CHAPTER 12

Autoimmune hemolytic anemias and paroxysmal nocturnal hemoglobinuria

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The hallmark of the autoimmune hemolytic anemias (AIHAs) and paroxysmal nocturnal hemoglobinuria (PNH) is shortened red blood cell survival. Although AIHA and PNH share the critical feature of shortened red cell survival, the mechanisms underlying this characteristic differ significantly between the two diseases. In the case of AIHA, autoantibodies directed against the patient’s own red blood cells lead to accelerated red cell destruction. In PNH, lack of surface complement-regulatory proteins as well as the loss of other surface proteins, leads to exquisite sensitivity of the red cells to complement-mediated lysis.

The AIHAs are classified by the characteristics of the responsible autoantibodies and their clinical settings (Table 12.1). Serologic differences in optimal temperature of reactivity allow for the differentiation of warm AIHA from cold AIHA; these broad divisions correlate with responsiveness to specific therapy and prognosis. Both warm and cold AIHAs can be further characterized as either a primary, idiopathic autoimmune phenomenon or a secondary process associated with an underlying clinical condition; this information also provides guidance on the most appropriate therapeutic approach. In contrast, PNH occurs in the context of a somatic gene mutation and some component of bone marrow dysfunction. This somatic mutation, which results in decreased glycosylphosphatidylinositol anchored proteins, ultimately leads to increased sensitivity to complement with effects of intravascular hemolysis and increased risk of thrombosis.

Autoimmune hemolytic anemias

Classification

The AIHAs are categorized into three broad subtypes: warm AIHA, cold AIHA, and drug-induced immune hemolytic anemia (Table 12.1). The distinction between warm and cold AIHAs is based on the causative antibody’s optimal temperature of reactivity in vitro. Warm AIHA is due to autoantibodies which optimally react at 37°C. Cold AIHA is associated with autoantibodies that optimally react at 0°C, but are also able to react at higher temperatures in cases with higher thermal amplitudes. Cold AIHA is further subdivided into the more common cold agglutinin disease (CAD) and the infrequent paroxysmal cold hemoglobinuria (PCH), based on serologic and clinical properties.

Because temperature of optimal reactivity and mechanism of action are known to vary amongst immunoglobulin classes, it is not surprising that warm and cold AIHA differ with respect to the most common immunoglobulin class of the causative autoantibody. Warm AIHA is typically due to an immunoglobulin G (IgG) autoantibody. CAD is usually associated with IgM autoantibodies. PCH is due to a unique type of IgG autoantibody called the Donath–Landsteiner antibody, which is also known as a biphasic cold hemolysin. This type of IgG can bind to red cells at low temperatures, activate complement, and subsequently lead to hemolysis at 37°C.

The AIHAs, both warm and cold, can be further characterized by their clinical associations. Primary AIHA is idiopathic. Secondary AIHA is associated with an underlying disorder, such as an autoimmune disease, an infection, or a malignancy, most commonly of the lymphoproliferative type. The underlying disorders most frequently associated with AIHAs vary between the warm and cold autoantibody types. The AIHAs also demonstrate variability in severity of clinical presentation, related to the rate of hemolysis and the time course of the development of anemia. Clinically, AIHAs may have an acute presentation with a sudden development of severe anemia. Alternatively, they may present as a chronic phenomenon with a gradual decrease in hemoglobin and hematocrit over a prolonged period of time. The AIHAs may also be transient, most often seen when associated with an infectious etiology.

In drug-induced immune hemolytic anemia, autoantibodies are not categorized by temperature of optimal reactivity or immunoglobulin type, but instead by their proposed pathophysiologic mechanisms, which include the drug adsorption mechanism, the immune complex mechanism, and the autoimmune induction mechanism. The antibodies in drug-induced immune hemolytic anemia frequently demonstrate reactivity at warm temperatures, and consequently, patients may present with symptoms that are indistinguishable from those of warm AIHA.

