Paroxysmal Nocturnal Hemoglobinuria

LARRY J SMITH

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal stem cell disorder resulting from a somatic mutation in the hematopoietic stem cell. It is characterized by intravascular hemolysis, cytopenias, frequent infections, bone marrow hypoplasia, and a high incidence of life-threatening venous thrombosis. An absent glycosylphosphatidylinositol (GPI)-anchored receptor prevents several proteins from binding to the erythrocyte membrane. These include the complement–regulatory proteins, CD55 and CD59, whose absence results in enhanced complement-mediated lysis. Patients present with anemia and hemoglobinuria. Laboratory diagnosis includes the sucrose hemolysis test, Ham acid hemolysis test, and fluorescent-activated cell analysis. There is considerable overlap between PNH, aplastic anemia, and myelodysplastic syndrome and some cases evolve into acute leukemia. Treatment is mainly supportive consisting of transfusion therapy, anticoagulation, and antibiotic therapy. Hematopoietic stem cell transplantation may be curative.

ABBREVIATIONS: DAF = decay accelerating factor; GPI = glycosylphosphatidylinositol; PNH = paroxysmal nocturnal hemoglobinuria; MIRL = membrane inhibitor of reactive lysis.

INDEX TERMS: paroxysmal nocturnal hemoglobinuria.

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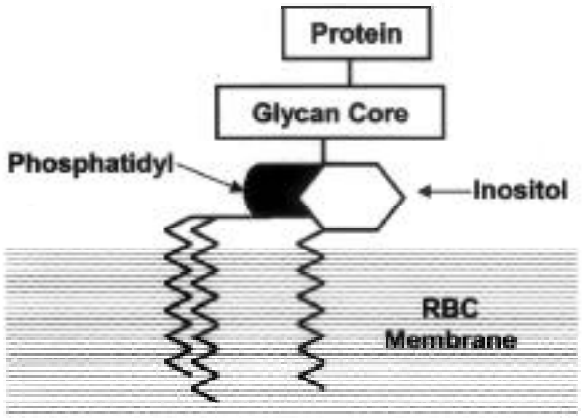
Paroxysmal nocturnal hemoglobinuria is an acquired stem cell disorder characterized by intravascular hemolysis, hemoglobinuria, and life-threatening thrombotic episodes.[1] PNH occurs with an incidence of two to six per million persons. In addition, it may be considered a chronic hemolytic anemia caused by a defect intrinsic to the erythrocyte. PNH is unique, in that it is the only hemolytic anemia due to an intrinsic defect that is acquired and not inherited. The disorder was described in 1882 by Strübing as a case of intermittent hemoglobinuria, wherein a patient presented with hemoglobinuria upon awakening.[2] Strübing went on to suggest that the hemoglobinuria was due to erythrocyte destruction within the blood vessels. Strübing's main contribution was to distinguish his patient from a common condition known as paroxysmal cold hemoglobinuria which was associated with syphilis. PNH is mainly a disease of adults; however, children and adolescents may be affected. Approximately 50% of PNH patients present with nocturnal hemoglobinuria, the hallmark of PNH. Darkening of the urine is most noticeable in the morning, either because the urine is more concentrated or there is increased hemolysis at night. Additional findings include hemosiderinuria, thrombocytopenia, decreased hematopoietic activity, and thrombosis. Thrombotic events often occur in unusual locations.

PATHOPHYSIOLOGY

PNH is an acquired stem cell disorder. Defective stem cells arise from the clonal expansion of a totipotent hematopoietic stem cell containing a somatic mutation in the phosphatidylinositol glycan complementation group A gene (PIG-A gene).[3] The PIG-A gene, located on the X chromosome, is responsible for the synthesis of the glycosylphosphatidylinositol anchor that serves to attach a number of proteins to the cell membrane surface (Figure 1). As a

