

35 - 105 Hemolytic Anemias

105 Hemolytic Anemias

Lucio Luzzatto, Lucia De Franceschi

Hemolytic Anemias ■ ■DEFINITIONS Turnover is typical of all blood cells, including erythrocytes, that have a finite life span. Hence, a logical, time-honored classification of anemias is in three groups: (1) decreased production of red cells, (2) increased destruction of red cells, and (3) acute blood loss. Decreased production is covered in Chaps. 102, 103, and 107; acute blood loss in Chap. 106; increased destruction is covered in this chapter. All patients who are anemic as a result of either increased destruction of red cells or acute blood loss have one important element in common: the anemia results from overconsumption of red cells from the peripheral blood, whereas the supply of cells from the bone marrow is normal (indeed, it is usually increased). However, with blood loss, as in acute hemorrhage, the red cells are physically lost from the body itself; this is fundamentally different from destruction of red cells within the body, as in hemolytic anemias (HAs). With respect to primary etiology, HAs may be inherited or acquired; from a clinical point of view, they may be more acute or more chronic, and they may vary from mild to very severe; the site of hemolysis may be predominantly intravascular or extravascular. With respect to mechanisms, HAs may be due to intracorpuseular causes or to extracorpuseular causes (Table 105-1). But before reviewing the individual types of HA, it is appropriate to consider what general features they have in common, in terms of clinical aspects and pathophysiology. ■ ■GENERAL CLINICAL AND LABORATORY FEATURES The clinical presentation of a patient with anemia is greatly influenced in the first place by whether the onset is abrupt or gradual, and HAs are no exception. A patient with autoimmune HA or with favism may be a medical emergency, whereas a patient with mild hereditary spherocytosis (HS) or with cold agglutinin disease (CAD) may be diagnosed after years. This is due in large measure to the remarkable ability of the body to adapt to anemia when it is slowly progressing (Chap. 66). What differentiates HAs from other anemias is that the patient has signs and symptoms arising directly from hemolysis (Table 105-2). At the clinical level, the main sign is jaundice; in addition, the patient may report discoloration of the urine. In many cases of HA, the spleen is enlarged because it is a preferential site of hemolysis; in some cases, the liver may be enlarged as well; and gallstones are common. In all severe congenital forms of HA, there may also be skeletal changes due to over activity of the bone marrow: they are never as severe as in thalassemia

TABLE 105-1 Classification of Hemolytic Anemias

EXTRACORPUSCULAR FACTORS

INTRACORPUSCULAR DEFECTS

Inherited Hemoglobinopathies Enzymopathies Membrane-cytoskeletal defects Familial (atypical) hemolytic-uremic syndrome Acquired Paroxysmal nocturnal hemoglobinuria (PNH) Mechanical destruction (microangiopathic) Toxic agents Drugs Infectious Autoimmune

Hereditary causes correlate with intracorpuseular defects because these defects are due to inherited mutations; the one exception is PNH because the defect is due to an acquired somatic mutation. Conversely, acquired causes correlate with extracorpuseular factors because

mostly these factors are exogenous; the one exception is familial hemolytic-uremic syndrome (HUS; often referred to as atypical HUS [aHUS]) because here an inherited abnormality permits complement activation triggered by exogenous factors to become excessive, with bouts of production of membrane attack complex capable of destroying normal red cells. Interestingly, in both PNH and aHUS, hemolysis is complement-mediated.

TABLE 105-2 Features Common to Most Patients with a Hemolytic Disorder

GENERAL EXAMINATION
JAUNDICE, PALLOR Other physical findings Spleen may be enlarged; bossing of skull in severe congenital cases
Hemoglobin level From normal to severely reduced
MCV, MCH Usually increased
Reticulocytes Usually increased
Bilirubin Almost always increased (mostly unconjugated)
LDH Increased (up to 10× normal with intravascular hemolysis)
Haptoglobin Reduced to absent if hemolysis is at least in part intravascular
Abbreviations: LDH, lactate dehydrogenase; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume.

major because ineffective erythropoiesis is less, or even absent. Since several forms of HA are inherited, it is important to include family history in the initial appraisal. The laboratory features of HA are related to (1) hemolysis per se and (2) the erythropoietic response of the bone marrow. In most cases, hemolysis is largely extravascular, and it produces an increase in unconjugated bilirubin and aspartate aminotransferase (AST) in the serum; urobilinogen will be increased in both urine and stool. If hemolysis is mainly intravascular, the telltale sign is hemoglobinuria (often associated with hemosiderinuria); in the serum, there is free hemoglobin, lactate dehydrogenase (LDH) is increased, and haptoglobin is reduced. In contrast, the serum bilirubin level may be normal or only mildly elevated. The main sign of the erythropoietic response by the bone marrow is an increase in reticulocytes (a test all too often neglected in the initial workup of a patient with anemia). Usually the increase will be reflected in both the percentage of reticulocytes (the more commonly quoted figure) and in the absolute reticulocyte count (the more definitive parameter). The increased number of reticulocytes is associated with an increased mean corpuscular volume (MCV) in the blood count. On the blood smear, this is reflected in the presence of macrocytes; polychromasia is also present, and sometimes one sees nucleated red cells. In most cases, a bone marrow aspirate is not necessary in the diagnostic workup; if it is done, it will show erythroid hyperplasia. In practice, once an HA is suspected, specific tests will usually be required for a definitive diagnosis of a specific type of HA.

CHAPTER 105 Hemolytic Anemias ■ ■ **GENERAL PATHOPHYSIOLOGY**

The mature red cell is the product of a developmental pathway that brings the phenomenon of differentiation to an extreme. An orderly sequence of events produces synchronous changes, whereby the gradual accumulation of a huge amount of hemoglobin in the cytoplasm (to a final level of 340 g/L, i.e., about 5 mM) goes hand in hand with the gradual loss of cellular organelles and of biosynthetic abilities. In the end, the erythroid cell undergoes a process that has features of apoptosis, including nuclear pyknosis and eventually extrusion of the nucleus. However, the final result is more altruistic than suicidal; the cytoplasmic body, instead of disintegrating, is now able to provide oxygen to all cells in the human organism for some remaining 120 days of the red cell life span. As a result of this unique process of differentiation and maturation, intermediary metabolism is drastically curtailed in mature red cells (Fig. 105-1); for instance, cytochrome-mediated oxidative phosphorylation has been lost with the loss of mitochondria (through a process of physiologic autophagy); therefore, there is no backup to anaerobic glycolysis, which in the red cell is the only provider of adenosine triphosphate (ATP). Also, the capacity of making protein has been lost with the loss of ribosomes. This places the cell's limited metabolic apparatus at risk, because if any protein component deteriorates, it cannot be replaced, as it would be in most other cells; and in

fact, the activity of most enzymes gradually decreases as red cells age. At the same time, during their long time in circulation, various red cell

Embden-Meyerhof pathway Hexose monophosphate shunt Glutathione reductase GSH GSSG glucose hexokinase ATP NADPH NADP⁺ ADP glucose-6-phosphate 6-phosphogluconate G6PD glucose phosphate isomerase fructose-6-phosphate ATP ADP phosphofructokinase fructose-1, 6-diphosphate aldolase glyceraldehyde-3-phosphate NAD⁺ glyceraldehyde 3-phosphate dehydrogenase HbFe²⁺ NADH HbFe³⁺ 2,3-bisphosphoglycerate mutase 1,3-bisphosphoglycerate ADP phosphoglycerate kinase 2,3-bisphosphoglycerate ATP 2,3-bisphosphoglycerate phosphatase 3-phosphoglycerate PART 4 Oncology and Hematology 3-phosphoglycerate mutase 2-phosphoglycerate enolase phosphoenolpyruvate pyruvate kinase ADP ATP pyruvate NADH lactate dehydrogenase NAD⁺ lactate FIGURE 105-1 Red blood cell (RBC) metabolism. The Embden-Meyerhof pathway (glycolysis) generates ATP required for cation transport and for membrane maintenance. The generation of NADH maintains hemoglobin iron in a reduced state. The hexose monophosphate shunt generates NADPH that is used to reduce glutathione, which protects the red cell against oxidant stress; the 6-phosphogluconate, after decarboxylation, can be recycled via pentose sugars to glycolysis. Regulation of the 2,3-bisphosphoglycerate level is a critical determinant of oxygen affinity of hemoglobin. Enzyme deficiency states in order of prevalence: glucose-6-phosphate dehydrogenase (G6PD) > pyruvate kinase > glucose-6-phosphate isomerase > rare deficiencies of other enzymes in the pathway. The more common enzyme deficiencies are encircled. components inevitably accumulate damage and become physically denser. The anion exchanger known as band 3 is the most abundant protein in the red cell membrane (Fig. 105-2 and Table 105-3), with about

1.2 million molecules per red cell. As red cells age and become denser, probability is increased that a region of the band 3 molecule becomes exposed on the cell surface and contributes to creating an antigenic site recognizable by low-avidity naturally occurring anti-band 3 IgG antibodies. This process might be enhanced by the clustering of band 3 molecules favored by the antibody itself and by the binding of hemichromes arising from hemoglobin degradation. Senescent red cells thus become opsonized, and this is the signal for phagocytosis by macrophages in the spleen, in the liver, and elsewhere. This process may become accelerated in various ways in HA. Another consequence of the relative simplicity of red cells is that they have a limited range of ways to manifest distress under hardship; in essence, any sort of metabolic failure will eventually lead either to structural damage to the membrane or to failure of the cation pump. In either case, the life span of the red cell is reduced, which is the definition of a hemolytic disorder. If the rate of red cell destruction exceeds the capacity of the bone marrow to produce more red cells, the hemolytic disorder will manifest as HA.

Thus, the essential pathophysiologic process common to all HAs is an increased red cell turnover; in many HAs, this is due at least in part to an acceleration of the senescence process described above. The gold standard for proving that the life span of red cells is reduced (compared to the normal value of ~120 days) is a red cell survival study, which can be carried out by labeling the red cells with ⁵¹Cr and measuring the fall in radioactivity over several days or weeks (this classic test can now be replaced by a methodology using the nonradioactive isotope ¹⁵N). If the hemolytic event is transient, it does not usually cause any long-term consequences, except for an increased requirement for erythropoietic factors, particularly folic acid. However, if hemolysis is recurrent or

persistent, the increased bilirubin production favors the formation of gallstones. If a considerable proportion of hemolysis takes place in the spleen, as is often the case, splenomegaly may become increasingly a feature, and hypersplenism may develop, with consequent neutropenia and/or thrombocytopenia. The increased red cell turnover has important consequences. In normal subjects, the iron from effete red cells is very efficiently recycled by the body; however, with chronic intravascular hemolysis, the persistent hemoglobinuria will cause considerable iron loss, needing replacement. With chronic extravascular hemolysis, the opposite problem, iron overload, is more common, especially if the patient needs frequent blood transfusions. Even without blood transfusion, when erythropoiesis is massively increased, the release of erythroferrone from erythroid cells suppresses hepcidin, causing increased iron absorption. In the long run, in the absence of iron-chelation therapy, iron overload will cause secondary hemochromatosis; this will cause damage particularly to the liver, eventually leading to cirrhosis, and to the heart muscle, eventually causing heart failure. Compensated Hemolysis versus Hemolytic Anemia Red cell destruction is a potent stimulus for erythropoiesis, which is mediated by erythropoietin (EPO) produced by the kidney. This mechanism is so effective that in many cases the increased output of red cells from the bone marrow can fully balance an increased destruction of red cells. In such cases, we say that hemolysis is compensated. The pathophysiology of compensated hemolysis is similar to what we have just described, except there is no anemia. This notion is important from the diagnostic point of view, because a patient with a hemolytic condition, even an inherited one, may present without anemia; and it is also important from the point of view of management because compensated hemolysis may become “decompensated,” i.e., anemia may suddenly appear in certain circumstances, for instance in pregnancy, folate deficiency, or renal failure interfering with adequate EPO production. Another general feature of chronic HAs is seen when any intercurrent condition, such as an acute infection, depresses erythropoiesis. When this happens, in view of the increased rate of red cell turnover, the effect will be predictably much more marked than in a person who does not have hemolysis. The most dramatic example is infection by parvovirus B19, which may cause a rather precipitous fall in hemoglobin—an occurrence sometimes referred to as aplastic crisis. ■ ■

