteiner hemolytic anemia, usually associated with an acute viral syndrome in children (relatively common)
          b. Associated with congenital or tertiary syphilis in adults

III. Mixed cold and warm autoantibodies
  A. Primary or idiopathic mixed AHA
  B. Secondary mixed AHA
      1. Associated with the rheumatic disorders, particularly SLE

IV. Drug-immune hemolytic anemia
  A. Hapten or drug adsorption mechanism
  B. Ternary (immune) complex mechanism
  C. True autoantibody mechanism
  D. Nonimmunologic protein adsorption (probably does not cause hemolysis)

Adapted from Packman CH. In: Lichtman, MA. *Williams Hematology*. 8th ed. New York, NY: McGraw-Hill; 2010.

AHA = autoimmune hemolytic anemia; SLE = systemic lupus erythematosus.

than random and when the AHA resolves with successful treatment of the underlying disease. The connection is strengthened when the underlying disease has a component of immunologic aberration. Using these criteria, primary (idiopathic) AHA and secondary AHA occur with approximately equal frequency.

Certain drugs also may cause immune destruction of RBCs by three different mechanisms. Some drugs induce formation of true autoantibodies directed against RBC antigens. The hapten-drug adsorption mechanism is characterized by the presence of antidrug antibodies in the blood. These antibodies bind only to RBC membranes that are coated with tightly bound drug. In a third type of drug-immune hemolytic anemia, antibodies recognize a neoantigen formed by a drug or its metabolite and an epitope of a specific membrane antigen. This is termed *ternary* or *immune complex mechanism*. In some, if not all, cases mediated by the ternary (immune) complex mechanism, antibodies may recognize both a drug or its metabolite and an epitope of a specific RBC antigen. The classification of the immune hemolytic anemias is shown in Table 7-7.

Pathophysiology

Warm AHA

The most common type of AHA is mediated by warm-reactive autoantibodies of the IgG isotype. Warm-reacting IgG antibodies bind optimally to antigens on RBCs at 37°C and may or may not fix complement, but they typically do not cause direct agglutination of RBCs because of their small size. Enhanced destruction of antibody-coated RBCs is mediated by Fc receptor–expressing macrophages, primarily located in the spleen. Partial phagocytosis results in the formation of spherocytes that may circulate for a time but eventually become entrapped in the spleen, resulting in enhanced RBC destruction.

Cold AHA

In contrast to warm-reactive autoantibodies, cold-reactive autoantibodies bind optimally to RBCs at temperatures <37°C. Cold autoantibodies are typically of the IgM isotype, and because of their large, pentameric conformation, they are able to span the distance between several RBCs to cause direct agglutination. Their ability to injure RBCs depends on their ability to fix complement. The consequence of complement fixation is clearance of C3b-coated cells by attachment to complement receptors on macrophages, primarily in the spleen, and Kupffer cells in the liver. Direct lysis by completion of the terminal complement sequence may also occur. Cold autoantibodies are characteristic of AHA associated

with *Mycoplasma* infection, as well as with Epstein-Barr virus-related disease. In addition, cold agglutinin disease is typically seen in the elderly, almost always associated with B-cell lymphoproliferative disorders; it is caused by a monoclonal IgM antibody that binds to carbohydrate I antigens or i antigens at temperatures below body temperature. Cold-reacting IgG (Donath-Landsteiner) autoantibodies, seen in paroxysmal cold hemoglobinuria, may cause significant intravascular lysis of RBCs as a result of their ability to fix complement. Donath-Landsteiner hemolytic anemia frequently was associated with congenital syphilis when that disease was common. Now, it is almost always idiopathic. Donath-Landsteiner hemolytic anemia accounts for almost one-third of AHA cases in children. The responsible autoantibodies bind to antigens in the P blood group system.

Mixed AHA

Some cases of AHA are associated with the presence of both IgM and IgG autoantibodies. Hemolysis is generally more severe in these cases. AHA due to IgA antibodies is rare. IgA autoantibodies usually are accompanied by IgG autoantibodies. The mechanisms for RBC destruction appear to be similar to those for IgG.

Drug-induced immune hemolytic anemia

The clinical and laboratory features of drug-induced and idiopathic hemolytic anemia are similar, so a careful history of drug exposure should be obtained in the initial evaluation. The number of drugs that can cause immune hemolytic anemia is large and encompasses a broad spectrum of chemical classes (Table 7-8). Three basic mechanisms of drug-induced immune RBC injury are recognized. A fourth mechanism may lead to nonimmunologic deposition of multiple serum proteins, including immunoglobulins, albumin, fibrinogen, and others, on RBCs, but RBC injury does not occur. The mechanisms of drug-induced immune-hemolytic anemia and positive DATs are summarized in Table 7-9. Second- and third-generation cephalosporins account for about 88% of drug-induced immune hemolytic anemia.

Hapten or drug adsorption mechanism

Hapten or drug adsorption mechanism applies to drugs that bind firmly to proteins on the RBC membrane. The classic setting is very high-dose penicillin therapy, but other drugs such as cephalosporins and semisynthetic penicillins also are implicated. The antibody responsible for hemolytic anemia by this mechanism is of the IgG class and is directed against

Table 7-8 Drugs associated with immune injury to RBCs or a positive direct antiglobulin test

Hapten or drug adsorption mechanism

Carbromal	Oxaliplatin
Cephalosporins	Penicillins
Cianidanol	Tetracycline
Hydrocortisone	Tolbutamide
6-Mercaptopurine	

Ternary-immune complex mechanism

Amphotericin B	Nomifensine
Antazoline	Oxaliplatin
Cephalosporins	Pemetrexed
Chlorpropamide	Probenecid
Diclofenac	Quinine
Diethylstilbestrol	Quinidine
Doxepin	Rifampicin
Etodolac	Stibophen
Hydrocortisone	Thiopental
Metformin	Tolmetin

Autoantibody mechanism

Cephalosporins	Lenalidomide
Cianidanol	Mefenamic acid
Cladribine	α-Methyldopa
Diclofenac	Nomifensine
L-DOPA (levodopa)	Oxaliplatin
Efalizumab	Pentostatin
Fludarabine	Procainamide
Glafenine	Teniposide
Latamoxef	Tolmetin

Nonimmunologic protein adsorption

Carboplatin	Cisplatin
Cephalosporins	Oxaliplatin

Uncertain mechanism of immune injury

Acetaminophen	Melphalan
p-Aminosalicylic acid	Mephenytoin
Carboplatin	Nalidixic acid
Chlorpromazine	Omeprazole
Efavirenz	Phenacetin
Erythromycin	Streptomycin
Fluorouracil	Sulindac
Ibuprofen	Temafloxacin
Insecticides	Thiazides
Isoniazid	Triamterene

epitopes of the drug. Other manifestations of drug sensitivity, such as hives or anaphylaxis, usually are not present. The antibody binds to drug molecules attached to the RBC membrane. Antibodies eluted from patients' RBCs or present in their sera react in the indirect antiglobulin test (IAT) only against drug-coated RBCs, which distinguishes these drug-dependent antibodies from true autoantibodies. Destruction of RBCs coated with drug and IgG antidrug antibody occurs mainly through sequestration by splenic macrophages.

Hemolytic anemia typically occurs 7-10 days after the drug is started and ceases a few days to 2 weeks after the patient discontinues taking the drug.

Ternary or immune complex mechanism: drug antibody-target cell interaction

Drugs in this group exhibit only weak direct binding to blood cell membranes. A relatively small dose of drug is capable of triggering destruction of blood cells. Blood cell injury is mediated by a cooperative interaction among three reactants to generate a ternary complex consisting of the drug or a drug metabolite, a drug-binding membrane site (an antigen) on the target cell, and a drug-dependent antibody. The drug-dependent antibody is thought to bind, through its Fab domain, to a compound neoantigen consisting of loosely bound drug and a blood group antigen intrinsic to the RBC membrane. The pathogenic antibody recognizes the drug only in combination with a particular membrane structure of the RBC (eg, a known alloantigen). Binding of the drug to the target cell membrane is weak until the attachment of the antibody to *both* drug and cell membrane is stabilized. Yet the binding of the antibody is drug dependent. RBC destruction occurs intravascularly after completion of the whole complement sequence, often resulting in hemoglobinemia and hemoglobinuria. The DAT is positive usually only for complement.

Autoantibody mechanism

Several drugs, by unknown mechanisms, induce the formation of autoantibodies reactive with RBCs in the absence of the instigating drug. The most studied drug in this category has been α-methyldopa, but levodopa and other drugs also have been implicated. Patients with chronic lymphocytic leukemia treated with pentostatin, fludarabine, or cladribine may have severe and sometimes fatal autoimmune hemolysis, although the mechanisms of autoantibody induction are likely different, most likely involving dysregulation of T lymphocytes.