result of this mutation, defective erythrocytes, leukocytes, and platelets are produced that are deficient in various surface membrane phosphatidylinositol-linked proteins such as CD14, CD16, CD24, CD48, CD55, CD58, CD59, CD66, CD67, and CD73.[4] The combined protein deficiencies consist of the following: 1) complement regulatory proteins such as CD55 and CD59, 2) membrane enzymes such as acetylcholinesterase and alkaline phosphatase, and 3) a group of miscellaneous membrane-associated proteins: CD14, CD16, CD55, and CD59 on granulocytes; CD48 and CD59 on lymphocytes; CD48 and CD95 on monocytes; and CD55 and CD59 on erythrocytes. Two very important proteins that are members of the complement regulatory protein family and are missing from the erythrocyte cell surface in PNH are the decay accelerating factor (DAF, CD55), and the membrane inhibitor of reactive lysis (MIRL, CD59).[5,6,7] These contribute to the normal red cell's ability to resist complement-mediated lysis. The importance of these two proteins has been demonstrated in experiments using antibodies to either DAF and/or MIRL.[8] Inhibition of MIRL results in greater hemolysis and is similar to the hemolysis seen in patients with a congenital defect in the MIRL molecule. In cells experimentally challenged with antibody to MIRL alone and with normal DAF expression, there is an increased susceptibility to complement-mediated lysis while in cells with antibody to DAF alone there is only a small increase in complement-mediated inhibition of erythrocytes.[8,9]

Figure 1. Structure of the glycosylphosphatidylinositol (GPI) anchor. Phosphatidylinositol is inserted into the lipid core of the membrane. An additional fatty acid, attached to the inositol, is inserted into the membrane. Various proteins such as CD55 and CD59 attach to the GPI.



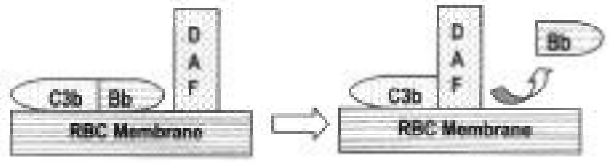
A better understanding of the pathologic basis of PNH can be seen by looking at the interaction of DAF, MIRL, GPI, and complement on the erythrocyte surface. In 1993, Miyata and colleagues cloned cDNA for the PIG-A gene and demonstrated its ability to restore the expression of GPI-linked proteins that were missing from lymphoblastoid cell lines derived from patients with PNH.[10] The actual gene product of the PIG-A gene is a glycosyltransferase that transfers N-acetylglucosamine from UDP-N-acetylglucosamine to phosphatidylinositol at the rough endoplasmic reticulum.[11] GPI anchors are complex glycolipid structures that when synthesized attach various proteins to the membrane.

Activation of the complement systems occurs via two pathways, classical and alternate. In normal individuals, when the complement system is activated by infections or immune reactions, various complement regulatory proteins are activated. DAF disrupts or inhibits the formation of the C3 and C5 convertase complexes (Figure 2).[12-14] In order to lyse an erythrocyte the complement pathways must be able to overcome the activity of DAF. [15,16] Cells that lack the DAF protein are unable to inhibit the alternative pathway of complement and therefore are more susceptible to lysis.

MIRL, on the other hand, binds to the C5b-8-C9 complex on the erythrocyte membrane and prevents the binding of additional C9 molecules, thus preventing the formation of the membrane attack complex (MAC) (Figure 3).[15,16] It appears that MIRL plays a more important role than DAF in protecting cells from complement-mediated lysis and may even be a contributing factor to some of the thrombotic episodes associated with PNH.

Several theories have been suggested to explain the pathophysiology of PNH.[12,13] One theory suggests that there is a

Figure 2. The interaction of DAF (CD55) with the C3bBb complex on the RBC membrane surface. When DAF binds to the C3bBb complex, it causes the dissociation of Bb from the complex preventing lysis of the erythrocyte membrane.