INHERITED HEMOLYTIC ANEMIAS The red cell has three essential components: (1) hemoglobin, (2) the membrane-cytoskeleton complex, and (3) the metabolic machinery necessary to keep hemoglobin and the membrane-cytoskeleton complex in working order. Diseases caused by inherited abnormalities of hemoglobin, or hemoglobinopathies, are covered in Chap. 103. Here we will deal with diseases of the other two components. Hemolytic Anemias due to Abnormalities of the Membrane-Cytoskeleton Complex The detailed architecture of the red cell membrane is complex, but its basic design is relatively simple (Fig. 105-2). The lipid bilayer incorporates phospholipids and cholesterol, and it is spanned by a number of proteins that have their hydrophobic transmembrane domain(s) embedded in the membrane; most of these proteins also extend to both the outside (extracellular domains) and the inside of the cell (cytoplasmic domains). Other proteins are tethered to the membrane through a glycosylphosphatidylinositol (GPI) anchor; these have only an extracellular domain. Membrane proteins include energy-dependent ion transporters, ion channels, receptors

Ankyrin complex RhAG AChE CD59 Rh KCNN4 ABCB6 Band 3 Band 3 CD47 PIEZO1 4.2 GPA Adducin -Spectrin 4.1R Ankyrin -Spectrin Tropomyosin Self-association site PIEZO1 KCNN4 ABCB6 Glut1 Band 3 HCO3⁻ Cl⁻ Na⁺ K⁺ Ca²⁺ Glucose

FIGURE 105-2 The red cell membrane and cytoskeleton schematic diagram. Within the membrane lipid bilayer, several integral membrane proteins are shown (see inset). Other proteins, e.g., acetylcholinesterase (AChE) and the two complement-

regulatory proteins CD59 and CD55, are tethered to the membrane through the glycosylphosphatidylinositol (GPI) anchor: in these cases, the entire polypeptide chain is extracellular. Many of the membrane proteins bear polypeptide and/or carbohydrate red cell antigens. Underneath the membrane, the α - β spectrin dimers, which associate head-to-head into tetramers, together with actin and other proteins, form most of the cytoskeleton. The ankyrin complex, which also involves the band 4.2 protein, and the junctional complex, which involves the band 4.1 protein and dematin, connect the membrane to the cytoskeleton. The ankyrin complex provides mainly radial (also called vertical) connections; the junctional complex provides mainly tangential (also called horizontal) connections. Pathogenic changes in the former can cause spherocytosis, whereas pathogenic changes in the latter can cause elliptocytosis; pathogenic changes in spectrin can cause either. Branched lines symbolize carbohydrate moiety of proteins. The various molecules are obviously not drawn to the same scale. Inset. Schematic diagram of membrane transporters, abnormalities of which underlie channelopathies. Band 3 (anion exchanger 1 [AE1]) is the most abundant. Mutations of SLC4A1 (encoding band 3) can inactivate anion exchange, causing cation leak or negatively affecting interactions with neighboring membrane proteins. PIEZO1 is a huge protein that is embedded in the membrane as a homo-trimer with a three-bladed, propeller-shaped structure; it is a mechano-sensitive cation channel. KCNN4 encodes a Ca^{2+} activated K^{+} channel, also known as the Gardos channel. PIEZO1 mutations can be associated with abnormal Ca^{2+} entry, resulting in overactivation of the Gardos channel; this promotes K^{+} efflux and red cell dehydration. Mutations on KCC4 induce alterations of channel properties (kinetic or ion trafficking), resulting again in abnormal K^{+} efflux and red cell dehydration. ABCB6 encodes a mitochondrial porphyrin transporter, but some mutations can cause increased K^{+} efflux from red cells and stomatocytosis. Glut1 facilitates glucose transport; it is encoded by SLC2A1, and mutations can block the glucose transport and induce Na^{+} , K^{+} leakage. (Modified from N Young et al: Clinical Hematology. Philadelphia, Elsevier, 2006; and from A Iolascon et al: Br J Haematol 187:13, 2019.) for complement components, and receptors for other ligands. The most abundant red cell membrane proteins are glycophorins and the so-called band 3, an anion transporter that is an integral membrane protein. The extracellular domains of many of these proteins are heavily glycosylated, and they carry antigenic determinants that correspond to blood groups. Underneath the membrane, and tangential to it, is a network of other proteins that make up the cytoskeleton. The main cytoskeletal protein is the spectrin tetramer, consisting of a head-to-head association of two α -spectrin- β -spectrin heterodimers. The cytoskeleton is linked to the membrane through the ankyrin complex (that includes also band 4.2) and the junctional complex (that includes adducin and band 4.1) (Fig. 105-2). These multiprotein complexes make membrane and cytoskeleton intimately connected to each other, thus supporting membrane stability and at the same time providing the erythrocyte with the important property of deformability.

The membrane-cytoskeleton complex has essentially three functions: it is an envelope for the red cell cytoplasm; it maintains the normal red cell shape; and it provides cross-membrane transport of electrolytes and of metabolites such as glucose and amino acids. In the membrane-cytoskeleton complex, the individual components are so intimately associated with each other that an abnormality of almost any of them will be disturbing or disruptive, causing mechanical instability of the membrane and/or reduced red cell deformability, ultimately causing hemolysis. These abnormalities are almost invariably inherited mutations; thus, diseases of the membrane-cytoskeleton complex belong to the category of inherited HAs. Before the red cells lyse, they often exhibit

more or less specific changes that alter the normal biconcave disk shape. Thus, the majority of the diseases in this group have been known for over a century as hereditary spherocytosis (HS) and hereditary elliptocytosis (HE). More recently, a third morphologic entity, whereby on a blood smear the roundshaped central pallor of a red cell is replaced by a linear-shaped central pale area, has earned the name stomatocytosis; because this abnormal shape is related to abnormalities of channel molecules, the underlying disorders are also referred to as channelopathies. From an understanding of the molecular basis of these disorders, it has emerged (Table 105-3) that, although these disorders are predominantly monogenic, no one-to-one correlation exists between a certain gene and a certain disorder. Rather, what has been regarded as a single disorder (e.g., HS) can arise through mutation of one of several genes; conversely, what have been regarded as different disorders can arise through different mutations of the very same gene (Fig. 105-3).

Junctional complex CD55 GPC Glut1 p55 Dematin Actin protofilament Tropomodulin CHAPTER 105 Hemolytic Anemias HEREDITARY SPHEROCYTOSIS This is most common among this group of HAs, with an estimated prevalence of 1:2000–1:5000 in populations of European ancestry. Its identification is credited to Minkowsky and Chauffard, who, at the end of the nineteenth century, reported families who had spherocytes in their peripheral blood (Fig. 105-4A). In vitro studies revealed that the red cells were abnormally susceptible to lysis in hypotonic media; indeed, the increase in osmotic fragility became the main diagnostic test for HS. Today we know that HS, thus defined, is genetically heterogeneous; i.e., it can arise from a variety of mutations in one of several genes (Table 105-3). It has been also recognized that the inheritance of HS is not always autosomal dominant (with the patient being heterozygous); indeed, some of the most severe forms are instead autosomal recessive (with the patient being homozygous). Clinical Presentation and Diagnosis The spectrum of clinical severity of HS is broad. Severe cases may present in infancy with severe anemia, whereas mild cases may present in young adults or even later in life. The main clinical findings are jaundice, an enlarged spleen, and often gallstones; indeed, it may be the finding of gallstones in a young person that triggers diagnostic investigations.

TABLE 105-3 Inherited Diseases of the Red Cell Membrane-Cytoskeleton Complex	CHROMOSOMAL LOCATION	PROTEIN PRODUCED	DISEASE(S) WITH CERTAIN MUTATIONS (INHERITANCE)	COMMENTS
GENE SPTA1	1q22-q23	α -Spectrin	HS (recessive) Rare	HE (dominant) Mutations of this gene account for about 65% of HE. More severe forms may be due to coexistence of an otherwise silent mutant allele.
SPTB	14q23-q24.1	β -Spectrin	HS (dominant) Rare	HE (dominant) Mutations of this gene account for about 30% of HE, including some severe forms.
ANK1	8p11.2	Ankyrin	HS (dominant)	May account for majority of HS.
SLC4A1	17q21 Band 3	Band 3; also known as AE (anion exchanger) or AE1	HS (dominant)	Mutations of this gene may account for about 25% of HS.
Southeast Asia ovalocytosis	(dominant)	Stomatocytosis (cryohydrocytosis)	EPB41	1p33-p34.2 Band 4.1 HE (dominant) Mutations of this gene account for about 5% of HE, mostly with prominent morphology but little/no hemolysis in heterozygotes; severe hemolysis in homozygotes.
EPB42	15q15-q21 Band 4.2	HS (recessive)	Mutations of this gene account for about 3% of HS.	
RHAG	6p21.1-p11	Rhesus-associated glycoprotein	Chronic nonspherocytic hemolytic anemia (recessive)	
PART 4 Oncology and Hematology	PIEZO1	16q23-q24	PIEZO1 (mechanosensitive ion channel component 1 channel)	Dehydrated hereditary stomatocytosis (dominant)
KCNN4	19q13.31	KCNN4	Intermediate conductance calcium-activated potassium channel protein 4 (Gardos channel)	Dehydrated hereditary stomatocytosis (dominant)
ABCB6	2q35-q36	ATP-binding cassette subfamily B member 6	Familial pseudohyperkalemia (dominant)	SLC2A1
1p34.2	GLUT1	glucose transporter		

Overhydrated hereditary stomatocytosis Note: PIEZO1, KCNN4, ABCB6, and GLUT1 are channel molecules; conditions associated with mutations in the respective genes are appropriately named channelopathies. Abbreviations: HE, hereditary elliptocytosis; HS, hereditary spherocytosis. The variability in clinical manifestations that is observed among patients with HS is largely due to the different underlying molecular lesions (Table 105-3). Not only are mutations of several genes involved, ANK1 EPB42 HS SPTA1 SPTB SLC4A1 EPB41 HE SLC2A1 RHAG PIEZO1 HSt KCNN4 ABCB6 FIGURE 105-3 Hereditary spherocytosis (HS), hereditary elliptocytosis (HE), and hereditary stomatocytosis (HSt) are three morphologically distinct forms of congenital hemolytic anemia. It has emerged that each one can arise from mutation of one of several genes and that different mutations of the same gene can give one or another form. (See also Table 105-3.) Genes encoding membrane proteins are in black; genes encoding cytoskeleton proteins are in green; genes encoding proteins in the junctional and ankyrin complexes are in purple.