Nonimmunologic protein adsorption

A small proportion (<5%) of patients receiving cephalosporin antibiotics, cisplatin and carboplatin, develop positive antiglobulin reactions because of nonspecific adsorption of plasma proteins to their RBC membranes. This process may occur within 1-2 days after the drug is instituted. Multiple plasma proteins, including immunoglobulins, complement, albumin, fibrinogen, and others, may be detected on RBC membranes in such cases. Hemolytic anemia resulting from this mechanism does not occur. This phenomenon, however,

Table 7-9 Immune hemolytic anemia and positive direct antiglobulin reactions caused by drugs

	Hapten-drug adsorption	Ternary-immune complex formation	Autoantibody formation	Nonimmunologic protein adsorption
Prototype drug	Penicillin	Third-generation cephalosporins	α-Methyldopa	Cephalothin
Role of drug	Binds to red cell membrane	Forms three-way complex with antibody and red cell membrane component	Induces antibody to native red cell antigen	Possibly alters red cell membrane
Drug affinity to cell	Strong	Weak	None demonstrated	Strong
Antibody to drug	Present	Present	Absent	Absent
Antibody class predominating	IgG	IgM or IgG	IgG	None
Proteins detected by direct antiglobulin test	IgG, rarely complement	Complement	IgG, rarely complement	Multiple plasma proteins
Dose of drug associated with positive antiglobulin test	High	Low	High	High
Mechanism of red cell destruction	Splenic sequestration	Direct lysis by complement plus splenic sequestration	Splenic sequestration	None

Modified from Packman CH. In: Lichtman, MA. *Williams Hematology*. 8th ed. New York, NY: McGraw-Hill; 2010.

may complicate cross-match procedures unless the drug history is considered.

Clinical manifestations and laboratory findings

Several clinical features of AHA are common to both warm- and cold-antibody types. Patients may present with signs and symptoms of anemia (eg, weakness, dizziness), jaundice, abdominal pain, and fever. Mild splenomegaly is common. Hepatomegaly and lymphadenopathy may be evident at presentation depending on the etiology. Anemia may vary from mild to severe, usually with either normocytic or macrocytic cells. Patients most frequently present with reticulocytosis. Reticulocytopenia, however, initially may be present up to one-third of the time as a result of intercurrent folate deficiency, infection, involvement of the marrow by a neoplastic process, or unidentifiable causes. Indirect bilirubin and LDH are elevated to varying degrees, and the haptoglobin is depressed. The blood smear often demonstrates spherocytes (Figure 7-14). Nucleated RBCs also may be present.

The onset of warm-antibody AHA may be rapid or insidious, but rarely is it so severe as to cause hemoglobinuria. Presenting symptoms usually are related to anemia or jaundice. In secondary cases, the presenting complaint usually is related to the underlying disease.

Patients with idiopathic or primary cold agglutinin disease usually have mild to moderate chronic hemolysis. Acute exacerbations can be associated with cold exposure. Spontaneous autoagglutination of RBCs at room temperature may be seen as clumps of cells on the blood smear (Figure 7-15). Occasionally spurious marked elevations in the MCV and
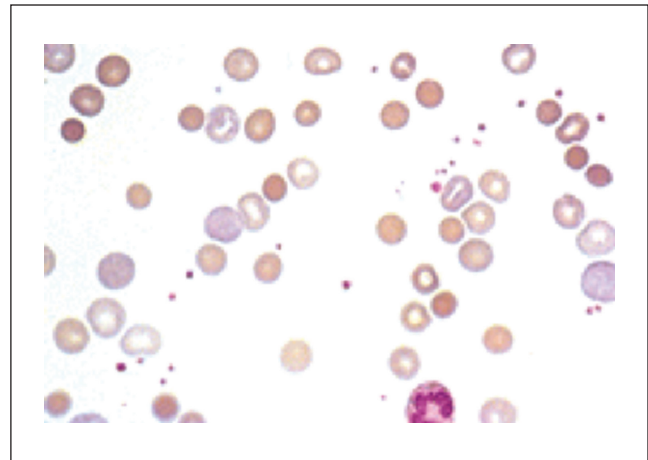

Figure 7-14 Warm-antibody autoimmune hemolytic anemia. Note the small round spherocytes and the large, gray polychromatophilic erythrocytes.

MCHC measurements and decrease in the RBC count are observed due to simultaneous passage of two or three agglutinated RBCs through the aperture of the automated cell counter.

Drug-immune hemolytic anemia due to the hapten or true autoantibody mechanism is usually mild. In contrast, hemolysis due to the ternary or immune complex mechanism can be acute in onset, severe, and sometimes fatal.

The DAT (Coombs test) is usually positive in AHA but may be negative in some patients. The threshold of detection of commercial antiglobulin reagents, which detect mainly IgG and fragments of C3, is approximately 200-500 antibody
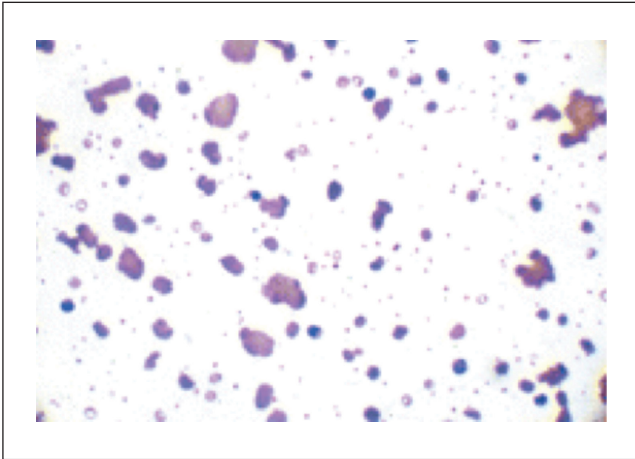
Figure 7-15 Cold agglutinin disease.

molecules per cell. However, <100 molecules of IgG per cell may significantly shorten RBC survival in vivo. IgM cold agglutinins are usually removed from RBCs during washing and usually are not detected. Most commercial reagents do not detect IgA. When monospecific anti-IgG and anti-C3 reagents are used, 30%-40% of patients with AHA will have only IgG on their RBCs; a slightly larger number will have both IgG and C3; and only approximately 10% will have C3 alone. The major reaction patterns of the DAT and their differential diagnosis are summarized in Table 7-10.

The strength of the direct Coombs test has poor clinical correlation with severity of hemolysis among patients, but in a given patient over time, the degree of hemolysis correlates fairly well with the current strength of the antiglobulin reaction. In the rare case of direct Coombs test–negative hemolytic anemia suspected of having an immune etiology,

Table 7-10 Differential diagnosis of reaction patterns of the direct antiglobulin test

Reaction pattern	Differential diagnosis
IgG alone	Warm antibody autoimmune hemolytic anemia
	Drug-immune hemolytic anemia: hapten/drug adsorption type or autoantibody type
Complement alone	Warm antibody autoimmune hemolytic anemia with subthreshold IgG deposition
	Cold-agglutinin disease
	Paroxysmal cold hemoglobinuria
	Drug-immune hemolytic anemia: ternary-immune complex type
IgG plus complement	Warm antibody autoimmune hemolytic anemia
	Drug-immune hemolytic anemia: autoantibody type (rare)

the diagnosis sometimes can be confirmed by using more sensitive assays for RBC-bound immunoglobulin, such as an enzyme-linked immunoadsorbent assay (ELISA) or radiolabeled anti-immunoglobulin. Specific assays for cell-bound IgA also may be worthwhile. In cold agglutinin disease, the DAT is positive with anti-C3 only.

Approximately 1 in 10,000 healthy blood donors have a positive DAT. The positive DAT in these individuals usually is due to warm-reacting IgG autoantibodies, indistinguishable from those occurring in AHA. Many of these individuals never develop AHA, but some do. It is not known how many of these normal individuals with a positive DAT eventually may develop AHA.

Treatment

Asymptomatic patients develop anemia over a period sufficient to allow for cardiovascular compensation and do not require RBC transfusions. For patients with symptomatic coronary artery disease or patients who rapidly develop severe anemia with circulatory failure, as in paroxysmal cold hemoglobinuria or ternary (immune) complex drug-immune hemolysis, transfusions can be lifesaving.

Transfusion of RBCs in immune hemolytic anemia is often problematic. Finding serocompatible donor blood is rarely possible because, in most cases, the autoantibody is a panagglutinin. It is most important to identify the patient's ABO type to find either ABO-identical or ABO-compatible blood for transfusion to avoid a hemolytic transfusion reaction. The difficult technical issue relates to detection of RBC alloantibodies masked by the presence of the autoantibody.