Warm autoimmune hemolytic anemia

Epidemiology and risk factors

The incidence of AIHA is approximately 1–3 cases in 100,000 individuals per year. Warm AIHA is the most common type, accounting for about 80% of all AIHAs. Individuals of all ages,
Pathophysiology

There are two distinct mechanisms of immune-mediated red cell destruction: extravascular hemolysis and intravascular hemolysis.9

Macrophages located in reticular tissue of the spleen and liver (known as the mononuclear phagocyte system or reticulo-endothelial system) are responsible for extravascular hemolysis. The spleen is the most common site of extravascular hemolysis,13 because there is increased interaction between antibody-coated red cells and macrophages created by the movement of red blood cells from the cords of Billroth through tiny gaps in sinusoidal walls to gain entry to the sinuses.14 However, the liver also participates in extravascular hemolysis when large amounts of IgG coat the red cells.13

The hemolysis of warm AIHA usually proceeds via the extravascular hemolysis mechanism.9 As previously discussed, warm autoantibodies are typically IgG autoantibodies that are optimally reactive at 37°C.9 Consequently, IgG autoantibodies bind to red blood cells at normal body temperature. The antibody-coated red cells then circulate to the spleen and liver where macrophages adhere to the Fc portion of the IgG bound to the red cells.13 Once bound to macrophages, the antibody-coated red cells are then subjected to either complete or partial phagocytosis by the macrophage. Complete phagocytosis results in removal of the antibody-coated red cells from circulation. Partial phagocytosis, which is more common,13 results in removal of a portion of the red cell membrane and the formation of spherocytes.9 Spherocytes are released back into the circulation but have a decreased life span.15 After a brief period of circulation, they pass back through the spleen where they become lodged in tiny gaps in sinusoidal walls as they attempt to reach the sinusoids, and they are destroyed.16

The degree of extravascular hemolysis depends on the subclass of IgG, quantity of bound IgG, and antibody specificity. There are four subclasses of IgG: IgG1, IgG2, IgG3, and IgG4. Macrophage Fc receptors have the highest affinity for IgG1 and IgG3 subclasses.17,18 Macrophages have variable affinity for IgG2, but they have the lowest affinity for IgG4. Not surprisingly, the most common subclasses of IgG found in warm AIHA are IgG1 and IgG3, followed by IgG2. Subjects with IgG4-coated red cells are not expected to demonstrate hemolysis.4 Based on macrophage affinity, one might suspect that the greatest degree of extravascular hemolysis would be observed when red cells are coated by IgG1 and IgG3 subtypes. Although this is partly true, in the case of IgG1, the quantity of immunoglobulin coating each red cell also plays a role in the degree of hemolysis. Studies suggest that at least 1200 IgG1 molecules17,18 must coat each red cell before macrophages can effectively bind and subsequently phagocytose them. Consequently, there is a demonstrated relationship between the quantity of IgG1 sensitizing the red cells and the severity of AIHA.19 In contrast, IgG3 is associated with hemolysis even when the quantity coating the red cells is too low to be detected by a direct antiglobulin test (DAT),1 which is an average of 335 +/− 72 molecules.20,21 In the case of IgG2, the situation is more complex. The gene that encodes the Fc receptor for IgG2 on macrophages has two alleles, resulting in differences in affinity. As a result, some people have macrophages with an Fc receptor that has a low-affinity for IgG2, and some are endowed with a receptor that has high affinity. Those individuals with high-affinity receptors for IgG2 have the capacity to destroy IgG2 coated red cells. However, in the case of IgG2 antibodies, destruction of red cells is also influenced by antigen specificity. For example, studies have shown that IgG2 alloantibodies against blood group A antigen lead to hemolysis, while hemolysis will not be seen due to IgG2 antibodies directed against Rh antigens.22 Despite the findings of differing Fc receptor affinity and IgG2 alloantibody reactivity, the role of IgG2 autoantibodies in autoimmune hemolysis remains unclear. Practically speaking, in AIHA, red cell destruction due to IgG antibodies is primarily observed when the subclass of bound IgG is either IgG1 or IgG3.1