Polymorphic mutation (deletion of nine amino acids); in heterozygotes, clinically asymptomatic and protective against *Plasmodium falciparum*. Certain specific missense mutations shift protein function from anion exchanger to cation conductance. Very rare; associated with total loss of all Rh antigens. One specific mutation in this gene entails loss of stomatin from the cell membrane, causing overhydrated stomatocytosis. Also known as xerocytosis with pseudohyperkalemia. Patients may present with perinatal edema. Clinical presentation similar to that of PIEZO1 mutants. Increased potassium leakage upon storage in blood bank condition: this can cause hyperkalemia in the recipient. ABCB6 mutation is present in 0.3% of blood donors. Associated with serious neurologic manifestations. but also different mutations of the same gene can give very different clinical manifestations. In milder cases, hemolysis is often compensated (see above), but changes in clinical expression may be seen even in the same patient because intercurrent conditions (e.g., pregnancy, infection) may cause decompensation. The anemia is usually normocytic with the characteristic morphology that gives the disease its name. An increased mean corpuscular hemoglobin concentration (MCHC >34 g/dL) and increased red cell distribution width (RDW

14%) associated with normal or slightly decreased MCV on an ordinary blood count report should raise the suspicion of HS. The spleen plays a key role in HS through a dual mechanism. On one hand, because HS red cells are less deformable, transit through the splenic circulation makes them more prone to vesiculate; on the other hand, like in many other HAs, the spleen itself is a major site of destruction through phagocytosis by macrophages. When there is a family history, it is usually easy to make a diagnosis based on features of HA and typical red cell morphology. However, family history may be negative for at least two reasons. First, the patient may have a *de novo* mutation, i.e., a mutation that has taken place in a germ cell of one of the patient's parents or early after zygote formation. Second, the patient may have a recessive form of HS (Table 105-3). In such cases, more extensive laboratory investigations are required, including osmotic fragility, the acid glycerol lysis test, the eosin-5'-maleimide (EMA)-binding test, sodium dodecyl sulfate (SDS)-gel electrophoresis of membrane proteins, and ektacytometry (testing red cell deformability as a function of shear stress at different osmolality); these tests are usually carried

out in laboratories with special expertise in this area. Sometimes a definitive diagnosis can be obtained only by molecular studies demonstrating a mutation in one of the genes underlying HS (Table 105-3).

TREATMENT Hereditary Spherocytosis We do not have a causal treatment for HS; i.e., no way has yet been found to correct the basic defect in the membrane-cytoskeleton structure. Given the special role of the spleen in HS (see above), splenectomy is often beneficial. Current recommendations are to proceed with splenectomy at the age of 4–6 years in severe cases, to delay splenectomy until puberty in moderate cases, and to avoid splenectomy in mild cases. Partial splenectomy can be considered in certain cases, and it is helpful to know about the outcome of splenectomy in the patient's affected relatives. Before splenectomy, vaccination against encapsulated bacteria (*Neisseria meningitidis* and *Streptococcus pneumoniae*) is imperative; penicillin prophylaxis after splenectomy is controversial. Along with splenectomy, cholecystectomy should not be carried out automatically, but it should be carried out, usually by the laparoscopic approach, whenever it is clinically indicated, mainly when gallstones are symptomatic. The most severe cases of HS (estimated at <10%) are transfusion dependent, and in infants with severe HS, erythropoietin may prove to be transfusion sparing.

HEREDITARY ELLIPTOCYTOSIS HE is at least as heterogeneous as HS, both from the genetic point of view (Table 105-3, Fig. 105-3) and from the clinical point of view. The global incidence of HE is 1:2000–4000 subjects. Again, it is the shape of the red cells (Fig. 105-4B) that gives the name to the condition, but there is no direct correlation between the elliptocytic morphology and clinical severity. In fact, some mild or even asymptomatic cases may have nearly 100% elliptocytes (or ovalocytes). Indeed, the diagnosis of HE is generally incidental, because hemolysis may be compensated and there may be no anemia, although this may become evident in the course of infection. One particular in-frame deletion of nine amino acids in the SLC4A1 gene encoding band 3 underlies the so-called Southeast Asia ovalocytosis (SAO): it is not a disease, but rather a polymorphism with a frequency of up to 5–7% in certain populations (e.g., Papua New Guinea, Indonesia, Malaysia, Philippines), presumably as a result of malaria selection. It is asymptomatic in heterozygotes and probably lethal in homozygotes. The cases of HE with the most severe HA are those with biallelic mutations of one of the genes involved (see Fig. 105-3), and these are said to have hereditary pyropoikilocytosis (HPP): here the instability of the cytoskeleton protein network may result from decreased tetramerization of spectrin dimers. The red cell volume is decreased (MCV: 50–60 fL), and all kinds of bizarre poikilocytes are seen on the blood smear (Fig. 105-4C). HPP patients have splenomegaly and often benefit from splenectomy.

Channelopathies These rare conditions (see Fig. 105-3) are characterized by abnormalities in red cell ion content and alteration of erythrocyte volume. Cation leak can cause hyperkalemia; in some cases, this leak is accelerated in the cold (the resulting spuriously high serum K⁺ is then referred to as pseudo-hyperkalemia). The less rare form, dehydrated stomatocytosis (DHS; also referred to as xerocytosis), is a (usually compensated) macrocytic hemolytic disorder, with increased MCHC (generally >36 g/dL) associated with mild jaundice. Mutations in either PIEZO1, encoding an ion channel activated by pressure (mechanoreceptor), or in KCCN4, encoding the Ca²⁺-activated K⁺ channel (Gardos channel) have been recognized to cause DHS (see Table 105-3). Another form is overhydrated stomatocytosis (OHS). OHS is also macrocytic (MCV >110 fL), but the MCHC is low (<30 g/dL). The underlying mutation is in the Rhesus gene RHAG, which encodes an ammonia channel. Yet other patients with

stomatocytosis (Table 105-3) have mutations in SLC4A1 (encoding band 3) and SLC2A1 (encoding the glucose transporter GLUT1). Mutations of the latter are responsible for cryohydrocytosis, a channelopathy in which the red cells swell and burst when they are cooled. In vivo hemolysis can vary from relatively mild to quite severe. Familial hyperkalemia has been recently linked to mutations in ABCB6, resulting in abnormal cation leak with extra cellular release of a large amount of K⁺ (hyperkalemia). Mutations in

A CHAPTER 105 Hemolytic Anemias B C FIGURE 105-4 Peripheral blood smear from patients with membrane-cytoskeleton abnormalities. A. Hereditary spherocytosis. B. Hereditary elliptocytosis, heterozygote. C. Pyropoikilocytosis, with both alleles of the α -spectrin gene mutated. ABCB6 have been identified in almost 0.3% of blood donors. However, splenectomy is contraindicated in stomatocytosis due to the significant proportion of severe thromboembolic complications observed in splenectomized DHS patients.

Laser diffraction analysis, or ektacytometry, can measure the deformability of red blood cells subjected to either increasing shear stress or to an osmotic stress. This technique has been used extensively to investigate membrane-cytoskeleton abnormalities, and it can differentiate stomatocytosis from spherocytosis.

Enzyme Abnormalities When an important defect in a component of the membrane-cytoskeleton complex is present, hemolysis is a direct consequence of the fact that the very structure of the red cell is compromised. Instead, when one of the enzymes is defective, the consequences will depend on the precise role of that enzyme in the metabolic machinery of the red cell. This machinery has two main functions: (1) to provide energy in the form of ATP, and (2) to prevent oxidative damage to hemoglobin and to other proteins by providing sufficient reductive potential; the key molecule for this is NADPH, required for regeneration of glutathione (GSH) and for degradation of H₂O₂.
 ABNORMALITIES OF THE GLYCOLYTIC PATHWAY Because red cells, in the course of their differentiation, have sacrificed not only their nucleus and their ribosomes but also their mitochondria, they rely exclusively on the anaerobic portion of the glycolytic pathway for producing ATP, most of which is required by the red cell for cation transport against a concentration gradient across the membrane. If this fails due to a defect of any of the enzymes of the glycolytic pathway

(Table 105-4), the result will be hemolytic disease. PART 4 Oncology and Hematology Pyruvate Kinase Deficiency Abnormalities of the glycolytic pathway are all inherited and all rare. Among them, deficiency of pyruvate kinase (PK) is the least rare, with an estimated prevalence in most populations of 1:10,000. However, recently, a polymorphic PK mutation (E277K) was found in some African populations with heterozygote frequencies of 1–7%, suggesting that this may be another malaria-related
 TABLE 105-4 Red Cell Enzyme Abnormalities Causing Hemolysis
 GENE SYMBOL; CHROMOSOMAL LOCATION PREVALENCE OF ENZYME DEFICIENCY (RANK) ENZYME (ACRONYM)
 Glycolytic Pathway Hexokinase (HK) HK1; 10q22 Very rare May benefit from splenectomy; BMTc
 Glucose-6-phosphate isomerase (G6PI) GPI; 19q31.1 Rare (4); at least 60 cases reporteda NM, CNS
 May benefit from splenectomy Phosphofructokinase (PFK)b PFKM; 12q13 Very rare Myopathy;
 myoglobinuria Aldolase ALDOA; 16q22-24 Very rare Myopathy Triose phosphate isomerase (TPI)
 TPI1; 12p13.31 Very rare CNS (severe), NM Glyceraldehyde 3-phosphate dehydrogenase (GAPD)
 GAPDH; 12p13.31 Very rare Myopathy Bisphosphoglycerate mutase (BPGM) BPGM; 7q33 Very rare

Erythrocytosis rather than hemolysis; some of the rare mutations are in the enzyme active site Phosphoglycerate kinase (PGK) PGK1; Xq21.1 Very rare CNS, NM May benefit from splenectomy; BMTc Pyruvate kinase (PK) PKLR; 1q22 Rare (2)a May benefit from splenectomy; BMTc Redox Glucose-6-phosphate dehydrogenase (G6PD) G6PD; Xq28 Common (1)a Very rarely granulocytes In almost all cases, only AHA from Glutathione synthase GSS; 20q11.22 Very rare CNS Glutathione reductase GSR; 8p12 Very rare Cataracts AHA from exogenous trigger (favism) γ -Glutamylcysteine synthase GCLC; 6p12.1 Very rare CNS Mutations affect catalytic subunit Cytochrome b5 reductase CYB5R3; 22q13.2 Rare CNS Methemoglobinemia rather than hemolysis Nucleotide Metabolism Adenylate kinase (AK) AK1; 9q34.11 Very rare CNS May benefit from splenectomy Pyrimidine 5' nucleotidase (P5N) NTSC3A; 7p14.3 Rare (3)a May benefit from splenectomy aThe numbers from (1) to (4) indicate the ranking order of these enzymopathies in terms of frequency. bPFK deficiency is associated with increased glycogen in muscle, and it is also known as glycogen storage disease type VII or Tarui's disease. cOccasional report of successful treatment of the hematologic manifestations by BMT. Abbreviations: AHA, acquired hemolytic anemia; BMT, bone marrow transplantation; CNS, central nervous system; NM, neuromuscular.

polymorphism. HA secondary to PK deficiency is an autosomal recessive disease (Fig. 105-5). The clinical picture of homozygous (or biallelic) PK deficiency is that of an HA that often presents in the newborn with severe neonatal jaundice, requiring nearly always phototherapy and frequently exchange transfusion; the jaundice often persists, and it is often associated with reticulocytosis. The anemia is of variable severity; sometimes it is so severe as to require regular blood transfusion treatment, whereas sometimes it is mild, bordering on a nearly compensated hemolytic disorder. As a result, the diagnosis may be delayed: in some cases, it is made, for instance, in a young woman during her first pregnancy, when the anemia may get worse. The delay in diagnosis may be caused in part by the fact that the anemia is often remarkably well tolerated because the metabolic block at the last step in glycolysis causes an increase in 2,3-bisphosphoglycerate (or DPG; Fig. 105-1), a major effector of the hemoglobin-oxygen dissociation curve; thus, for a certain level of hemoglobin, the oxygen delivery to the tissues is enhanced, a remarkable compensatory feat. **TREATMENT** Pyruvate Kinase Deficiency Until recently, the management of PK deficiency has been supportive. Folic acid supplements should be given constantly. Blood transfusion should be used as necessary, and iron chelation may be required even in some patients who, though not receiving blood transfusion, may be developing iron overload (see "General Pathophysiology" above). About one-half of patients sooner or later undergo splenectomy, which usually provides a modest but significant increase in hemoglobin (paradoxically, reticulocytes also often increase, because they were previously trapped in the spleen). **CLINICAL MANIFESTATIONS** EXTRA-RED CELL COMMENTS exogenous trigger