Clinicians and blood bank physicians speak of identifying "least incompatible" blood for transfusion, but this is a misnomer because all units will be serologically incompatible. Units incompatible because of autoantibody are less dangerous to transfuse, however, than units incompatible because of alloantibody. Patients with a history of pregnancy, abortion, or prior transfusion are at risk of harboring an alloantibody. Patients who have never been pregnant or transfused with blood products are unlikely to harbor an alloantibody. Consultation between the clinician and the blood bank physician should occur early to allow for informed discussion and confident transfusion of mismatched blood if the situation demands. Clinicians must understand that the dropping hemoglobin often in the setting of reticulocytopenia is a life-threatening situation, and delay in transfusion over concerns about red cell incompatibility can lead to a patient's demise.

The selected RBCs should be transfused slowly while the patient is monitored carefully for signs of a hemolytic transfusion reaction. Even if transfused cells are rapidly destroyed, the increased oxygen-carrying capacity provided by the

transfused cells may maintain the patient during the time required for other modes of therapy to become effective.

In AHA, therapy is aimed at decreasing the production of autoantibody and at decreasing clearance of RBCs from the circulation. For warm-antibody IgG-mediated hemolysis, glucocorticoids such as prednisone usually are the first-line treatment in all but drug-induced syndromes (for which removal of the offending agent is the principal treatment). Glucocorticoids decrease the ability of macrophages to clear IgG- or complement-coated erythrocytes and reduce auto-antibody production. After remission is achieved with pred-nisone at approximately 60-100 mg/d (or 1 mg/kg/d), the dose should be decreased by 10 mg/d each week until a dose of 30 mg/d is reached. Subsequent dose reduction should then proceed more slowly (at 5 mg/d per week), with the goal of either maintaining remission with prednisone at 20-40 mg every other day or complete weaning of predni-sone if the DAT becomes negative; this goal is not always achievable. Approximately two-thirds of adult patients respond to prednisone, with approximately 20% achieving complete remission. Pulses of high-dose glucocorticoids (eg, 1 g methylprednisolone intravenously) are effective in some patients in whom standard therapy has failed.

Splenectomy is often considered if hemolysis remains severe for 2-3 weeks at prednisone doses of 1 mg/kg, if remis-sion cannot be maintained on low doses of prednisone, or if the patient has intolerable adverse effects or contraindica-tions to glucocorticoids. Removing the spleen results in a reduced rate of clearance of IgG-coated cells. Although not usually recommended in children, splenectomy in patients past adolescence appears relatively safe. Patients should receive pneumococcal, *H. influenzae*, and meningococcal vaccines before splenectomy. Approximately two-thirds of patients will have complete or partial remission with sple-nectomy, but relapses are common.

Other therapies may be effective for patients with refrac-tory hemolysis or for those who relapse after glucocorticoids or splenectomy. Standard-dose (375 mg/m^2) and low-dose (100 mg/m^2) monoclonal anti-CD20 (rituximab) has been useful in refractory cases. Adults and children respond equally well with response rates ranging from 40% to 100%. Immu-nosuppressive drugs, such as cyclophosphamide, azathio-prine, mycophenolate mofetil, and cyclosporine, as well as the nonvirilizing androgen, danazol, have been used with varying degrees of success. Intravenous immunoglobulin has been less successful in treatment of AHA than in immune TTP.

For patients with idiopathic cold agglutinin disease, main-taining a warm environment may be all that is needed to avoid symptomatic anemia. Cold agglutinin disease usually does not respond to glucocorticoids. Recently, rituximab has demonstrated efficacy in treating cold agglutinin disease, with response rates approaching 50%. Chlorambucil and

cyclophosphamide have been beneficial in selected cases. Chemotherapy is indicated if the disorder is associated with a lymphoproliferative disorder. Splenectomy usually is not indicated because cells typically are cleared by intravascular hemolysis or hepatic Kupffer cells. Intravenous immuno-globulin does not have a role in management. Plasmaphere-sis may be temporarily effective in acute situations by removing IgM cold agglutinin from the circulation. Recently, a combination of fludarabine and rituximab has been used with success, but toxicity is a concern.

AHA during childhood tends to occur suddenly, during, or after an acute infection. As many as one-third of cases are associated with intravascular hemolysis because of a Donath-Landsteiner antibody directed against the erythrocyte P anti-gen. Usually these patients exhibit only a single paroxysm of hemolysis. In warm-antibody hemolytic anemia, acute man-agement is similar to that for adults. Approximately two-thirds of children recover completely within a matter of weeks. Only a small percentage of children (but a larger pro-portion of adolescents) exhibit more chronic refractory dis-ease that warrants consideration of other pharmacologic agents or splenectomy.

Clinical case (continued)

The patient presented in this section has cold agglutinin disease, likely secondary to underlying lymphoma. Automated tech-niques reveal the red cell count is artifactually low, and the MCV and MCHC are falsely elevated secondary to red cell agglutina-tion. Warming of the blood tube with immediate measurement and slide preparation will minimize agglutination. The direct antiglobulin test is positive only for complement. Lymphopro-liferative disorders are well-identified underlying etiologies. The patient should be maintained in a warm environment. Ameliora-tion of the anemia can be anticipated with cytotoxic therapy for the lymphoma.

Key points

- Warm-antibody-induced immune hemolytic anemia is typically IgG mediated and results in spherocytic red cells.
- Cold agglutinin disease is IgM mediated with associated complement activation. The peripheral blood smear reveals red cell agglutination and spherocytes.
- A variety of drugs cause immune hemolytic anemia. Clinical laboratory support of the diagnosis may not be available. Discontinuation of the suspected offending drug is indicated.
- Symptoms resulting from autoimmune hemolytic anemia are typically indistinguishable from other causes of hemolysis.
- The direct antiglobulin test is the primary tool for diagnosing autoimmune hemolytic anemia. It is rarely positive in healthy individuals and may be negative in autoimmune hemolytic anemia.

Key points (continued)

• Warm-antibody-mediated autoimmune hemolytic anemia is treated with glucocorticoids, other immunosuppressive agents such as rituximab, and splenectomy.

• Avoidance of cold environments may be sufficient to avoid complications of cold agglutinin disease. Chemotherapy and rituximab have a role, and plasmapheresis occasionally can be helpful in the acute and temporary management of symptomatic cases by physically removing the antibody.

• Immune-mediated hemolytic anemia is uncommon in children. Most cases are acute and transient, following viral infection.

• Transfusion therapy can be difficult in patients with autoimmune hemolytic anemia. Consultation with the blood bank is important. A history of prior pregnancy, abortion, or transfusion of blood products should be obtained, as these patients are at risk to harbor dangerous alloantibodies. No patient with AHA should be allowed to die because serologically "compatible" RBCs are not available.

Paroxysmal nocturnal hemoglobinuria

Clinical case

A previously healthy 37-year-old female is admitted to the hospital for evaluation of severe abdominal pain. Workup reveals mesenteric vein thrombosis. The patient is treated with thrombolytic therapy and anticoagulated with heparin, leading to clinical improvement. She has no prior or family history of thrombosis. She currently is taking an oral contraceptive. Her examination is significant for mild scleral icterus and jaundice. There is no abdominal tenderness. Mild splenomegaly is noted. Laboratory studies are significant for a hematocrit of 32% with a corrected reticulocyte count of 8%. White count and platelet count are slightly depressed. Indirect bilirubin is elevated at 4 mg/dL, but AST, ALT, and alkaline phosphatase are normal. LDH is also increased at 1,024 U/L. Blood bank evaluation confirms a Coombs-negative hemolytic anemia. A bone marrow aspirate and biopsy are hypocellular and reveal findings concerning for early myelodysplasia.

Paroxysmal nocturnal hemoglobinuria (PNH) should be considered in the patient with unexplained hemolysis, pancytopenia, or unprovoked thrombosis. PNH is an acquired clonal disorder of hematopoietic stem cells occurring in both children and adults with no apparent familial predisposition.

Pathophysiology

Hemolysis in PNH is due to the action of complement on abnormal RBCs. Compared with normal RBCs, PNH RBCs lyse more readily in the presence of activated complement. Earlier tests to diagnose PNH (eg, Ham test or acid hemolysis test; sucrose hemolysis test) were based on this property of PNH RBCs. It is now known that PNH granulocytes and platelets are sensitive to complement as well.