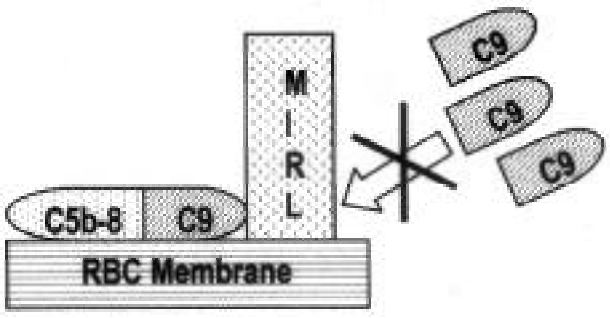
GPI-negative population of cells in normal marrow that have a growth disadvantage in the normal hematopoietic inductive microenvironment. If another bone marrow failure syndrome, such as aplastic anemia, develops, these GPI-negative clones may acquire a growth advantage over the normal clone of hematopoietic precursors and may become the predominant source of hematopoiesis.

CLINICAL FINDINGS

PNH has three cardinal clinical manifestations: 1) hemolysis and hemoglobinuria, 2) thrombosis, and 3) bone marrow failure that is likely to be immune-mediated. Hemolysis results from the action of activated complement on the GPI-deficient erythrocytes. Other blood cells deficient in the GPI anchor are lymphocytes, granulocytes, and platelets. Patients presenting with PNH usually manifest most of the clinical signs and symptoms of patients with chronic hemolytic anemia, however the severity varies depending on the degree of hemolysis. Symptoms include hemoglobinemia, general fatigue, pallor, dyspnea, and splenomegaly. Splenomegaly occurs when there has been splenic, hepatic, or portal vein occlusion. Varying degrees of iron deficiency may be present as a result of iron loss due to hemosiderinuria and hemoglobinuria. Plasma hemoglobin levels are elevated as a result of intravascular hemolysis. Patients with PNH generally do not become iron-overloaded.

An increase in venous thrombotic episodes has been noted in patients with PNH. Thrombosis-related death occurs in approximately 40% of patients. These thrombotic episodes can occur in any venous site throughout the body; however, the hepatic veins and veins of the portal system are particularly affected.[17,18] The Budd-Chiari syndrome is a serious manifestation of hepatic venous thrombosis, characterized by obstruction in hepatic venous outflow. Valla and colleagues reported that five of 40 patients with Budd-Chiari syndrome had PNH. The authors went on to suggest that PNH should be considered in any patient with hepatic vein thrombosis.[19,20] The increased thrombotic tendencies have been attributed to a number of factors including platelet activation via complement, an increase in circulating procoagulant activity resulting from erythrocyte membrane destruction, and the release of adenosine diphosphate (ADP).[20] Furthermore, receptors for urokinase and tissue factor pathway inhibitor are GPI-linked and abnormal expression of these proteins may result in a hypercoagulable state.[21,22]

Figure 3. The interaction of MIRL (CD59) with the C5b-9 complex. The attachment of MIRL to the C5b-8-C9 prevents the binding of additional C9 units needed to form the membrane attack complex (MAC). As a result of this interaction MAC-mediated lysis of the erythrocyte membrane is inhibited.