PK deficiency G6PD deficiency (autosomal) (X-linked) Homozygous normal Heterozygous Homozygous deficient **FIGURE 105-5** Different phenotypes of heterozygotes for red cell enzymopathies. In a heterozygote for deficiency of pyruvate kinase (PK), encoded by an autosomal gene (see Table 105-4), the level of enzyme is about one-half of normal in all red cells. Because this level of enzyme is sufficient, there are no clinical consequences, i.e., PK deficiency is recessive. In a heterozygote for deficiency of glucose-6-phosphate dehydrogenase (G6PD), encoded by an X-linked gene, the situation is quite different: X-chromosome inactivation generates red cell mosaicism, whereby some red cells are entirely normal and others are G6PD deficient. Therefore, G6PD deficiency is expressed in heterozygotes; it is not recessive. spleen). Cholecystectomy may

also be required. A major advance has been the introduction of mitapivat, an allosteric activator of PK, and the first drug for a red cell enzymopathy, approved on grounds of a significant increase in hemoglobin in one-half of PK-deficient patients—those in whom either one or both PKLR mutations are of the missense type. Some patients with severe disease have received bone marrow transplantation (BMT) from human leukocyte anti gen (HLA)-identical PK-normal sibling. Prenatal diagnosis has been carried out in a mother who had already had an affected child. Rescue of inherited PK deficiency through lentiviral-mediated human PK gene transfer has been successful in mice and is currently undergoing a clinical trial in patients. Other Glycolytic Enzyme Abnormalities All of these defects are rare to very rare (Table 105-4), and most of them cause HA with varying degrees of severity. It is not unusual for the presentation to be in the guise of severe neonatal jaundice, which may require exchange transfusion; if the anemia is less severe, it may present later in life, or it may even remain asymptomatic and be detected incidentally when a blood count is done for unrelated reasons. The spleen is often enlarged. When other systemic manifestations occur, they can involve the central nervous system (sometimes entailing severe intellectual disability, particularly in the case of triose phosphate isomerase deficiency), the neuromuscular system, or both (see Table 105-4). This is not altogether surprising if we consider that these are housekeeping genes, i.e., expressed in all tissues. The diagnosis of HA is usually not difficult, thanks to the triad of normo-macrocytic anemia, reticulocytosis, and hyperbilirubinemia. Enzymopathies should be considered in the differential diagnosis of any chronic Coombs-negative HA. Unlike with membrane disorders, in most cases of glycolytic enzymopathies, morphologic abnormalities are conspicuous by their absence. A definitive diagnosis can be made only by demonstrating the deficiency of an individual enzyme by quantitative assays; these are carried out in only a few specialized laboratories. If a particular molecular abnormality is already known in the family, then one could test directly for that defect at the DNA level, thus bypassing the need for enzyme assays. Of course, the time may be getting nearer when a patient will present with their exome already sequenced, and we will need to concentrate on which genes to look up within the file. The principles for the management of these conditions are similar as for PK deficiency. In isolated clinically severe cases of glycolytic enzyme abnormalities, BMT has been carried out successfully, although unfortunately, nonhematologic manifestations, if any, are not reversed.

ABNORMALITIES OF REDOX METABOLISM • Glucose-6-Phosphate

Dehydrogenase (G6PD) Deficiency G6PD is a housekeeping enzyme

critical in the redox metabolism of all aerobic cells (Fig. 105-1). In red cells, its role is even more critical because it is the only source of NADPH, which directly and via GSH defends these cells against oxidative stress (Fig. 105-6). G6PD deficiency-related HA is a prime example of an HA due to interaction between an intracorporeal cause and an extracorporeal cause; indeed, in the vast majority of cases, hemolysis is triggered by an exogenous agent. Although the G6PD activity is decreased in most tissues of G6PD-deficient subjects, in other cells, the decrease is much less pronounced than in red cells, and it does not seem to impact on clinical expression.

■ ■ **GENETIC CONSIDERATIONS** The G6PD gene is X-linked, and this has important implications. First, because males have only one G6PD gene (i.e., they are hemizygous for this gene), they must be either normal or G6PD deficient. By contrast, females, who have two G6PD genes, can be either normal or deficient (homozygous) or intermediate (heterozygous). Second, as a result of the phenomenon of X chromosome inactivation, heterozygous females are genetic mosaics (see Fig.

105-5), with a highly variable ratio of G6PD-normal to G6PD-deficient cells and an equally variable degree of clinical expression; some heterozygotes can be just as affected as hemizygous males. The enzymatically active form of G6PD is either a dimer or a tetramer of a single protein subunit of 514 amino acids. G6PD-deficient subjects have been found invariably to have mutations in the coding region of the G6PD gene. Almost all of the over 230 different mutations known are single missense point mutations, entailing single amino acid replacements in the G6PD protein. In most cases, these mutations cause G6PD deficiency by decreasing the in vivo stability of the protein; thus, the physiologic decrease in G6PD activity that takes place with red cell aging is greatly accelerated. In some cases, an amino acid replacement can also affect the catalytic function of the enzyme. The genetic heterogeneity of G6PD deficiency is clinically important, and for the variants that are widespread, it also has public health implications. Therefore, the World Health Organization (WHO) has worked out a classification (Table 105-5).

CHAPTER 105 Hemolytic Anemias Among these mutations, those underlying chronic nonspherocytic hemolytic anemia (CNSHA; see below) are a discrete subset. This much more severe clinical phenotype can be ascribed in some cases to adverse qualitative changes (e.g., a decreased affinity for the substrate glucose-6-phosphate) or simply to the fact that the enzyme deficit is more extreme because of a more severe instability of the enzyme. For instance, a cluster of mutations map at or near the dimer interface, and clearly, they compromise severely the formation of the dimer.

Epidemiology G6PD deficiency is widely distributed in tropical and subtropical parts of the world (Africa, southern Europe, the Middle East, Southeast Asia, and Oceania) (Fig. 105-7) and wherever people from those areas have migrated. A conservative estimate is that at least 500 million people have a G6PD deficiency gene. In several of these areas, the frequency of a G6PD deficiency gene may be as high as 20% or more. It would be quite extraordinary for a trait that causes significant pathology to spread widely and reach high frequencies in many populations without conferring some biologic advantage. Indeed, G6PD is one of the best-characterized examples of genetic polymorphisms in the human species. Clinical field studies and in vitro experiments strongly support the view that G6PD deficiency has been selected by *Plasmodium falciparum* malaria because it confers a relative resistance against this highly lethal infection. As in other cases of balanced polymorphism, it is heterozygotes, therefore females, who are protected. Different G6PD variants (class B) underlie most of the prevalence of G6PD deficiency in different parts of the world. Examples of widespread variants are G6PD Mediterranean on the shores of that sea, in the Middle East, and elsewhere; G6PD A- in Africa, in the Middle East, and in southern Europe; G6PD Orissa in India; G6PD Viangchan and G6PD Mahidol in Southeast Asia; G6PD Kaiping and G6PD Canton in China; and G6PD Union worldwide. The heterogeneity of polymorphic G6PD variants is proof of their independent origin, further supporting the notion of selection by a common environmental

Divicine (fava beans) Primaquine ROS from neutrophils O_2^- Superoxide dismutase Rasburicase H_2O_2 Uric acid GSH Catalase Glutathione reductase Prx_2 -SHGSSG NADPH H_2O Glutathione peroxidase Prx_2 -S-SA Divicine (fava beans) Primaquine OXIDATIVE DAMAGE ROS from neutrophils O_2^- – PART 4 Oncology and Hematology Superoxide dismutase Rasburicase H_2O_2 Uric acid GSH Catalase Glutathione reductase Prx_2 -SHGSSG NADPH H_2O Glutathione peroxidase Prx_2 -S-SB

FIGURE 105-6 The role of glucose-6-phosphate dehydrogenase (G6PD) in protecting red cells from oxidative damage. A. In G6PD-normal red cells, G6PD and 6-phosphogluconate dehydrogenase—two of the enzymes of the pentose phosphate pathway—provide ample supply of NADPH, which in turn regenerates glutathione (GSH) when this is oxidized by reactive oxygen species (e.g., O_2^- and H_2O_2). Thus when O_2^- (meant here to represent itself and other reactive

oxygen species [ROS]) is produced by pro-oxidant compounds such as primaquine, or the glucosides in fava beans (divicine), or the oxidative burst of neutrophils, these ROS are rapidly neutralized; similarly, when rasburicase administered to degrade uric acid produces an equimolar amount of hydrogen peroxide, this is rapidly degraded by the combined action of glutathione peroxidase, catalase, and Prx2 (peroxiredoxin-2; all three mechanisms are NADPH dependent). B. In G6PD-deficient red cells, where the enzyme activity is reduced, NADPH production is limited, and it may not be sufficient to cope with the excess ROS generated by pro-oxidant compounds and the consequent excess hydrogen peroxide. This diagram also explains why a defect in GSH reductase has very similar consequences to G6PD deficiency. agent, namely malaria, in keeping with the concept of convergent evolution (Fig. 105-7). Clinical Manifestations The vast majority of people with G6PD deficiency remain clinically asymptomatic throughout their lifetime; however, all of them have an increased risk of developing neonatal jaundice (NNJ) and a risk of developing acute HA (AHA) when challenged by a number of oxidative agents. NNJ related to G6PD deficiency is rarely present at birth; the peak incidence of clinical onset is between day 2 and day 3, and in most cases, the anemia is not severe. However, TABLE 105-5 Current World Health Organization Classification of Glucose 6-Phosphate Dehydrogenase (G6PD) Variants

G6PD VARIANT	CLASS	MEDIAN OF G6PD ACTIVITY (% OF NORMAL)	ASSOCIATED CLINICAL MANIFESTATIONS
Aa	<20%	Chronic hemolytic anemia	
Bc	<45%	Neonatal jaundice; acute hemolytic anemia triggered by certain medicines, fava beans, and infections	
Ca			

60% None reported
 Ud Any Uncertain clinical significance
 aClass A corresponds to former class I; class C corresponds to former class IV. bA variant with <20% activity will be in class A only if it is associated with chronic hemolytic anemia. cIn view of the extensive overlap in enzyme activity and in clinical expression of variants in former class II and class III, these have been merged into class B. dA temporary assignment for variants for which there is currently insufficient information regarding clinical manifestations.