The biochemical basis of complement sensitivity was initially elusive. Early on, PNH blood cells were found to be deficient in leukocyte alkaline phosphatase and erythrocyte acetylcholinesterase. Neither of these deficiencies, however, explained the complement sensitivity or the clinical manifestations in PNH. Subsequently, two complement regulatory proteins, CD55 (decay accelerating factor [DAF]) and CD59 (homologous restriction factor or membrane inhibitor of reactive lysis [MIRL]), were found to be missing from PNH blood cells, helping to explain the unusual sensitivity of RBCs to the hemolytic action of complement. Of these, CD59, whose action is to inhibit the terminal complement sequence leading to hemolysis, seems to be the most important.

The approximately 20 proteins missing from the hematopoietic cells in PNH are all attached to the membrane by a glycosylphosphatidylinositol (GPI) anchor. Defective synthesis of the GPI anchor is due to somatic mutations in the *pig-A* gene in hematopoietic stem cells. Whereas a *pig-A* gene mutation appears to be necessary for the development of PNH and its clinical manifestations, it is not sufficient because *pig-A* mutations can be found in small numbers of hematopoietic stem cells in normal individuals. Patients with aplastic anemia exhibit a larger proportion of stem cells with *pig-A* mutations.

A multistep process seems necessary for PNH to develop. It is thought that in aplastic anemia and likely in PNH that immunologic processes suppress proliferation of normal hematopoietic precursors more efficiently than proliferation of precursors lacking GPI-anchored proteins. Resistance to apoptotic death may partly explain the survival advantage of these GPI-negative cells. The abnormal clones thus are able to expand until the numbers of abnormal progeny are sufficient to cause the clinical manifestations of PNH.

Two missing GPI-linked proteins may contribute to the increased incidence of thrombosis in PNH: (i) urokinase plasminogen activator receptor, the lack of which may decrease local fibrinolysis; and (ii) tissue factor pathway inhibitor, the lack of which may increase the procoagulant activity of tissue factor. PNH platelets, which are sensitive to the lytic activity of complement, are hyperactive. RBC phospholipids released during intravascular hemolysis also may initiate clotting.

Most of the clinical manifestations of the disease are due to the lack of the complement-regulating protein CD59. The monoclonal antibody eculizumab, which binds the complement component C5, thereby inhibiting terminal

complement activation, decreases hemolysis of RBCs and the tendency to thrombosis as well. The drug does not seem to alter the defect in hematopoiesis. Thus, although deficient hematopoiesis is probably related to deficiency of GPI-anchored proteins, it is not related to complement sensitivity.

Laboratory findings

There are no specific morphologic abnormalities of the RBCs in PNH. RBCs may be macrocytic, normocytic, or microcytic, the latter occurring when iron deficiency develops because of chronic urinary iron loss from intravascular hemolysis. With or without iron deficiency, the reticulocyte count may not be as elevated as expected for the degree of anemia. This is due to underlying bone marrow dysfunction that often accompanies the PNH. Leukopenia and thrombocytopenia often are present. Serum LDH usually is elevated and may suggest the diagnosis in the patient with minimal anemia. Iron loss may amount to 20 mg/d, and urine hemosiderin often is identified. Bone marrow examination reveals erythroid hyperplasia unless there are associated bone marrow disorders.

Laboratory diagnosis

The laboratory diagnosis of PNH formerly relied on the demonstration of abnormally complement-sensitive erythrocyte populations. Ham first described the acidified serum lysis test in 1938. In that test, acidification of the serum activates the alternative pathway of the complement, and increased amounts of C3 are fixed to RBCs lacking complement regulatory proteins. Complement sensitivity of PNH RBCs also can be demonstrated in high-concentration sucrose solutions, the basis for the "sugar water" or sucrose hemolysis test. These tests are primarily of historical interest and are not used routinely in the clinical laboratory because flow cytometry techniques aimed specifically at demonstrating the deficiency in expression of GPI-anchored proteins in PNH are readily available. Using commercially available monoclonal antibodies, blood cells can be analyzed for expression of the GPI-anchored proteins CD55 (DAF) and CD59 (MIRL). These methods have the sensitivity to detect small abnormal populations; because monocytes and granulocytes have short half-lives and their numbers are not affected by transfusion, analysis of GPI-anchored proteins on neutrophils or monocytes rather than RBCs is preferred.

A new assay is being used increasingly to detect GPI-deficient blood cells. The fluorescein-labeled aerolysin (FLAER) assay exploits a property of aerolysin, the principle virulence factor of the bacterium *Aeromonas hydrophila*, which binds selectively with high affinity to the GPI anchor of most cell lineages. FLAER is most useful to assay the GPI anchor on granulocytes because aerolysin binds weakly to glycophorin on RBCs.

Clinical manifestations

Although chronic hemolytic anemia is a common manifestation, only a minority of patients report nocturnal hemoglobinuria. The degree of anemia seen in PNH varies in affected individuals from minimal to quite severe. Symptoms related to episodes of hemolysis include back and abdominal pain, headache, and fever. Exacerbations of hemolysis can occur with infections, surgery, or transfusions. Several symptoms in PNH may be related to the ability of free plasma hemoglobin to scavenge nitric oxide. These include esophageal spasm, male erectile dysfunction, renal insufficiency, thrombosis, and pulmonary hypertension.

Aplastic anemia has been diagnosed both before and after the identification of PNH. PNH clones are present in approximately 20% of patients with severe aplastic anemia. Approximately 20% of patients with myelodysplastic syndromes have PNH clones. Hemolysis in the setting of bone marrow hypoplasia or myelodysplastic or myeloproliferative disorders should suggest the diagnosis of PNH. Infections associated with leukopenia and bleeding due to thrombocytopenia contribute to increased mortality. An increased incidence of acute leukemia also has been reported.

Patients frequently have thrombotic complications that can be life threatening and may represent the initial manifestation of PNH. In addition to venous thrombosis involving an extremity, there is a propensity for thrombosis of unusual sites such as hepatic veins (Budd-Chiari syndrome), other intra-abdominal veins, cerebral veins, and venous sinuses. Thus, complaints of abdominal pain or severe headache should alert the clinician to the consideration of thrombosis in the patient with PNH. The thrombotic tendency is particularly enhanced during pregnancy.

Treatment

The clinical manifestations of PNH are highly variable among patients. For patients with PNH clones numbering <10%, no clinical intervention is needed. Because expansion of the clone may occur, however, the size of the clone should be monitored every 6-12 months. Anemia is often the dominant issue in PNH. Glucocorticoids can reduce complement activation and decrease the hemolysis; however, high doses are frequently necessary, and every-other-day administration has been recommended to reduce the adverse effects. Iron may be required to replace the large urinary losses seen in PNH. Folate supplementation usually is recommended as well. Erythropoietin (10,000-20,000 U three times weekly)

may be helpful for patients with inadequate reticulocyte responses. Transfusion may be necessary when these measures fail to maintain adequate hemoglobin levels.

Eculizumab is a humanized monoclonal antibody that was engineered to reduce its immunogenicity; importantly, it is unable to bind Fc receptors on cells or to activate complement. It binds to C5 and blocks the terminal complement sequence. The US Food and Drug Administration approved its use in PNH to treat hemolysis based on efficacy in two phase 3 clinical trials. Eculizumab reduces intravascular but not extravascular hemolysis, eliminates or reduces transfusion requirement in almost all patients, improves quality of life, improves pulmonary hypertension, and decreases the risk of thrombosis. It does not treat the marrow failure. It must be used indefinitely because it does not treat the underlying cause of PNH.

Although eculizumab generally is well tolerated, its most serious complication is sepsis due to *Neisseria* organisms. Patients congenitally lacking one of the terminal complement components, C5 to C9, are known to be at risk for *Neisseria* infection. Patients receiving eculizumab are at risk because of its inhibition of the terminal complement sequence. Vaccination against *Neisseria meningitidis* is recommended 2 weeks before starting therapy. Revaccination every 3-5 years may be important because eculizumab is given for an indefinite period. Because vaccination does not eliminate the risk completely, patients should be told to seek medical attention for any symptoms consistent with *Neisseria* infection.

Allogeneic hematopoietic stem cell transplantation is the only known cure for PNH. Because of the high risk for serious complications including death, however, such treatment should be reserved for patients with severe pancytopenia or the rare individuals whose hemolysis or thrombosis is not controlled by eculizumab. For patients with PNH and marrow failure who lack an HLA-matched sibling donor, immunosuppressive therapy may be attempted.