LABORATORY FINDINGS

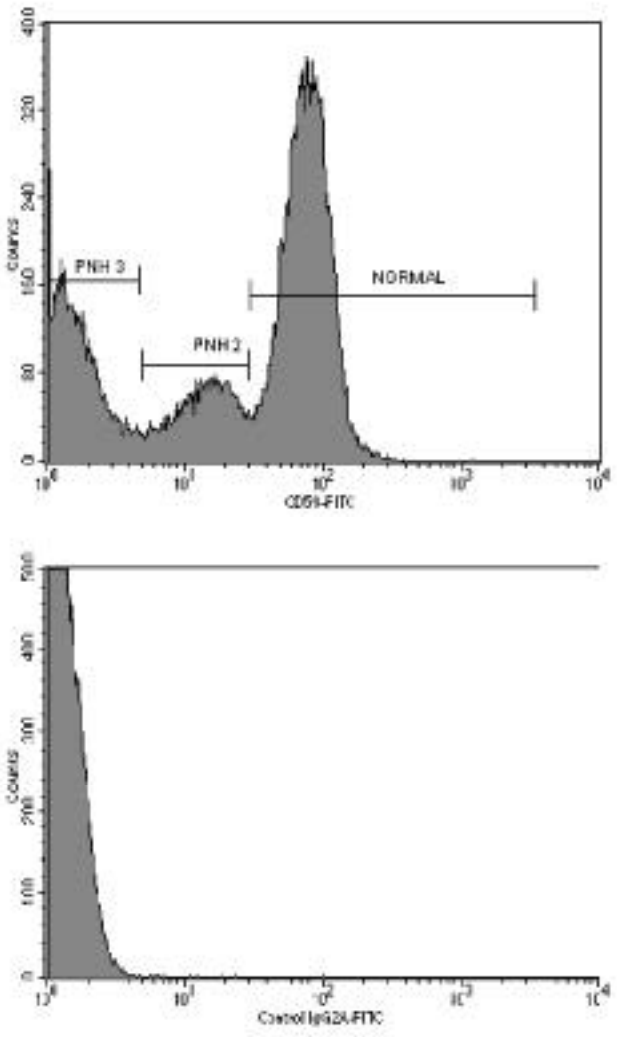
The severity of anemia varies depending on the degree of hemolysis. This depends on the relative proportion of PNH cells present, and any clinical event that triggers the release of complement, such as an infection. Reticulocytosis is generally present ranging to greater than 80,000/µL (20,000-80,000/µL reference interval) however, the reticulocyte percentage in PNH is less than that seen in other hemolytic anemias. Patients in an aplastic phase can have low reticulocyte production as well as hemolysis contributing to the anemia. On the peripheral blood smear, erythrocytes may appear normocytic or macrocytic. When macrocytes are present, their numbers may vary depending on the degree of reticulocytosis. Macrocytosis is seen in aplastic anemia, myelodysplastic syndrome (MDS), and PNH even if the reticulocyte count is low. Microcytosis and hypochromia may also be present depending on the degree of iron deficiency that occurs due to the loss of iron via hemoglobinuria. Nucleated red blood cells may also be present. Leukopenia is a characteristic finding with granulocytopenia in patients with bone marrow failure. Leukocyte alkaline phosphatase scores are generally low.

The bone marrow cellularity ranges from hypercellular to hypocellular and may have a patchy cellularity. In addition, there is often an erythroid hyperplasia. Patients presenting with bone marrow failure syndromes, particularly aplastic anemia, may be at risk for PNH when they have a modest reticulocyte count. The bone marrow in advanced stages of PNH is indistinguishable from the bone marrow in aplastic anemia. However, PNH can be distinguished from aplastic anemia by the presence of reticulocytosis, hemoglobinuria,

and flow cytometry. Morphological abnormalities and chromosomal abnormalities are frequently seen in PNH and do not indicate that the patient has MDS.

The sucrose-hemolysis test is a screening assay based on complement-mediated lysis of erythrocytes in a low ionic-strength solution. The test is performed by mixing the patient's washed red cells (0.1mL of a 50% suspension) with fresh ABO-compatible normal serum in a 10% isotonic sucrose solution. The tube is incubated at room temperature for 30 minutes, centrifuged, and the percent hemolysis in the supernatant is determined. PNH cells, due to their increased sensitivity to complement, will lyse, whereas normal red cells will not. In patients with PNH lysis is usually greater than 10%. Even though the sucrose-hemolysis assay is still performed by some institutions, greater specificity may be achieved through the use of fluorescence activated cell sorter (FACS) analysis in the diagnosis of PNH.

Figure 4. Top panel: there is a trimodal distribution of red cells, PNH type 3 cells, PNH type 2 cells (indicating the presence of PNH positive cells with intermediate sensitivity to complement), and a normal population of cells. This finding is diagnostic of PNH. Bottom panel is the positive control.