Glucose 6-phosphate NADP Glucose 6-phosphate dehydrogenase 6-Phosphoglucono-δ-lactone 6-Phosphoglucono lactonase 6-Phosphogluconate 6-Phosphogluconate dehydrogenase Ribulose 5-phosphate Glucose 6-phosphate NADP Glucose 6-phosphate dehydrogenase 6-Phosphoglucono-δ-lactone 6-Phosphoglucono lactonase 6-Phosphogluconate 6-Phosphogluconate dehydrogenase Ribulose 5-phosphate NNJ can be very severe in some G6PD-deficient babies, especially in association with prematurity, infection, and/or environmental factors (e.g., naphthalene-camphor balls, which may be used in babies' bedding and clothing); and the risk of severe NNJ is also increased by the coexistence of a monoallelic or biallelic mutation in the uridyl transferase gene (UGT1A1; the same mutations are associated with Gilbert's syndrome). It is imperative to manage promptly NNJ associated with G6PD deficiency because it can produce kernicterus and permanent neurologic damage. AHA can develop as a result of three types of triggers: (1) fava beans, (2) infections, and (3) drugs (Table 105-6). Typically, a hemolytic attack starts with malaise, weakness, and abdominal or lumbar pain. Within a time frame of several hours to 2–3 days, the patient develops jaundice and often dark urine. The onset can be extremely abrupt, especially with favism in children. The anemia is moderate to extremely severe, usually normocytic and normochromic, and due partly to intravascular hemolysis; hence, it is associated with hemoglobinemia,

hemoglobinuria, high LDH, and low or absent plasma haptoglobin. The blood film shows anisocytosis, polychromasia, and spherocytes; in addition, the most typical feature of G6PD deficiency is the presence of bizarre poikilocytes, with red cells that appear to have unevenly distributed hemoglobin ("hemighosts") and red cells that appear to have had parts of them bitten away ("bite cells" or "blister cells") (Fig. 105-8). A classical test, now rarely carried out, is supravital staining with methyl violet, which, if done promptly, reveals the presence of Heinz bodies (consisting of precipitates of denatured hemoglobin and hemichromes), which are regarded as a signature of oxidative damage to red cells (they are

A- Canton Coimbra Kaiping Kalyan Mahidol Med Orissa Union Viangchan No data <1% 1-4.9% 5-9.9% 10-14.9% 15-19.9%

20% FIGURE 105-7 Epidemiology of glucose-6-phosphate dehydrogenase (G6PD) deficiency throughout the world. Each country on the map is shaded in a color based on the best estimate of the mean frequency of G6PD deficiency allele(s) in that country (this is the same as the frequency of G6PD-deficient males). The small panel on the left gives the key to color shadings corresponding to each country. The larger panel gives a color-coded list of 10 common G6PD variants associated with G6PD deficiency: asterisk-shaped symbols in the corresponding colors are shown in the countries where these variants have been observed (for graphic reasons, symbols could not be inserted in all countries). (Reproduced with permission from L Luzzatto, M Ally, R Notaro. Glucose-6-phosphate dehydrogenase deficiency. 136:1225, 2020.) also seen with unstable hemoglobins). Since there is also a substantial component of extravascular hemolysis, unconjugated bilirubin is high and there is often clinical icterus. The most serious threat from AHA in adults is the development of acute renal failure (this is exceedingly rare in children). Once the threat of acute anemia is over and in the TABLE 105-6 Drugs That Carry Risk of Clinical Hemolysis in Persons with Glucose 6-Phosphate Dehydrogenase Deficiency RISK OF ACUTE HEMOLYTIC ANEMIA DRUG CLASS HIGH MEDIUM TO LOW Antimalarials Primaquine Tafenoquine Chloroquine Hydroxychloroquine Quinine Sulphonamides/ sulphones Dapsone Sulfadimidine Sulfamethoxazole Sulfasalazine Antibacterial/ antibiotics Chloramphenicol Ciprofloxacin Cotrimoxazole Nalidixic acid Nitrofurantoin Norfloxacin p-Aminosalicylic acid Anti-helminth Niridazole Antipyretic/ analgesics Acetylsalicylic acid high dose (>3 g/d) Acetaminophen Acetanilide Phenacetin Phenazopyridine Other Rasburicase Pegloticase Methylene blue Toluidine blue Ascorbic acid (>1 g) Doxorubicin Probenecid Vitamin K analogues

CHAPTER 105 Hemolytic Anemias absence of comorbidity, full recovery from AHA associated with G6PD deficiency is the rule. It was primaquine (PQ)-induced AHA that led to the discovery of G6PD deficiency, but this drug has not been very prominent subsequently because it is not necessary for the treatment of life-threatening *P. falciparum* malaria. Today there is a revival in the use of PQ for two reasons. First, it is the only effective agent for eliminating the gametocytes of *P. falciparum*

(thus preventing further transmission): a small single dose (0.25 mg/kg) is required, and it is safe for G6PD-deficient persons. Second, a 14-day course of PQ is the standard treatment for eliminating the hypnozoites of *Plasmodium vivax* (thus preventing endogenous relapse) (Chap. 231). In countries aiming to eliminate malaria, there may be a call for mass administration of PQ; this ought to be made mandatory before giving either of these drugs because fatal cases have been reported upon using one of these drugs, which generate hydrogen peroxide, in newborns with kidney injury and in children and adults with tumor lysis syndrome.

(From MA Lichtman et al: Lichtman's Atlas of Hematology: <http://www.accessmedicine.com>. Copyright © The McGraw-Hill Companies, Inc. All rights reserved.)

to be associated with G6PD testing. At the other end of the historic spectrum, the latest additions to the list of potentially hemolytic drugs (Table 105-6) are rasburicase and pegloticase; again, G6PD testing ought to be made mandatory before giving either of these drugs because fatal cases have been reported upon using one of these drugs, which generate hydrogen peroxide, in newborns with kidney injury and in children and adults with tumor lysis syndrome.

Although drug-induced AHA has been prominent in the study of G6PD deficiency, the most common clinical manifestations are in fact NNJ and favism, both of which are of public health importance in many populations. Contrary to beliefs that are still widespread, fava bean pollen inhalation does not cause favism, and other beans are safe. A very small minority of subjects with G6PD deficiency, those who have a class A variant, suffer from CNSHA of variable severity. The patient is nearly always a male, usually with a history of NNJ, who may present with anemia, unexplained jaundice, or gallstones later in life. The spleen may be enlarged. The severity of anemia ranges in different patients from borderline to transfusion dependent. The anemia is usually normo-macrocytic, with reticulocytosis. Bilirubin and LDH are increased. Although hemolysis is, by definition, chronic in these patients, they are also vulnerable to acute oxidative damage, and therefore, the same agents that can cause AHA in people with the ordinary type of G6PD deficiency will cause severe exacerbations in people with CNSHA associated with G6PD deficiency. In some cases of CNSHA, the deficiency of G6PD is so severe in granulocytes that it limits their capacity to produce an oxidative burst, with consequent increased susceptibility to some bacterial infections.

PART 4 Oncology and Hematology Laboratory Diagnosis The suspicion of G6PD deficiency can be confirmed by semiquantitative methods often referred to as screening tests, which are suitable for population studies and can correctly classify male subjects, in the steady state, as G6PD normal or G6PD deficient. However, in clinical practice, a diagnostic test is usually needed when the patient has had a hemolytic attack, whereby the oldest, most G6PD-deficient red cells have been selectively destroyed, and young red cells, having higher G6PD activity, are being released into the circulation. Under these conditions, only a quantitative test can give a definitive result. In males, this test will identify normal hemizygotes and G6PD-deficient hemizygotes; among females, some heterozygotes will be missed, but those who are at most risk of hemolysis will be identified. Of course, G6PD deficiency also can be diagnosed by DNA testing. Currently easy-to-use point-of-care tests for G6PD deficiency are becoming available, geared especially to the prospect of mass administration of PQ or of the newly introduced derivative tafenoquine.

TREATMENT G6PD Deficiency The AHA of G6PD deficiency is largely preventable by avoiding exposure to triggering factors of previously screened subjects. Of course, the practicability and cost-effectiveness of screening depend on the prevalence of G6PD deficiency in each individual community. Favism is entirely preventable in G6PD-deficient subjects by not eating fava beans. Drug-induced hemolysis can be prevented by testing for G6PD deficiency before prescribing; in many cases, one can use

alternative drugs. When AHA develops and once its cause is recognized, no specific treatment is needed in most cases. However, if the anemia is severe, it may be a medical emergency, especially in children, requiring immediate action, including blood transfusion. This has been the case with an antimalarial drug combination containing dapsone (called Lapdap, introduced in 2003) that has caused severe acute hemolytic episodes in children with malaria in several African countries; after a few years, the drug was taken off the market. If there is acute renal failure, hemodialysis may be necessary, but if there is no previous kidney disease, recovery is the rule. The management of NNJ associated with G6PD deficiency is no different from that of NNJ due to other causes. In cases with CNSHA, if the anemia is not severe, regular folic acid supplements and regular hematologic surveillance will suffice.

It will be important to avoid exposure to potentially hemolytic drugs, and blood transfusion may be indicated when exacerbations occur, mostly in concomitance with intercurrent infection. In rare patients, regular blood transfusions may be required, in which case appropriate iron chelation should be instituted. Unlike in HS, there is no evidence of selective red cell destruction in the spleen; however, in practice, splenectomy has proven beneficial in severe cases.

Abnormalities of the Redox System As mentioned previously, GSH is a key player in the defense against oxidative stress. Inherited defects of GSH metabolism are exceedingly rare, but each one can give rise to chronic HA (Table 105-4). A rare, peculiar, and severe but usually self-limited HA occurring in the first month of life, called infantile poikilocytosis, may be associated with deficiency of glutathione peroxidase (GSHPX) due not to an inherited abnormality, but to transient nutritional deficiency of selenium, an element essential for the activity of GSHPX.

PYRIMIDINE 5'-NUCLEOTIDASE (P5N) DEFICIENCY P5N is a key enzyme in the catabolism of nucleotides arising from the degradation of nucleic acids that takes place in the final stages of erythroid cell maturation. How exactly its deficiency causes HA is not well understood, but a highly distinctive feature of this condition is a morphologic abnormality of the red cells known as basophilic stippling. The condition is rare, but it probably ranks third in frequency among red cell enzyme defects (after G6PD deficiency and PK deficiency). The anemia is lifelong, is of variable severity, and may benefit from splenectomy.

Familial (Atypical) Hemolytic-Uremic Syndrome (aHUS)

This term is used to designate a group of rare disorders, mostly affecting children, characterized by microangiopathic HA with presence of fragmented erythrocytes in the peripheral blood smear, thrombocytopenia (usually mild), and acute renal failure. (The word atypical in this phrase should be consigned to history; it was introduced originally to distinguish this condition from the hemolytic-uremic syndrome [HUS] caused by infection with *Escherichia coli* producing the Shiga toxin, regarded as typical.) The disease is caused by dysregulation of the complement alternative pathway: in some cases, this results from anti-factor H autoantibodies, but in the majority of cases, it is a consequence of mutations in genes encoding proteins that are components or regulators of the complement system, i.e., C3 (encoding complement component C3), CFB (encoding complement factor B), CFH (encoding complement factor H), CD46 (encoding the membrane cofactor protein), CFI (encoding complement factor I), THBD (encoding thrombomodulin), and rarely others. Patients may have abnormalities in one, two, or even three of the above genes. Pathogenic variants of these genes predispose to HUS with autosomal dominant inheritance; because clinical aHUS requires a triggering factor, most commonly infection, the penetrance is incomplete. Thus, whereas all other inherited HAs are due to intrinsic red cell abnormalities, this group is unique in that hemolysis results from an inherited defect external to red cells (Table 105-

1). Because the regulation of the complement cascade has considerable redundancy, in the steady state, any of the above abnormalities can be tolerated. However, when an intercurrent infection or some other trigger briskly activates complement, the deficiency of one of the complement regulators becomes critical. Endothelial cells get damaged, especially in the kidney. At the same time, and partly as a result of this, there will be brisk hemolysis; thus, the more common Shiga toxin-related HUS (Chap. 172) can be regarded as a phenocopy of aHUS. aHUS is a severe disease; before anticomplement therapy was available, mortality was up to 15% in the acute phase, and up to 50% of cases progressed to end-stage renal disease (ESRD). Not infrequently, aHUS undergoes spontaneous remission. Because it is an inherited abnormality, it is not surprising that, given renewed exposure to a trigger, the syndrome will tend to recur; when it does, the prognosis is always serious. The traditional treatment has been plasma exchange, which will supply the deficient complement regulator and clear complement activation products. This has changed since the introduction of the anti-C5 complement inhibitor eculizumab (see “Paroxysmal Nocturnal Hemoglobinuria”), which was found to greatly ameliorate the microangiopathic picture, with improvement in platelet counts and in

renal function, thus abrogating the need for plasma exchange, which is not always effective and not free of complications. Once a full remission is obtained, with urine dipstick negative for hemoglobinuria, within 3–6 months, eculizumab can be discontinued. Given the genetic background of aHUS, relapses are possible. Fortunately, patients who relapsed after discontinuing eculizumab have responded again. No current evidence supports continuing eculizumab indefinitely. For the diagnosis of inherited red cell abnormalities, including those of the membrane-cytoskeleton, channelopathies, and enzymopathies, DNA sequencing of a gene panel (e.g., next-generation sequencing [NGS]) has become increasingly popular. This approach (which may or may not be more expensive) has the advantage of being potentially comprehensive and of providing definitive data. On the other hand, the mutations identified may be of uncertain significance, in which case, the diagnosis must be still confirmed by conventional methodology. Thus, the use of NGS is specially useful with unsolved cases of hereditary HAs. ■ ■ACQUIRED HEMOLYTIC ANEMIA

Mechanical Destruction of Red Cells Although red cells are characterized by the remarkable deformability that enables them to squeeze through capillaries narrower than themselves for thousands of times in their lifetime, there are at least two situations in which they succumb to shear, if not to wear and tear; the result is intravascular hemolysis, resulting in hemoglobinuria (Table 105-7). One situation is acute and self-inflicted, march hemoglobinuria. Why sometimes a marathon runner may develop this complication, whereas on another occasion, this does not happen, we do not know (perhaps their footwear needs attention). A similar syndrome may develop after prolonged barefoot ritual dancing or intense playing of bongo drums. The other situation is chronic and iatrogenic (it has been called microangiopathic hemolytic anemia). It takes place in patients with prosthetic heart valves, especially when paraprosthetic regurgitation is present. If the hemolysis consequent on mechanical trauma to the red cells is mild, and if the supply of iron is adequate, the loss may be largely compensated; if more than mild anemia develops, reintervention to correct regurgitation may be required.