Thrombosis is the leading cause of death in PNH patients. Thrombosis should be treated promptly with anticoagulation. Thrombolytic therapy may be considered as well, depending on the extent and location of the clot. In contrast to anticoagulation as treatment, prophylactic anticoagulation is controversial. In one large, nonrandomized trial, primary prophylaxis with warfarin decreased the risk of thrombosis in patients with large PNH clones (>50% PNH granulocytes). Because eculizumab also decreases the risk of thrombosis, however, prophylactic anticoagulation is not indicated in these patients based on the current state of knowledge. The bigger question concerns prophylaxis in patients who do not require eculizumab; in general, lacking a randomized trial, it is probably not indicated until further studies are available. The exception may be pregnant women

who are at particularly increased risk for thrombosis; low–molecular weight heparin may be useful in these patients during pregnancy and the puerperal period. Eculizumab is a pregnancy category C pharmaceutical; however, there are recent case reports of its apparent safe use in pregnancy. Also, patients with PNH undergoing surgery should receive prophylactic anticoagulation in the perioperative period. The recommended duration of either prophylactic or therapeutic anticoagulation has not been established.

Prognosis

The median survival for PNH is 10-15 years. Thrombotic events, progression to pancytopenia, and age >55 years at diagnosis are poor prognostic factors. The development of a myelodysplastic syndrome or acute leukemia markedly shortens survival. Patients without leukopenia, thrombocytopenia, or other complications can anticipate long-term survival.

Clinical case (continued)

The patient presented in this section likely has PNH. She has evidence of hemolysis and marrow failure. The diagnosis can be confirmed by flow analysis for CD55 and CD59 on granulocytes, revealing a population of cells with absence of GPI-linked proteins. Treatment is aimed at the major clinical presentation. Eculizumab is effective in decreasing hemolysis and thrombosis, but not marrow failure. Thrombosis is treated with anticoagulation; thrombolytic therapy may be employed if the thrombosis is acute. There are no randomized studies to support anticoagulation for prophylaxis of thrombosis, but it is prudent to employ prophylaxis in high-risk situations for thrombosis, such as pregnancy or surgery. If pancytopenia is marked, immunosuppressive therapies, such as antithymocyte globulin and cyclosporine, have been used. Allogeneic marrow transplantation has been performed in selected cases, primarily those with severe marrow failure and an HLA-matched sibling donor. Marrow transplantation is the only potentially curative therapy of PNH.

Key points

• PNH is an acquired clonal hematopoietic stem cell disorder caused by a somatic mutation of the *pig-A* gene that results in hematopoietic cells lacking GPI-linked proteins.
• Patients may experience chronic hemolytic anemia, cytopenias, or a thrombotic tendency.
• Flow cytometric techniques to identify cell populations lacking GPI-linked proteins (CD55 and CD59) have replaced the sucrose hemolysis and Ham tests.
• PNH clones have been identified in individuals without hematologic abnormalities.

Fragmentation hemolysis

Clinical case

A 63-year-old male is referred for evaluation of anemia. His past history is significant for oxygen-dependent chronic obstructive pulmonary disease, coronary artery disease, a mechanical aortic valve placed in 1986, and mild heart failure. On examination, he has distant breath sounds and a grade III/VI systolic ejection murmur heard at the left upper-sternal border. Mild scleral icterus is noted. Laboratory data are significant for a hematocrit of 21% (normal 2 years prior). Corrected reticulocyte count is elevated at 3%, LDH 1,686 IU/dL, and indirect bilirubin 3.4 mg/dL. Examination of the blood smear reveals schistocytes, hypochromic RBCs, and a few cigar-shaped RBCs.

Fragmentation hemolysis takes place within the vasculature. Laboratory features common to both intra- and extravascular hemolysis include increased concentrations of plasma bilirubin and LDH and decreased concentration of plasma haptoglobin. Additional features characteristic of intravascular as opposed to extravascular hemolysis include the presence of free Hb in the plasma and urine, resulting in red urine and pink plasma. If the hemolysis is chronic, urine hemosiderin may be present. In fragmentation hemolysis, schistocytes are a prominent feature of the blood smear (Figure 7-16). The differential diagnosis of microangiopathic hemolytic anemia (MAHA) is summarized in Table 7-11.
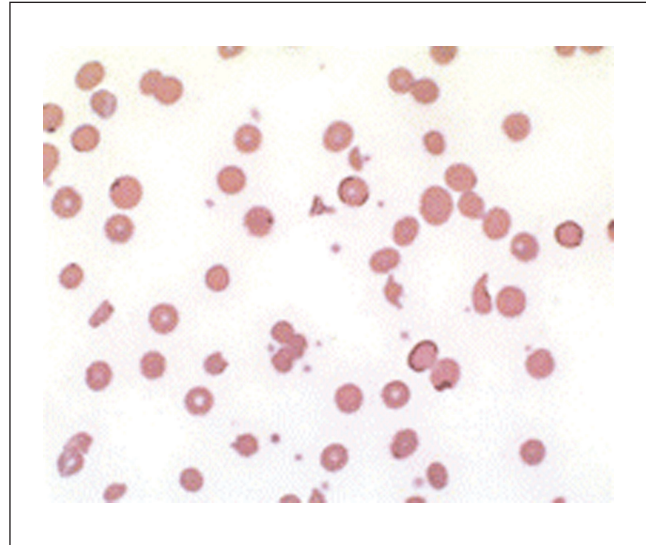

Figure 7-16 Schistocytes.

Pathophysiology

Among the several causes of fragmentation hemolysis, the common thread is mechanical damage to RBCs, resulting in the presence of fragmented RBCs or schistocytes on the blood smear. When microvascular or endothelial injury is present, the process is termed *microangiopathic hemolytic anemia*. When thrombosis is part of the picture, the term *thrombotic microangiopathy* is used. In disseminated intravascular coagulation (DIC), the microangiopathic hemolytic anemia is accompanied by activation and consumption of soluble clotting factors, resulting in prolongation of the prothrombin time and activated partial thromboplastin time, whereas TTP-HUS is associated with activation of platelets but not soluble clotting factors.

Injury to blood vessel endothelium, intravascular clotting, and primary platelet activation all result in formation of fibrin strands in the circulation. The shearing force generated as the RBCs pass through the fibrin strands causes the RBCs to be cut into small irregular pieces. RBCs may be broken into pieces by direct mechanical trauma as may occur in

Table 7-11 Differential diagnosis of microangiopathic hemolytic anemia

Thrombotic thrombocytopenia purpura
Atypical hemolytic uremic syndrome
Disseminated intravascular coagulation
HELLP syndrome
Cardiac valve disease
Malignancy
Vasculitis
Malignant hypertension
Scleroderma renal crisis

march hemoglobinuria or with a dysfunctional mechanical heart valve in which high-velocity jets of blood strike an unendothelialized surface. The resulting small RBC fragments are self-sealing and continue to circulate, albeit with shortened survival. This is due in part to their decreased deformability, which results in accelerated removal by the spleen.

Etiology

Cardiac valve hemolysis

Hemolysis may occur with calcific or stenotic native heart valves, although it is usually very mild and well compensated in the absence of severe valvular disease. Mechanical heart valves have a smaller diameter than the native heart valve. Normally, the hemodynamic consequences are minimal. Prosthetic valve dysfunction or perivalvular regurgitation may result in intravascular hemolysis, however. An aged or damaged valve surface may become irregular, leading to thrombus formation. In a high-flow state, such as exists across the aortic valve or across a regurgitant mitral valve, the formation of jets and turbulent flow results in high shear stress that may exceed the stress resistance of the normal RBC. Hemolysis may be made worse with concomitant cardiac failure or high-output states. Recently designed bioprosthetic heart valves have a significantly decreased risk of thrombus formation and a lower rate of traumatic hemolysis. A recent prospective study reported a 25% rate of mild subclinical hemolysis with a mechanical prosthesis and a 5% rate with a bioprosthesis.

Ruptured chordae tendinae, aortic aneurysm, and patch repair of cardiac defects, as well as intraventricular assist devices and aortic balloon pumps used in the management of severe heart failure, have been associated with traumatic hemolysis. Intravascular hemolysis has been described after cardiopulmonary bypass and is thought to be secondary to both physical damage and complement activation.

Anemia is variable in patients with prosthetic valve hemolysis. The blood smear may include abnormal erythrocytes with schistocytes and cells with abnormal membrane projections.

With chronic hemolysis, hemoglobin is lost in the urine, leading to iron deficiency. Iron-deficient RBCs are mechanically fragile, which can worsen hemolysis, exacerbate anemia, and lead to further hemodynamic compromise that may increase the rate of hemolysis. At times, this cycle may be abated by correction of iron deficiency or by RBC transfusion. The addition of erythropoietin to increase RBC production may compensate for ongoing hemolysis. If anemia is severe or fails to respond to the conservative measures, valve replacement may become necessary.