Flow cytometry involves immunophenotyping directed at various GPI-anchored proteins, including CD55 and CD59. This methodology is diagnostic, highly sensitive, and specific for PNH and has greatly improved the ability to diagnose PNH. In addition, immunophenotyping allows one to differentiate between different populations of PNH cells. Type I PNH cells are GPI+ cells which circulate in the blood of PNH patients. They are very similar to normal erythrocytes in that they demonstrate normal levels of CD59 expression; however, unlike normal red cells, type I PNH cells arise from an abnormal clone of cells in the bone marrow. Type II PNH cells demonstrate a slight increase in sensitivity to complement-mediated lysis over type I PNH cells and have lower CD59 expression than type I PNH cells. Type III PNH cells demonstrate high sensitivity to complement-mediated lysis and do not express CD59. The percentage of type III PNH positive cells can be correlated with the degree and severity of clinical symptoms in PNH (Figure 4). Patients with fewer than 20% type III PNH cells have mild hemolysis, patients with 20% to 50% type III PNH cells have moderate hemolysis which could be considered episodic or sleep-induced hemolysis, while patients with greater than 50% type III cells have severe hemolysis. The PNH phenotype can be detected by flow cytometry on peripheral blood granulocytes and erythrocytes. Detection on the erythrocytes, however, is affected by transfusion and acute hemolytic events. In practice both erythrocytes and granulocytes should be analyzed.

DIFFERENTIAL DIAGNOSIS

A provisional diagnosis of PNH should be considered in all patients presenting with pancytopenia of unknown origin with an accompanying reticulocytosis. PNH has been associated with bone marrow failure syndromes such as aplastic anemia. Recent studies have demonstrated the presence of granulocytes of the PNH phenotype in untreated patients with aplastic anemia.[20] PNH can be differentiated from other hemolytic anemias by the presence of hemosiderinuria which does not usually occur in other hemolytic anemias.[21]

TREATMENT

The only known cure for PNH is hematopoietic stem cell transplantation. Transplantation replaces the abnormal clone(s) present in the marrow. Supportive therapy includes transfusion and anticoagulation. Acute thrombosis can be reversed with tissue plasminogen activator and bone marrow failure can respond to immunosuppression, as it does in aplastic anemia. Iron therapy may have some value in replenishing iron stores, especially in young women who are iron deficient. As in hemolytic anemia, the patient should be given folic acid supplementation, 5 mg daily. Erythropoietin is probably helpful only in those patients whose kidneys have not responded appropriately to anemia. Eculizumab, a recently developed complement inhibitor has entered clinical trials and has been shown to be safe and effective at reducing hemolysis in PNH patients.[24]

The clinical course of PNH is quite variable and ranges from death within a few months of diagnosis to several years after diagnosis. Due to the presence of an active hypercoagulable state, oral contraception and female hormone replacement therapy should be avoided. Pregnancy should be considered only with great caution with prophylactic injections of anticoagulants in a high risk clinic.[25] The clinical course in children and adolescents is probably more severe than in adults. Patients with PNH may go on to develop aplastic anemia or myelodysplasia and acute leukemia. Conversely, after long follow-up the PNH clone can disappear.

The relationship between PNH and aplastic anemia was first described by Dacie and Lewis in 1944.[26] The evolution of PNH in patients with aplastic anemia treated with immunosuppressive therapy has been reported to be 10% to 13%.[12] In addition, it was reported by Jenkins and colleagues that in about 2% to 3% of patients with PNH, acute myelogenous leukemia (AML) will evolve.[17] In studies performed by Ware and Hillmen the transformation to AML was rare, zero out of 80 patients and one out of 24 patients, respectively.[13,27] Additional studies performed by Meletis suggest an association between PNH and MDS. In their study, simultaneous CD55 and CD59 deficiency was noted in 17.8% of patients with MDS and occurred more frequently in patients with advanced stages of MDS – 22.2% with refractory anemia with excess blasts (RAEB) and refractory anemia with excess blasts in transformation (RAEB-t).[28]

SUMMARY

PNH is an acquired clonal stem cell disorder that results from a somatic mutation in the PIG-A gene. It is characterized by the clonal expansion of stem cells with an absent or deficient GPI anchor protein on the erythrocyte membrane that leads to enhanced complement-mediated lysis. In addition, it is an acquired hemolytic anemia with chronic hemolysis, hemosiderinuria, and an increased risk of thrombosis. Venous thrombosis is probably the most common cause of death in these patients. It has also been associated with aplastic anemia and myelodysplastic syndrome, and in rare cases, with acute leukemia.

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