Infection By far, the most frequent infectious cause of HA in endemic areas is malaria (Chap. 231). In other parts of the world, the most frequent direct cause is probably Shiga toxin-producing *E. coli* O157:H7, now recognized as the main etiologic agent of HUS, which is more common in children than in adults (Chap. 166). Life-threatening intravascular hemolysis, due to a toxin with lecithinase activity, occurs with *Clostridium perfringens* sepsis, particularly following open wounds, septic abortion, or as a disastrous accident

due to a contaminated blood unit. Rarely, and if at all in children, HA is seen with sepsis or endocarditis from a variety of organisms. In addition, bacterial and viral infections can cause HA by indirect mechanisms (see Table 105-7). TABLE 105-7 Diseases and Clinical Situations in Which Hemolysis Is Largely Intravascular

ONSET/TIME COURSE	MAIN MECHANISM	
Mismatched blood transfusion	Abrupt	Nearly always ABO incompatibility
Paroxysmal nocturnal hemoglobinuria (PNH)	Chronic with acute exacerbations	Complement (C)-mediated destruction of CD59(–) red cells
Paroxysmal cold hemoglobinuria (PCH)	Acute	Immune lysis of normal red cells
Septicemia	Very acute	Exotoxins produced by <i>Clostridium perfringens</i>
Microangiopathic	Acute or chronic	Red cell fragmentation
Red cell morphology on blood smear	March hemoglobinuria	Abrupt
Mechanical destruction	Targeted history taking	Has been reported after extreme ritual dancing
Favism	Acute	Destruction of older fraction of G6PD-deficient red cells

The trigger of acute hemolytic anemia, often with hemoglobinuria, can be infection or a drug (see Table 105-5) rather than fava beans. Hemoglobinuria may or may not be reported by patient, but it is often macroscopic, i.e., recognizable by simple inspection of urine. Abbreviation: G6PD, glucose 6-phosphate dehydrogenase.

Immune Hemolytic Anemias These can arise through at least two distinct mechanisms. First, when an antibody directed against a certain molecule (e.g., a drug) reacts with that molecule, red cells may get caught in the reaction (the so-called innocent bystander mechanism; see later section “Hemolytic Anemia from Toxic Agents and Drugs”), whereby they are damaged or destroyed. Second, and more frequently, a true autoantibody is directed against a red cell antigen, i.e., a molecule present on the surface of red cells. Autoimmune HAs have been originally classified into two types, depending on the thermal amplitude of the autoantibodies involved; this classification is valid because the two types have different pathophysiologic and clinical features.

AUTOIMMUNE HEMOLYTIC ANEMIA, WARM TYPE (WAIHA; FOR SIMPLICITY, WE WILL USE THE ACRONYM AIHA) AIHA has an estimated incidence in the United States of about 1–3:100,000 per year, and a prevalence of 17:100,000. AIHA can be serious because even with appropriate management the mortality is ~5–10%. **Clinical Features and Diagnosis** The onset is often abrupt and can be dramatic. The hemoglobin level may drop, within days, to as low as 4 g/dL; the massive red cell removal will produce jaundice, and sometimes the spleen is enlarged. When this triad is present, the suspicion of AIHA must be high. The reticulocyte count is typically elevated, except when erythroid precursors are also targeted by the autoantibody attack. LDH may also be elevated. In some cases, AIHA can be associated, on first presentation or subsequently, with autoimmune thrombocytopenia. This double autoimmune condition, referred to as Evans syndrome, may be a manifestation of common variable immune deficiency, and in children, it may suggest one of several primary immune deficiency syndromes. Evans syndrome signals high-risk disease. Other predictors of the outcome and of the probability of relapse of AIHA are severe anemia (hemoglobin <6 g/dL), certain characteristics of the antibody, acute renal failure, and infection.

CHAPTER 105 Hemolytic Anemias There are few situations in hematology where one laboratory test is as informative as the direct antiglobulin test developed in 1945 by R. R. A. Coombs and known since then by this name. The currently recommended version of this test uses in the first instance a “broad-spectrum” reagent, i.e., one that will detect not only immunoglobulins (Ig) but also complement (C) components (usually C3 fragments) bound to the surface of the patient’s red cells. If the test is positive, it is, almost on its own, diagnostic of AIHA. False positives may occur as a result of previous blood transfusion or, much more rarely, following organ

transplantation procedures or administration of antilymphocyte or immunoglobulin products. One can then determine, by using specific reagents, whether Ig or C or both are implicated. The sensitivity of the Coombs test varies depending on the techniques that are used. In general, the test is positive if there is an average of at least 400 molecules of Ig and/or C on each red cell; but with more advanced techniques involving flow cytometry analysis or enzyme-linked radio labeled tests, the sensitivity can be pushed to as low as 30–40 molecules.

APPROPRIATE DIAGNOSTIC PROCEDURE COMMENTS

Repeat cross-match Flow cytometry to display a CD59(–) red cell population

Exacerbations due to C activation through any pathway

Test for Donath-Landsteiner antibody

Often triggered by viral infection

Blood cultures

Other organisms may be responsible

Different causes ranging from endothelial damage to hemangioma to leaky prosthetic heart valve

G6PD assay

Triggered by ingestion of large dish of fava beans

per red cell. Therefore, liaison with a specialized laboratory is desirable; a dual direct antiglobulin test has also been developed. In the past, the diagnosis of “Coombs-negative AIHA” was regarded as a last resort, but it is important to know that a patient with this label may have severe AIHA, because if the antibody is powerful (high affinity/avidity), few molecules may be sufficient to opsonize red cells. Based on the Coombs test findings as well as on the thermal characteristics and the antigenic specificities of the autoantibodies (Table 105-8), AIHA has been classified into subtypes.

In AIHA, the autoantibody reacts best at 37°C, and it is usually Rhesus specific (sometimes specifically anti-e). The main mechanism of hemolysis in AIHA is that the Fc portion of the IgG antibody bound to red cells is recognized by the Fc receptor of macrophages. This will trigger erythrophagocytosis wherever macrophages are abundant, i.e., in the liver, in the bone marrow, but especially in red pulp of the spleen (Fig. 105-9), which, also because of its special anatomy, is often the predominant site of red cell destruction. AIHA may be seen in isolation (and it is then called idiopathic) or as secondary to other disorders such as systemic autoimmune disorders (systemic lupus erythematosus [SLE]; sometimes, AIHA may be the first manifestation that leads to a diagnosis of SLE) or lymphoproliferative disorders (Table 105-8). Like all autoimmune diseases, AIHA must arise from a dysregulation of immunity. It is therefore not surprising that it is increasingly being recognized in chronic lymphocytic

PART 4 Oncology and Hematology

TABLE 105-8 Classification of Acquired Immune Hemolytic Anemias

TYPE OF ANTIBODY COLD, MOSTLY IgM, OPTIMAL TEMPERATURE 4°C–30°C WARM, MOSTLY IgG, OPTIMAL TEMPERATURE 37°C; OR MIXED

CLINICAL SETTING

Primary CAD AIHA (idiopathic)

Secondary to viral infection EBV CMV Other Parvovirus B19 HIV HCV EBV Viral vaccines

Secondary to other infection Mycoplasma infection: paroxysmal cold hemoglobinuria Babesia

Secondary to/ associated with other disease CAD in: Waldenström’s disease Lymphoma

AIHA in: SLE, scleroderma, RA CLL Lymphoproliferative disorders Multiple myeloma Other malignancy Chronic inflammatory disorders (e.g., IBD) Thyroiditis (including Hashimoto)

After allogeneic HSCT Common variable immunodeficiency After immune checkpoint modulating drugs

Secondary to drugs: drug-induced immune hemolytic anemia

Small minority (e.g., with lenalidomide) Majority: currently most common culprit drugs are cefotetan, ceftriaxone, piperacillin, methyldopa, fludarabine

Drug-dependent: antibody destroys red cells only when drug present (e.g., rarely penicillin)

Drug-independent: antibody can destroy red cells even when drug no longer present (e.g., methyldopa)

Associated with Pregnancy

Abbreviations: AIHA, autoimmune hemolytic anemia; CAD, cold agglutinin disease; CLL, chronic lymphocytic leukemia; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HCV, hepatitis C virus; HIV,

human immunodeficiency virus; HSCT, hematopoietic stem cell transplantation; IBD, inflammatory bowel disease; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis.

leukemia (CLL), whether treated or untreated; after BMT; and after solid organ transplantation entailing immunosuppressive treatment. Recently, warm antibody AIHA has also occurred as a side effect of the use of immune checkpoint inhibitors, such as nivolumab, in patients with various types of cancer. **TREATMENT Warm Antibody Autoimmune Hemolytic Anemia** Severe acute AIHA can be a medical emergency. The immediate treatment almost invariably includes transfusion of red cells. This may pose a special problem because many or all of the blood units cross-matched may be incompatible. In these cases, it is often correct, if paradoxical, to transfuse ABO-matched but incompatible blood, with the rationale being that the transfused red cells will be destroyed no less—but no more—than the patient's own red cells, and in the meantime, the patient stays alive. A situation like this requires close liaison and understanding between the clinical unit treating the patient and the blood transfusion/serology lab. Whenever the anemia is not immediately life-threatening, blood transfusion should be withheld (because compatibility problems may increase with each unit of blood transfused) and medical treatment started immediately with prednisone (1 mg/kg per day), which will produce a remission promptly in at least one-half of patients. Rituximab (anti-CD20), previously regarded as second-line treatment, is increasingly being used at a relatively low dose (100 mg/week $\times$ 4), together with prednisone as part of first-line treatment. It is especially encouraging that this approach seems to reduce the rate of relapse, a common occurrence in AIHA. For patients who do relapse or are refractory to medical treatment, additional therapeutic strategies are now available. Splenectomy does not cure the disease, but it can produce significant benefit by removing a major site of hemolysis, thus improving the anemia and/or reducing the need for other therapies (e.g., the dose of prednisone); of course, splenectomy is not free of risk, as it entails increased risk of sepsis and of thrombosis. The response rates to splenectomy and rituximab are similar. Since the introduction of rituximab, azathioprine, cyclophosphamide, cyclosporine, mycophenolate, and intravenous immunoglobulin have become second- or third-line agents. In very rare severe refractory cases, one may have to consider a high dose of cyclophosphamide (50 mg/kg

per day for 4 days), followed by a myelo-stimulating agent, or the anti-CD52 agent alemtuzumab. Fostamatinib, an inhibitor of intracellular cell signaling (Syk kinase) mediating erythrophagocytosis, has shown beneficial effects in an open-label clinical study, becoming an interesting third-line therapeutic option (Fig. 105-9). When severe anemia is associated with inadequate reticulocyte output, the use of EPO may help to reduce or avoid the requirement for transfusion of red cells. Several new agents, including inhibitors of the neonatal Fc receptor and others, are currently under investigation. **PAROXYSMAL COLD HEMOGLOBINURIA (PCH)** PCH is a rare form of AIHA occurring mostly in children, usually triggered by a viral infection, usually self-limited, and characterized by the so-called Donath-Landsteiner antibody. In vitro, this antibody has unique serologic features; it has usually anti-P specificity, and it binds to red cells only at a low temperature (optimally at 4°C), but when the temperature is shifted to 37°C, lysis of red cells takes place in the presence of complement. Consequently, in vivo, there is intravascular hemolysis, resulting in hemoglobinuria. Clinically the differential diagnosis must include other causes of hemoglobinuria (Table 105-7), but the presence of the Donath-Landsteiner antibody will prove PCH. Active supportive treatment, including blood transfusion, may be needed to control the anemia; subsequently, recovery is the rule. **COLD AGGLUTININ DISEASE** This designation indicates the other

main type of AIHA, which has quite different features when compared with warm antibody AIHA. First, cold agglutinin disease (CAD) is a