Thrombotic thrombocytopenic purpura

TTP is due to the deposition of platelet microthrombi along the endothelium of small vessels of multiple organs. The classic clinical presentation consists of microangiopathic hemolytic anemia and thrombocytopenia. In advanced stages, fever, renal failure, and CNS involvement are seen. TTP may be confused with eclampsia, HUS, the HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome, and acute fatty liver of pregnancy (AFLP), all of which can present with microangiopathic anemia. A critical distinguishing feature between TTP and DIC is the presence of consumptive coagulopathy in the latter. Malignant hypertension and renal crisis in scleroderma may resemble TTP, presenting with microangiopathic hemolysis, thrombocytopenia, and renal insufficiency. Rapid control of the hypertension is important in these patients. (TTP and the HUS are covered in detail in Chapter 10.)

Certain drugs, especially antineoplastic agents, can cause microangiopathic hemolysis that resembles TTP. Mitomycin, a chemotherapeutic agent used in the treatment of gastrointestinal malignancies, has been best described. Gemcitabine, another chemotherapeutic agent, also has been implicated. The mechanism has been proposed to be direct endothelial injury. Both tacrolimus and cyclosporine used to prevent and treat graft-versus-host disease can cause a similar syndrome. Both ticlopidine and clopidogrel, antiplatelet agents, have been associated paradoxically with TTP.

Hemolytic uremic syndrome

HUS is characterized by red cell fragmentation, thrombocytopenia, and renal failure. HUS has been divided into typical and atypical forms. The typical form is usually caused by infection with *Escherichia coli*. The atypical form of HUS (aHUS) is due to dysregulation of the complement alternative pathway. aHUS is becoming increasingly recognized with the ability to distinguish this cause of MAHA and thrombocytopenia from TTP with the use of ADAMTS13 testing. Individuals with aHUS do not always have thrombocytopenia as seen in a French registry of patients where 16% did not have a low platelet count at presentation. It is now thought that aHUS occurs in individuals with an underlying complement risk factor who have a secondary trigger. Triggers can include infection, pregnancy, drug exposure, and malignancy.

Distinguishing TTP or other MAHA from aHUS is crucially important, as therapy with eculizumab has been shown to be more effective than plasma exchange in the management of aHUS. Although mostly based on case reports, eculizumab has been shown to result in full recovery of baseline renal function in 80% of children and 31% of adults. With the significant morbidity associated with aHUS, a French working group recommended the use of eculizumab as a

first-line therapy in children with aHUS. In a patient with MAHA and thrombocytopenia with normal ADAMTS13 levels, eculizumab therapy should be considered.

Disseminated intravascular coagulation

DIC is associated with many disorders, including sepsis, obstetrical catastrophes, and malignancy. The disorder is characterized by activation of coagulation and generation of excess thrombin leading to deposition of fibrin strands in arterioles, venules, and capillaries. MAHA may be present but often is not severe enough to cause morbidity. Disseminated malignancy presents with microangiopathic anemia and DIC in approximately 5% of cases. Fibrin deposition and vascular disruption by the malignancy itself have both been noted. Mucin-producing adenocarcinomas are frequent offenders. Promyelocytic leukemia characteristically presents with DIC due, at least in part, to the release of tissue factor from promyelocytic granules. If treatment is effective at reversing the underlying condition causing DIC, hemolysis and the coagulopathy often resolve.

HELLP syndrome

The HELLP syndrome (microangiopathic hemolytic anemia, elevated liver enzymes, and a low platelet count) is a serious complication of late pregnancy that is part of a spectrum with preeclampsia. Thrombocytopenia and MAHA with or without renal failure may also occur in pregnancy due to TTP-HUS and AFLP. It is important to distinguish TTP, HUS, and AFLP from HELLP and preeclampsia for therapeutic reasons. The clinical features are quite similar, however, and the correct diagnosis is often elusive.

Although not absolute, the timing of onset during the pregnancy may be helpful. In general, TTP, and aHUS occurs earlier in gestation than AFLP, preeclampsia, or HELLP; approximately two-thirds of TTP cases in pregnancy occur in the first or second trimester. Most cases of AFLP, preeclampsia, and HELLP occur after 20 weeks of gestation, the great majority in the third trimester. A history of proteinuria and hypertension before the onset of hemolysis, liver abnormalities, and thrombocytopenia favors the diagnosis of preeclampsia or HELLP, whereas a high LDH level with only modest elevation of AST favors TTP. Severe liver dysfunction or liver failure favor AFLP.

The characteristics of the coagulopathy are different as well. Whereas both TTP and HELLP are characterized by thrombocytopenia in HELLP and more so in AFLP, DIC may also be present with evidence of consumptive coagulopathy. In TTP, only thrombocytopenia is seen without evidence of consumption of soluble clotting factors. Treatment of HELLP and AFLP consist of prompt delivery of the fetus. The use of dexamethasone in HELLP, previously supported by small studies, has not proven helpful in subsequent randomized trials.

Kasabach-Merritt syndrome

Kasabach-Merritt syndrome is characterized by consumptive coagulopathy occurring in the capillaries of a large kaposiform hemangioendothelioma. Microangiopathic hemolytic anemia accompanies evidence of DIC. A number of treatments, including glucocorticoids, chemotherapy, interferon-alfa, embolization, and surgical removal have been tried with some success.

Foot strike hemolysis

Foot strike hemolysis, also known as march hemoglobinuria, has been described in soldiers subjected to long foot-stomping marches in rigid-soled boots, long-distance runners, conga drummers, pneumatic hammer operators, and karate enthusiasts. Hemoglobinuria occurs shortly after the episode of exercise. The hemolysis is caused by direct trauma to RBCs in the blood vessels of the extremities. This condition has become much less common as shoe technology has improved. Cessation of the activity always leads to resolution of the hemolysis.

Clinical case (continued)

The patient presented in this section has evidence of a moderate hemolytic anemia. The blood smear is consistent with both traumatic hemolysis and iron deficiency, as schistocytes and hypochromic and cigar-shaped cells were noted on review of the peripheral blood smear. Valve structure and function should be investigated with an echocardiogram or other imaging studies. Other causes for hemolysis should be ruled out. The patient should be evaluated for iron deficiency. If further evaluation confirms iron deficiency, the patient should receive oral iron. Erythropoietin administration also should be considered. He appears to be a poor surgical candidate, but valve replacement may become necessary if conservative treatment fails.

Hemolytic anemia due to chemical or physical agents

Clinical case

A 23-year-old female is referred for evaluation of mild anemia noted during a workup of liver function test abnormalities. Her recent history has been significant for bizarre schizophrenic-like behavior and arthritis. She has not had a menstrual period in several months. Recent slit-lamp examination by an ophthalmologist revealed golden brown pigmentation of the cornea. Physical examination is otherwise unremarkable. Laboratory data suggest a Coombs-negative hemolytic anemia. Liver enzymes are moderately elevated. A ceruloplasmin level returns low at 11 mg/dL.

The use of primaquine and dapsone to prevent or treat *Pneumocystis jirovecii* in acquired immunodeficiency syndrome (AIDS) patients has become fairly common. Both drugs may cause methemoglobinemia in high doses in normal patients and may precipitate hemolysis in patients with G6PD deficiency. Most AIDS clinics screen their patients for G6PD deficiency before starting either of these drugs. Methemoglobinemia and G6PD deficiency are covered in detail earlier in this chapter.

Ribavirin, used to treat HCV infection, is a frequent cause of hemolysis by an unknown mechanism. The hemolysis is dose dependent and decreases or resolves with decreased ribavirin dose or discontinuation of the drug. The rate of sustained HCV response, however, also decreases with dose reduction. Erythropoietin has been used as an adjunct to maintain ribavirin therapy at full dose.

Phenazopyridine is a bladder analgesic that is used to treat the symptoms of cystitis. In high doses, it has been associated with oxidative hemolysis. It is recommended that patients be treated for no more than 2 days. Overdoses, prolonged administration, and renal insufficiency have led to methemoglobinemia and severe hemolysis, occasionally severe enough to induce acute renal failure.

Lead intoxication can lead to a modest shortening of RBC life span, although the anemia more often is due to an abnormal heme synthesis and decreased production of erythrocytes. On the blood smear, RBCs are normocytic, hypochromic, with prominent basophilic stippling in young polychromatophilic cells.