IgG Warm Ab Rituximab Alemtuzumab CD20 CD52 Spleen Fc receptor BTK B cell Venetoclax Ibrutinib Complement blockers (C1s) Plasma cell Complement Bortezomib IgM Cold Ab

FIGURE 105-9 Basic mechanisms involved in warm antibody- and cold antibody-mediated autoimmune hemolytic anemias. Top. With warm antibodies (usually IgG), opsonized red cells are removed by Fc receptor-bearing macrophages, largely in the spleen (extravascular hemolysis); fragmentation and spherocyte formation also play a role, with the spleen again being the main site. Bottom. In cold agglutinin disease (CAD), the antibody is IgM, which, once bound to red cells, causes complement activation through the classic pathway, with consequent intravascular hemolysis. In addition, C3b-opsonized red cells will undergo erythrophagocytosis by Kupffer cells in the liver, with consequent extravascular hemolysis. The inset on the left illustrates the B cells that make these autoantibodies: polyclonal in the case of warm antibody-mediated autoimmune hemolytic anemia and monoclonal in the case of CAD. The new therapeutic approaches in addition to glucocorticoids for autoimmune hemolytic anemias are shown in red. The inset highlights drugs targeting immune cells involved in generation of antibodies. Ab, antibody; BTK, Bruton's tyrosine kinase; Syk, spleen tyrosine kinase.

chronic and more frequently indolent condition—in contrast to the abrupt onset of warm antibody AIHA. Second, the term cold refers to the fact that the autoantibody involved reacts with red cells poorly or not at all at 37°C, whereas it reacts strongly at lower temperatures. As a result, hemolysis is more prominent the more the body is exposed to the cold. Third, the antibody is produced by a clone of autoreactive B lymphocytes. Sometimes the antibody concentration in the serum is high enough to show up as a spike in plasma protein electrophoresis, thus qualifying CAD as an IgM monoclonal gammopathy; however, it differs from Waldenström macroglobulinemia by not having the characteristic MYD88 mutation (see Chap. 116); instead, a somatic mutation in the KMT2D gene, encoding a lysine histone methylase, is present in the B-cell clone of a majority of CAD patients: this seems to favor proliferation. The antibody produced by the B-cell clone is IgM; usually it has an anti-I specificity (the I antigen is present on the red cells of almost everybody), and it may have a very high titer (1:100,000 or more has been observed). IgM, when bound to red cells, is a powerful activator of the complement cascade, with ultimate formation of the membrane attack complex (see Fig. 105-9); this will directly cause destruction of red cells (intravascular hemolysis; indeed, CAD patients may present with hemoglobinuria). In addition, once complement is activated, C3b will bind to red cells that, thus opsonized, will be destroyed by macrophages (extravascular hemolysis); unlike in AIHA, the spleen is not predominant in this process. In mild forms of CAD, avoidance of exposure to cold may be all that is needed to enable the patient to have a reasonably comfortable quality of life; but in more severe forms, the management of CAD is not easy. Plasma exchange will remove antibodies and is, therefore, in theory, a rational approach in severe cases. However, the management of CAD has changed significantly with the advent of the anti-CD20 antibody rituximab; up to 60% of patients respond. If remission is followed by relapse, a new course of rituximab may be again effective, and remissions may be more durable with a combination of rituximab with either

Fostamatinib Spherocytes Syk Erythroid microparticles Extravascular hemolysis Liver Extravascular hemolysis Complement activation with formation of membrane attack complex CHAPTER 105 Intravascular hemolysis Hemolytic Anemias fludarabine or bendamustine, in particular in CAD associated with a clinically manifested lymphoproliferative disorder. Therefore, even in the absence

of a formal trial, rituximab has become de facto firstline treatment, especially since previously used immunosuppressive/

cytotoxic agents, although they can reduce the antibody titer, have limited clinical efficacy and, in view of the chronic nature of CAD, their side effects may prove unacceptable. Unlike in AIHA, prednisone and splenectomy are ineffective. In the management of CAD in relapse, the B-cell receptor inhibitors venetoclax and ibrutinib, as well as for the proteasome inhibitor bortezomib, are emerging as effective agents. A different approach targeting complement inhibitors has been also explored by using sutimlimab (anti-C1s); a limitation of this approach is that hemolysis will be curbed only for as long as this agent is administered (Fig. 105-9). In terms of supportive treatment, blood transfusion may be helpful, despite the fact that red cells from the donor, being I positive, will survive no longer than those of the patient. Both the blood bag and the patient's extremities must be kept warm during transfusion. Hemolytic Anemia from Toxic Agents and Drugs A number of chemicals with oxidative potential, whether medicinal or not, can cause hemolysis even in people who are not G6PD deficient (for which, see above). Examples are hyperbaric oxygen (or 100% oxygen), nitrates, chlorates, methylene blue, dapsone, cisplatin, and numerous aromatic (cyclic) compounds. Other chemicals may be hemolytic through nonoxidative, largely unknown mechanisms; examples include arsine, stibine, copper, and lead. The HA caused by lead poisoning is characterized by basophilic stippling; it is in fact a phenocopy of that seen in P5N deficiency (see above), suggesting it is mediated at least in part by lead inhibiting this enzyme. In these cases, hemolysis appears to be mediated by a direct chemical action on red cells. But drugs can cause hemolysis through at least two other mechanisms. (1) A drug can behave as a hapten and induce

antibody production; in rare subjects, this happens, for instance, with penicillin. Upon a subsequent exposure, red cells are caught, as innocent bystanders, in the reaction between penicillin and antipenicillin antibodies. Hemolysis will subside as soon as penicillin administration is stopped. (2) A drug can trigger, perhaps through mimicry, the production of an antibody against a red cell antigen. The best-known example is methyldopa, an antihypertensive agent no longer in use, which in a small fraction of patients stimulated the production of the Rhesus antibody anti-e. In patients who have this antigen, the anti-e is a true autoantibody, which then causes true AIHA (see above). Usually this will gradually subside once methyldopa is discontinued.

Severe intravascular hemolysis can be caused by the venom of certain snakes (cobras and vipers), and HA can also follow spider bites. Paroxysmal Nocturnal Hemoglobinuria (PNH) PNH is an acquired chronic HA characterized by persistent intravascular hemolysis with occasional or frequent recurrent exacerbations. In addition to (1) hemolysis, there may be (2) pancytopenia and (3) a distinct tendency to venous thrombosis. This triad makes PNH a truly unique clinical condition; however, when not all of these three features are manifest on presentation, the diagnosis is often delayed, although it can always be made by appropriate laboratory investigations (see below). PNH is encountered in all populations throughout the world, but it is a rare disease, with an estimated prevalence of 5–10 per million (it may be somewhat less rare in Southeast Asia and in the Far East). PNH has about the same frequency in men and women. PNH is not inherited, and it has never been reported as a congenital disease, but it can present in small children or as late as in the seventies, although most patients are young adults. PART 4 Oncology and Hematology CLINICAL FEATURES When seeking medical attention, the patient may report that one morning, they “passed blood instead of urine.” This distressing or frightening event may be

regarded as the classic presentation; however, more frequently, this symptom is not noticed or not reported. Indeed, the patient often presents simply as a problem in the differential diagnosis of anemia, whether symptomatic or discovered incidentally. Sometimes the anemia is associated from the outset with neutropenia, thrombocytopenia, or both, thus signaling an element of bone marrow failure (see below). Some patients may present with recurrent attacks of severe abdominal pain eventually found to be related to thrombosis in abdominal veins, or attributable to nitric oxide depletion associated with intravascular hemolysis. When thrombosis affects the hepatic vein, it may produce acute hepatomegaly and ascites, i.e., a full-fledged Budd-Chiari syndrome, which, in the absence of liver disease, ought to raise the suspicion of PNH. The natural history of PNH can extend over decades. In the past, with supportive treatment only, the median survival was estimated to be about 10–20 years, with the most common cause of death being venous thrombosis, followed by infection secondary to severe neutropenia and hemorrhage secondary to severe thrombocytopenia. Rarely (estimated 1–2% of all cases), PNH may terminate in acute myeloid leukemia. On the other hand, full spontaneous recovery from PNH has been documented, albeit rarely.

LABORATORY INVESTIGATIONS AND DIAGNOSIS The most consistent blood finding is anemia, which may range from mild to moderate to very severe. The anemia is usually normo-macrocytic, with unremarkable red cell morphology. If the MCV is high, it is usually largely accounted for by reticulocytosis, which may be quite marked (up to 20%, or up to 400,000/ μ L). The anemia may become microcytic if the patient is allowed to become iron deficient as a result of chronic iron loss through hemoglobinuria. Unconjugated bilirubin is mildly or moderately elevated; LDH is typically markedly elevated (values in the thousands are common); and haptoglobin is usually undetectable. All of these findings make the diagnosis of HA compelling. Hemoglobinuria may be overt in a random urine sample; if it is not, it may be helpful to obtain serial urine samples because hemoglobinuria can vary dramatically from day to day and even from hour to hour. The bone marrow is usually cellular, with marked to massive erythroid hyperplasia, often with mild to moderate dyserythropoietic features

(these overlap with those seen in myelodysplastic syndromes, but PNH remains a separate entity). At some stage of the disease, the marrow may become hypocellular or even frankly aplastic (see below). The definitive diagnosis of PNH must be based on the demonstration that a substantial proportion of the patient's red cells are deficient in proteins (notably CD59 and CD55) that normally protect the red cells from activated complement (C). The sucrose hemolysis test is unreliable; in contrast, the acidified serum (Ham) test is highly reliable. The gold standard today is flow cytometry, which can be carried out on granulocytes as well as on red cells and has a very high sensitivity. In PNH, characteristically, one sees a bimodal distribution of cells, with a discrete population that is CD59 and CD55 negative. Although very small populations of CD59(–) cells are of interest in terms of pathophysiology (particularly of aplastic anemia [AA]), no patient should be diagnosed with PNH unless the proportion is substantial: in first approximation, at least 5% of the total red cells and at least 20% of the total granulocytes.