Copper causes hemolysis through direct toxic effects on RBCs and has been observed in association with hemodialysis. Copper accumulates in RBCs and disrupts normal metabolic function through a variety of mechanisms, including oxidation of intracellular reduced glutathione, hemoglobin, and NADPH and inhibition of multiple cytoplasmic enzymes. Wilson disease, due to a mutation of the ATP7B gene, leads to absence or dysfunction of a copper-transporting ATPase encoded on chromosome 13. This subsequently results in lifelong copper accumulation. Hemolytic anemia may be an early manifestation. The hemolytic process in Wilson disease varies in severity and duration. Kayser-Fleischer rings due to the deposition of copper around the periphery of the cornea are a key diagnostic finding. Diagnosis can be made by quantitative ceruloplasmin measurements or by liver biopsy with assessment of the copper concentration. Treatment consists of penicillamine, which mobilizes copper stores. Acute hemolysis in Wilson disease has been treated successfully with plasmapheresis.

Certain spider bites may be associated with traumatic RBC fragmentation. In the southern United States, the brown recluse spider (*Loxosceles reclusa*) is the most common species causing hemolysis. The toxin proteolyzes the RBC membrane through damage to protein band 3 and other integral proteins. In the northwestern United States, hemolysis has been noted after hobo spider (*Tegenaria agrestis*) bites. Microangiopathic hemolysis may occur after the bite of pit vipers (eg, rattlesnakes, cottonmouth moccasins, copperheads) associated with DIC induced by the venom. Cobra venom contains phospholipases that may cause hemolysis. Massive bee and wasp stings rarely have been associated with intravascular hemolysis.

Fragmentation hemolysis has been described after injury from a variety of physical agents. Thermal injury can lead to severe intravascular hemolysis. This is best described in patients suffering from extensive third-degree burns. At temperatures above 47°C, irreversible injury occurs to the RBC membrane. Shortened RBC survival has been noted after ionizing radiation exposure.

Clinical case (continued)

The patient presented in this section displays the classic historical and physical findings of Wilson disease. The low ceruloplasmin level is diagnostic. Hemolytic anemia has been well described in this disease. Once the severity of her liver disease is further evaluated, treatment with penicillamine should be considered. The hemolytic anemia is likely to resolve as excess copper is removed.

Hemolytic anemia due to infection

Clinical case

A 21-year-old man went to the emergency department of his local hospital complaining of fever and shaking chills. He had just returned from a 6-month deployment in eastern Afghanistan with the US Army. He has been home for 2 weeks on leave before reporting for his next duty assignment in the United States. He states that he faithfully took his malaria prophylaxis consisting of mefloquine 250 mg weekly while in Afghanistan; he was instructed to continue the weekly mefloquine for four more doses postdeployment, plus primaquine 15 mg daily for the first 2 weeks. On examination, he appeared acutely ill. His vital signs were BP 126/66, pulse 110, respirations 20, and temperature 39°C. The remainder of the examination was unremarkable. There was no splenomegaly. A Wright-Giemsa stained thick blood smear confirmed the diagnosis of *Plasmodium vivax* malaria.

Infection may lead to hemolysis through a variety of mechanisms. Parasites may directly invade RBCs, leading to premature removal by macrophages of the liver and spleen. Alternatively, hemolytic toxins may be produced by the organism and lead to damage of the RBC membrane. Development of antibodies to RBC surface antigens has been well

described with certain viral and bacterial illness, especially infectious mononucleosis and *Mycoplasma pneumoniae* infections. Hypersplenism may ensue, which can further decrease RBC life expectancy. In addition, the antibiotic drugs used to treat a variety of these infections may lead to further hemolysis in G6PD-deficient individuals. Anemia that occurs with concomitant acute or chronic infection is likely to be multifactorial, with the anemia of chronic inflammation often coexisting and predominating.

RBC membrane injury caused by bacteria

Clostridial sepsis

Clostridial sepsis is seen in patients with anaerobic subcutaneous infections, in body areas of impaired circulation, after trauma, after septic abortion or postpartum sepsis, and in patients with acute cholecystitis with gangrene of the gallbladder or bowel necrosis. Severe neutropenia of any cause may be a significant risk factor. The α toxin of *Clostridium* is a lecithinase (phospholipase C) that disrupts the lipid bilayer structure of the RBC membrane, leading to membrane loss and hemolysis. Brisk intravascular hemolysis with spherocytosis seen on the peripheral blood smear is accompanied by hemoglobinemia, hemoglobinuria, and severe anemia. The plasma may be a brilliant red color, and there may be dissociation between the hemoglobin and hematocrit values because of the plasma hemoglobin levels reaching several grams per deciliter. Acute renal failure may ensue secondary to excessive hemoglobinuria, but the exact mechanism remains disputed. Renal failure and hepatic failure contribute to the high mortality in clostridial sepsis.

Hemolytic anemias with Gram-positive and Gram-negative organisms

Septicemia and endocarditis caused by Gram-positive bacteria, such as streptococci, staphylococci, *S. pneumoniae*, and *Enterococcus faecalis* are often associated with hemolytic anemia. The anemia in patients with infections due to Gram-positive cocci appears to result from the direct toxic effect of a bacterial product on erythrocytes. *Salmonella typhi* infection may be accompanied by severe hemolytic anemia with hemoglobinemia. In typhoid fever, the onset of hemolysis may occur during the first 3 weeks of illness, with anemia lasting from several days to 1 week. *Salmonella* and other microorganisms can cause direct agglutination of RBCs in vitro, but it is not known whether this phenomenon contributes to in vivo hemolysis. In approximately one-third of patients with typhoid fever, a positive DAT develops, but hemolytic anemia is not manifest in all cases.

Immune hemolysis associated with infections

Pneumonia caused by *M. pneumoniae* can be associated with production of cold agglutinins, IgM antibodies directed against the RBC I antigen. Hemolytic anemia associated with *M. pneumoniae* may occur during the second or third week of the illness. The onset of the hemolysis may be rapid, usually occurring after recovery from respiratory symptoms. The clinical presentation often includes dyspnea or fatigue and the presence of pallor and jaundice. The blood smear shows RBC agglutination with or without spherocytosis and with polychromatophilia (Figure 7-14). When ethylenediaminetetraacetic acid–anticoagulated blood is cooled in a test tube, RBC agglutination can be seen; disagglutination occurs when the blood is warmed. Cold agglutination titers at the onset of hemolysis usually exceed 1:256 and may reach higher levels, although they are typically lower than in monoclonal cold agglutinin disease. The DAT is positive for complement deposition on RBCs. The hemolytic anemia associated with *Mycoplasma* pneumonia is self-limited, transient, and usually mild, although severe cases requiring corticosteroid therapy or plasmapheresis have been reported.

Infectious mononucleosis caused by Epstein-Barr virus infection may be associated with hemolytic anemia due to cold agglutination. The cold agglutinin in this case is an IgM antibody directed against the i antigen. Severe hemolytic anemia associated with infectious mononucleosis is unusual, although anti-i antibodies frequently are present. When hemolytic anemia occurs, the mechanism involves fixation of complement on the RBC membrane by IgM antibodies. Hemolysis proceeds either by completion of the complement cascade through C9 or by opsonization of RBCs with fragments of C3 leading to phagocytosis of RBCs by macrophages in the liver or spleen.

Several other viral infections have been associated with AHA. These include cytomegalovirus, herpes simplex, rubeola, varicella, influenza A, and HIV. Postviral acute hemolytic anemia in children may be due to the formation of a cold-reactive hemolytic IgG antibody of the Donath-Landsteiner type, which induces complement lysis of RBCs.

Microangiopathic hemolytic anemias associated with infection include bacteremia with Gram-negative organisms, staphylococci, meningococci, and pneumococci, all of which can lead to DIC with endothelial damage and fibrin thrombi within the microcirculation. RBC injury results from mechanical fragmentation by fibrin strands in the vasculature. Microvascular damage induced by meningococcal and rickettsial infections (eg, Rocky Mountain spotted fever) may be associated with DIC, thrombocytopenia, microvascular thrombi, and fragmentation hemolytic anemia. Patients with either congenital or tertiary syphilis may develop paroxysmal cold hemoglobinuria. Whereas paroxysmal cold hemoglobinuria

used to be fairly common in the late 19th and earlier 20th centuries, it is rare in the 21st century due to the disappearance of congenital and tertiary syphilis.

Hemolytic anemia associated with parasitic infestation of RBCs

Malaria

Malaria is the most common cause of hemolytic anemia worldwide. Transmitted by the bite of an infected female *Anopheles* mosquito, sporozoites that are injected from the mosquito make their way to liver cells. Merozoites enter into the bloodstream 1-2 weeks later. Hemolysis in malaria results directly from erythrocytic infestation by *Plasmodium* organisms (Figures 7-17 and 7-18). Noninfected RBCs may be hemolyzed by poorly understood mechanisms. Infested erythrocytes are selectively removed from the circulation by the spleen, with some RBCs reentering circulation after splenic pitting of parasites. Previously infested erythrocytes manifest membrane and metabolic abnormalities along with decreased deformability. In addition, the *Plasmodium* species digests the host RBC hemoglobin for its own use as a nutrient.