PATHOPHYSIOLOGY Hemolysis in PNH is mainly intravascular and is due to an intrinsic abnormality of the red cell, which makes it exquisitely sensitive to activated C, whether C is activated through the alternative pathway or through an antigen-antibody reaction (classic pathway). The former mechanism is mainly responsible for chronic hemolysis in PNH; the latter explains why the hemolysis can be dramatically exacerbated in the course of a viral or bacterial infection. Hypersusceptibility to C is due to deficiency in the red cell membrane of several protective proteins (Fig. 105-10), among which CD59 is the most important because it is able to hinder the insertion into the membrane of C9

polymers (the so-called membrane attack complex [MAC]). The molecular basis for the deficiency of these proteins has been pinpointed not to a defect in any of the respective genes, but rather to the shortage of a unique glycolipid molecule, GPI (Fig. 105-2), which, through a peptide bond, anchors these proteins to the surface membrane of cells. The shortage of GPI is due in turn to a somatic mutation in an X-linked gene, called PIGA, required for an early step in GPI biosynthesis. As a result, the patient's marrow is a mosaic of mutant and nonmutant cells, and the peripheral blood always contains both GPI-negative (PNH) cells and GPI-positive (nonPNH) cells; in most cases, the former prevail. Thrombosis is one of the most immediately life-threatening complications of PNH and yet one of the least understood in its pathogenesis. It could be that deficiency of CD59 on the PNH platelet causes inappropriate platelet activation; however, other mechanisms are possible. In very rare cases, PNH can be caused by biallelic mutations of the autosomal PIGT gene, which maps to chromosome 20q, in the absence of a PIGA mutation. In these cases, because GPI is produced but cannot bind to proteins, the clinical picture is further complicated by the coexistence of a chronic inflammatory state.

BONE MARROW FAILURE (BMF) AND RELATIONSHIP BETWEEN PNH AND AA

It is not unusual that patients with firmly established PNH have a previous history of AA, sometimes well documented; in many cases, BMF preceding overt PNH may have passed unnoticed, but it is probably the rule rather than the exception. On the other hand, some times a patient with PNH becomes less hemolytic and more pancytopenic and ultimately has the clinical picture of AA. The relationship between PNH and AA manifested in the clinical course of patients reflects a close link in pathogenesis. AA is thought to be an organ-specific autoimmune disease, in which T cells cause damage to hematopoietic stem cells via an as yet unidentified molecular target. The same may be true of PNH, and in this condition, the target might be the GPI molecule itself. This would explain why GPI-negative (PNH) stem cells are spared; PIGA mutations can be demonstrated in normal people. Thus, PNH results from the combined action of two factors: failure of normal hematopoiesis and massive expansion of a PNH clone. There is evidence from mouse models that PNH stem cells do not expand on their own, and there is evidence from human patients that expansion is associated with negative selection against GPI-positive cells by GPI-specific T cells. Thus, PNH is a prime example of a clonal disease that is not malignant.

Classic pathway Lectin pathway C5 C3 C4b2a C4b2aC3b C5 convertase C3 convertase fD
 Amplification loop Amplification loop fB C3(H₂O)Bb, C3bBb C3 convertases C3BbC3b C5 convertase
 C5b C3b Alternative pathway C6 C7 CD55 C8 C9 CD59 Normal blood B A Classic pathway Lectin
 pathway C5 C3 C3 C4b2aC3b C5 convertase C4b2a C3 convertase C4b2a C3 convertase
 ECULIZUMAB fD Amplification loop Amplification loop fB RAVULIZUMAB C3(H₂O)Bb, C3bBb C3
 convertases C3BbC3b C5 convertase C5b C3b Alternative pathway C6 C7 C8 Macrophages C9
 EXTRAVASCULAR HEMOLYSIS PNH patient, C5 blockade D C

FIGURE 105-10 The complement cascade and the fate of red cells.

A. In normal blood, when complement is activated, red cells are protected from lysis in several ways: primarily by the two glycosylphosphatidylinositol (GPI)-linked surface proteins CD55 (prevents binding of C3 fragments) and CD59 (prevents the membrane attack complex [MAC] from inserting into the membrane). B. Paroxysmal nocturnal hemoglobinuria (PNH) red cells are deficient in CD55 and CD59 because the glycosylphosphatidylinositol (GPI) biosynthetic pathway is blocked as a result of a PIGA mutation; therefore, C3 fragments, particularly C3d, bind to their surface, and the red cells are rapidly lysed by the action of the MAC. C. With drugs (monoclonal antibodies) that bind to C5 and prevent it from splitting into C5a and C5b, the entire distal pathway from C5 onward is blocked, MAC is not formed, and intravascular hemolysis (IVH) is abrogated. However, red cells opsonized by C3d will be destroyed in the spleen.

and elsewhere; this drug-induced extravascular hemolysis (EVH) varies in severity between patients. The Coombs test, which is characteristically negative in PNH, becomes positive (provided that a “broad spectrum” or an anticomplement reagent is used). D. With a drug that targets C3, C3b formation is inhibited, and the distal pathway is not triggered by C3b. Therefore, again, no MAC is formed (abrogating IVH), and at the same time, opsonization of red cells by C3d is prevented, so that EVH is also curbed. The same is largely true for drugs that target factor B or factor D, although C3b can still be formed through the classical pathway. (Reproduced with permission from L Luzzatto: Control of hemolysis in patients with PNH. Blood 138:1908, 2021.)

TREATMENT Paroxysmal Nocturnal Hemoglobinuria Until around 20 years ago, there were essentially two treatment options for PNH: either allogeneic BMT, providing a definitive cure at the cost of nonnegligible risks; or continued supportive treatment for what, unlike other acquired HAs, may be a lifelong condition. A major advance has been the introduction in 2007 of a humanized

Classic pathway Lectin pathway C5 C3 C4b2aC3b C5 convertase C4b2a C3 convertase fD fB C3(H2O)Bb, C3bBb C3 convertases C3BbC3b C5 convertase C5b C3b Alternative pathway C6 C7 C8 C9 MAC MAC INTRAVASCULAR HEMOLYSIS PNH patient CHAPTER 105 Classic pathway Lectin pathway PEGCETACOPLAN C5 Hemolytic Anemias C4b2aC3b C5 convertase fD DANICOPAN fB IPTACOPAN C3(H2O)Bb, C3bBb C3 convertases C3BbC3b C5 convertase C3b C5b Alternative pathway C6 C7 Macrophage C8 C9 MAC MAC PNH patient, C3 blockade monoclonal antibody, eculizumab, which binds to the complement component C5 near the site that, when cleaved, will trigger the distal part of the complement cascade leading to formation of the MAC. With C5 blocked by anti-C5, the patient is relieved of intra vascular hemolysis and of its attendant consequences, including hemoglobinuria, with a reduced risk of thrombosis. In the majority of patients who needed regular blood transfusion, the transfusion requirement is either abolished or significantly reduced. For many

PNH patient, untreated PNH patient on eculizumab PNH patient on pegcetacoplan A C5 blockade C3 C5 C5 C5 MAC MAC MAC C3 C5 C5 C5 MAC MAC MAC Escape from C5 blockade C5 C5 C5 MAC MAC MAC C3 C5 C5 C5 MAC MAC MAC B PART 4 Oncology and Hematology FIGURE 105-11 Impact and implications of anticomplement therapy in paroxysmal nocturnal hemoglobinuria (PNH). A. The three vertical bars symbolize red cell composition in examples of PNH patients. In the untreated patient, severe anemia is present with ~30% PNH red cells. In the patient on eculizumab, PNH red cells are protected from intravascular hemolysis, although at the expense of iatrogenic extravascular hemolysis (see text), and therefore, mild anemia is still present and can become severe. In the patient on pegcetacoplan, both intravascular and extravascular hemolysis are prevented, and the anemia is completely corrected. Note that the increase in red cells in the treated patients consists entirely of PNH red cells. B. Schematics of complement action and inhibition. In the untreated patient (left), the membrane attack complex (MAC) produces intravascular hemolysis. On eculizumab (middle), C5 blockade prevents MAC formation; if blockade is incomplete, some MAC is formed, with consequent breakthrough hemolysis. On pegcetacoplan (right), C5 convertase cannot be formed, and therefore, MAC formation is equally prevented; however, since there is an enzymatic cascade upstream of C5 cleavage, if blockade is incomplete, MAC formation will be more abundant with consequently more severe breakthrough hemolysis, potentially more massive as the PNH red cells are greatly increased in both relative and absolute terms. RBC, red blood cell. PNH patients, eculizumab has meant a real transformation in the quality of life, as well as a decrease in complications, particularly thrombosis; thrombosis may still occur,

but much more rarely. At the same time, it is important to know that in patients on eculizumab, the PNH red cells, now protected from being lysed through the MAC, do still bind C3 fragments and thus become opsonized; thus, the Coombs test becomes positive and hemolysis persists, which is now iatrogenic and mainly extravascular. The extent to which this happens depends in part on a genetic polymorphism of the complement receptor CR1. Based on its half-life, eculizumab must be administered intravenously every 14 days. Ravulizumab, a derivative of eculizumab with longer life in circulation, is administered at 8-week intervals, with obvious practical advantage. Patients who, upon C5 blockade, are still receiving blood transfusions are at risk of iron overload. Persistent blood transfusion requirement and extravascular hemolysis in PNH patients on C5 blockade therapy have been a stimulus to developing agents that may inhibit complement activation more upstream, at the level of C3. This has been found not only to prevent intravascular hemolysis but also to avoid C3 opsonization of red cells, thus not causing extravascular hemolysis. Pegcetacoplan, an inhibitor of C3, and iptacopan, an inhibitor of factor B (which, once activated, becomes part of the alternative pathway C3 convertase) are already approved drugs; and danicopan, an inhibitor of factor D (required for the activation of factor B), has given promising results in clinical trials. Thus, several drug therapy options are available for an ultra-rare disease (Fig. 105-11). Compared to C5 blockade, C3 blockade has a greater chance to completely correct anemia. We must consider that correction of anemia takes place by the buildup of a much larger population of PNH red cells than would ever be possible in an untreated PNH patient, and this population may undergo sudden lysis if complement blockade is abrogated, whether by omission or otherwise. Eculizumab and the other complement inhibitors are very expensive and, for this reason, not accessible to patients in many parts of the world. Therefore, the management of PNH by supportive

PNH RBC Normal RBC C3 blockade C3 Escape from C3 blockade C5 C5 C5 MAC MAC MAC C3 treatment is still very important. Folic acid supplements (at least

3 mg/d) are mandatory; the serum iron should be checked periodically, and iron supplements should be administered as appropriate. Transfusion of white cell-free red cells should be used whenever necessary, which, for some patients, means quite frequently. Long-term glucocorticoids are not indicated because there is no evidence that they have any effect on chronic hemolysis; in fact, they are contraindicated because their side effects are considerable. A short course of prednisone may be useful when an inflammatory process exacerbates hemolysis. Any PNH patient who has had venous thrombosis or who has been found after a thrombophilia screen to have a genetic risk factor should be on regular anticoagulant prophylaxis. With thrombotic complications that do not resolve otherwise, thrombolytic treatment with tissue plasminogen activator may be indicated. Where anti-C5 therapy is available, the proportion of PNH patients receiving BMT has decreased significantly. However, when an HLA-identical sibling is available, BMT should be taken into consideration for any young patient with severe PNH, and for patients with the so-called PNH-AA syndrome, since C inhibitors have no effect on BMF. For these patients, immunosuppressive treatment with antithymocyte globulin and cyclosporine A may be an alternative, and it may be compatible with concurrent administration of eculizumab. ■ ■ FURTHER READING Berentsen S, Barcellini W: Autoimmune hemolytic anemias. *N Engl J Med* 385:1407, 2021. Brodsky RA: Warm autoimmune hemolytic anemia. *N Engl J Med* 381:64, 2019. Dacie J: *The Haemolytic Anaemias*. London, Churchill Livingstone, volumes 1-5, 1985-1999. De Franceschi L et al: Acute hemolysis by hydroxycloquine was observed in G6PD-deficient patient with severe COVID-19 related lung injury. *Eur J Intern Med* 77:136, 2020. Grace RF et al: Safety and efficacy of mitapivat in pyruvate kinase deficiency. *N Engl J Med* 381:933, 2019.

Revision #1

Created 2026-01-06 16:31:58 UTC by Omar Ayman

Updated 2026-01-06 16:31:58 UTC by Omar Ayman