The severity of the hemolytic process is often out of proportion to the degree of parasitemia. *P. vivax* and *Plasmodium ovale* invade only reticulocytes, whereas *Plasmodium malariae* invades only mature erythrocytes. *P. falciparum* invades erythrocytes of all ages and is associated with more severe hemolysis. In areas where malaria has been a frequent cause of death for many centuries, a number of genetic polymorphisms are prevalent, including G6PD deficiencies, thalassemias, and hemoglobinopathies. These polymorphisms have in common the ability to interfere with the ability of the malaria parasites to invade RBCs.
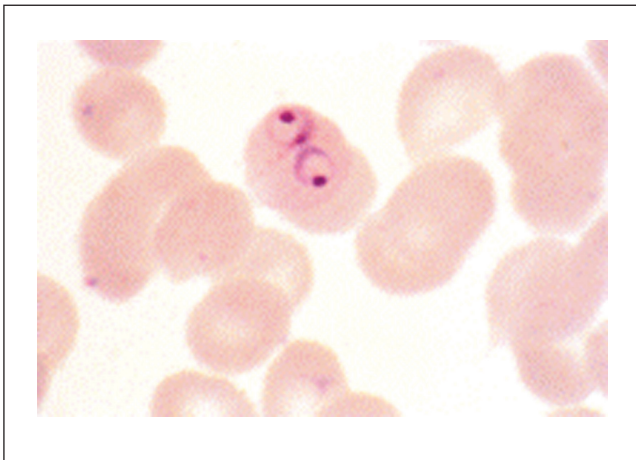

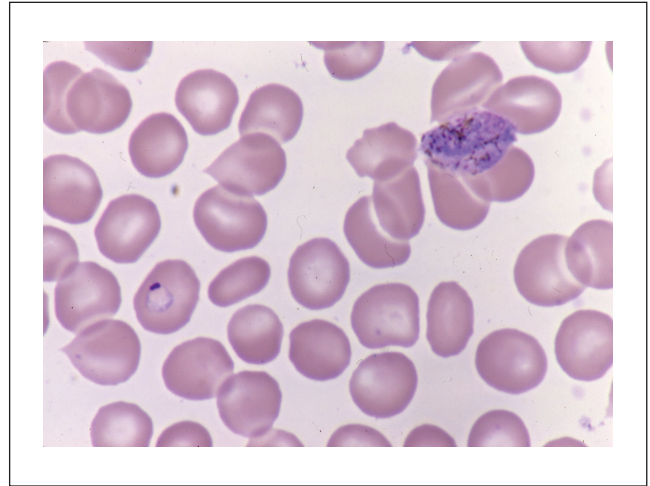**Figure 7-17** Intraerythrocyte parasite *Plasmodium falciparum*.

Figure 7-18 Intraerythrocyte parasite *Plasmodium vivax*: trophozoite (ring form) and female gametocyte.

With *P. falciparum* infection, intravascular hemolysis may be severe and associated with hemoglobinuria (blackwater fever). Another potentially lethal complication of *P. falciparum* infection, cerebral malaria, results from expression of *P. falciparum* erythrocyte protein 1 on the membranes of infected RBCs. These RBCs adhere to receptors on vascular endothelium in various organs, including the central nervous system, resulting in vaso-occlusion and neurologic manifestations.

Diagnosis of malaria is based on identification of parasite-infected RBCs on a thick Wright-stained blood smear. The distinction of *P. falciparum* infection from the other species is important because its treatment may constitute a medical emergency. The findings of two or more parasites per RBC and infestation of >5% of RBCs are characteristic of *P. falciparum* infection.

Chemoprophylaxis should be offered to all people planning travel to known endemic areas. The hemolytic anemia of malaria resolves after successful therapy with quinine, chloroquine, artemisinin, and other drugs, depending on the species of malaria. Many of these agents may be associated with drug-induced hemolysis in patients with G6PD deficiency.

Babesiosis

Babesiosis is a protozoan infection caused by *Babesia microti*. Once thought to be rare, outbreaks have been described with increasing frequency on Nantucket Island, in Cape Cod, in northern California, and in several other North American locations. The organism is transmitted by the bite of the *Ixodes* tick, which infects many species of wild birds and domestic animals and occasionally humans. Babesiosis rarely may be transmitted by transfusion with fresh or
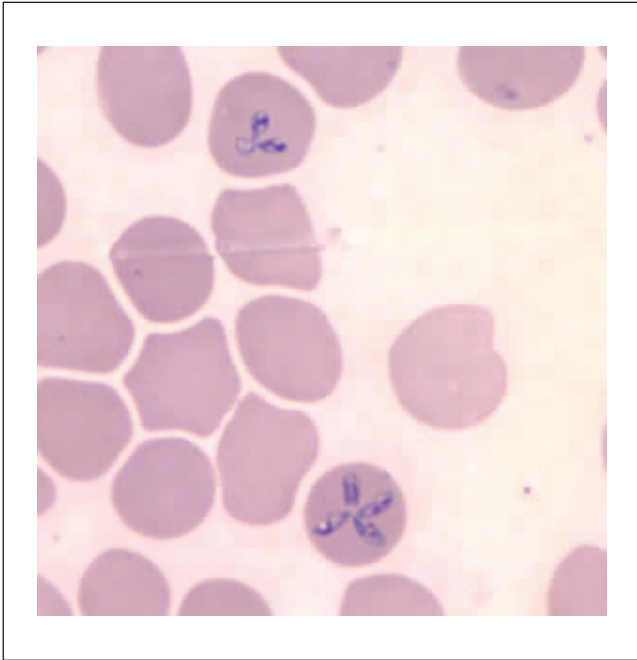
Figure 7-19 Intraerythrocyte parasite *Babesia microti*.

frozen-thawed RBCs. Infection leads to a clinical syndrome of fever, lethargy, malaise, and hemoglobinuria 1-4 weeks after the bite. Hemolytic anemia due to intravascular hemolysis occurs, and renal and liver function tests are frequently abnormal. The disease is often asymptomatic in people with intact spleens; patients who have undergone splenectomy are at high risk for severe symptomatic infection. *Babesia* infection can be diagnosed by demonstrating typical intraerythrocytic parasites on a thin blood smear (Figure 7-19). Standard treatment has consisted of clindamycin and quinine. Recent studies have suggested that atovaquone plus azithromycin is an equally efficacious regimen, yet better tolerated.

Bartonellosis

Bartonellosis, caused by *Bartonella bacilliformis*, manifests in two clinical stages: an acute hemolytic anemia and a chronic granulomatous phase. The microorganism enters the blood following the bite of an infected sand fly. The infective *Bartonella* agent adheres to the membrane of RBCs that are then removed by the spleen. The hemolytic anemia of bartonellosis develops rapidly and may be severe, with hemoglobinemia and hemoglobinuria. When untreated, this disorder is associated with high mortality. Survivors manifest a second stage of the disease with cutaneous granulomas. Bartonellosis is common in South America and has been reported in the Peruvian Andes and parts of Brazil, where it is also known as

Oroya fever. On Giemsa-stained blood films, red-violet rods of varying lengths can be identified on RBCs and represent the bacteria. Effective treatment exists and consists of penicillin, streptomycin, chloramphenicol, or tetracycline.

Clinical case (continued)

The patient was admitted for treatment. The CDC Malaria Hotline (1-770-488-7788) was called, and the regimen of chloroquine and primaquine was recommended for vivax malaria acquired in Afghanistan. He made a full recovery. He ultimately admitted that he had forgotten to take his prophylactic medications after leaving Afghanistan. The most common cause of failure of malaria prophylaxis in military or civilian populations is noncompliance. Because of the importance of primaquine in terminal prophylaxis and treatment of vivax malaria, it is currently the policy of the US military to screen all personnel for G6PD deficiency.

Key points

- Red cell fragmentation syndromes are diverse in etiology.
- In all suspected cases of hemolytic anemia, the blood smear should be examined carefully for schistocytes. Their presence can direct differential diagnosis.
- Red cell destruction can be at the macrovascular or microvascular (microangiopathic) level of the circulatory system. Classic examples include heart valve hemolysis, DIC, and TTP.
- Various chemical exposures or physical agents can cause fragmentation hemolysis.
- Infection can cause accelerated RBC destruction through a variety of mechanisms, including direct invasion, toxin production, and immune mechanisms.
- Malaria, the most common infectious disease worldwide, causes hemolysis through both direct parasitic invasion of RBCs and through alterations in noninfected cells. Malaria can be diagnosed by thorough review of a thick Wright-stained peripheral blood smear.

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