Although warm AIHA usually proceeds via the extravascular hemolysis mechanism, in some individuals presenting with severe hemolysis there may also be evidence of intravascular hemolysis,9 which occurs when antibody-coated red cells are capable of activating complement. Red cell destruction occurs by the classical
complement pathway, with formation of a membrane attack complex (MAC), forming pores in the red cell membrane leading to cell swelling and lysis. The ability of antibody-coated red cells to activate complement depends on many things, including the subclass and quantity of IgG coating the red cells. Subclasses IgG1 and IgG3 are the most commonly implicated in intravascular hemolysis, as they are thought to be more efficient at activating complement than other IgG subclasses. In addition, studies have demonstrated that a high density of cell-bound IgG is required for efficient binding and activation of complement, likely due to the need for two IgG molecules to be in close proximity to allow for binding and activation of the complement system. Since a high number of IgG molecules are required to coat the red cells before, by chance, two are in close proximity, the overall density would be expected to correlate with complement activation.13,25

Despite attempts to divide hemolysis discretely into extravascular and intravascular mechanisms for the purpose of understanding the pathophysiology of these disorders, they do not always occur discretely in clinical situations. Many cases of warm AIHA demonstrate both mechanisms of hemolysis. There may be a predominance of one type over another, but laboratory and clinical findings may ultimately overlap.9

Clinical features
Warm AIHA has a variable clinical presentation. Severe anemia as a result of hemolysis can present with varying degrees of fatigue, fever, dizziness, angina, shortness of breath (with exertion or even at rest), and flank pain. The severity of symptoms depends largely on the rate of hemolysis and the rate of decrease in hematocrit and hemoglobin. In chronic hemolytic anemia, the pace of the disease may allow for physiologic compensation and, therefore, less severe symptomatology. In addition, the presence of underlying conditions, such as cardiopulmonary disease, affects the ability to compensate for anemia, even when the hemolytic pace is slow. Patients may also develop jaundice, pallor, or dark urine consistent with hemoglobinuria. Hepatosplenomegaly is present in approximately half of all warm AIHA cases.7,26 Patients with idiopathic AIHA have been shown to be at increased risk for venous thromboembolism.7,27-29 It is important to note that approximately half of warm AIHA cases are due to secondary causes, and the clinical presentation of the underlying cause may predominate.

Diagnosis
The diagnosis of warm AIHA is based on laboratory findings indicative of shortened red cell survival and serologic evidence of autoantibody directed against red cell antigens, optimally reactive at 37 °C.

Laboratory findings
Complete blood counts will demonstrate typical features of anemia, with hemoglobin and hematocrit as low as 5 g/dL and 15%, respectively.30 The anemia is typically macrocytic, due to concomitant bone marrow compensation and marked reticulocytosis, related to decreased red cell survival.31 However, reticulocytopenia may also be seen in as many as one-third of cases. Reticulocytopenia should be treated as a hematologic emergency as it is often associated with the development of life-threatening anemia.31 In the case of Evan’s syndrome (warm AIHA associated with autoimmune thrombocytopenia), the platelet count is also low.12 Other findings include hemoglobinemia, hemoglobinuria, elevated lactate dehydrogenase and indirect bilirubin, and decreased haptoglobin.2,10

The peripheral blood smear typically shows evidence of extravascular hemolysis, demonstrable by the presence of spherocytes.32 Due to the relatively short lifespan of spherocytes, their presence in the peripheral blood should be taken as evidence for ongoing extravascular hemolysis.16 Indirect evidence of bone marrow compensation, in the form of polychromatophilic macrocytes (which are suggestive of reticulocytes) or even nucleated red blood cells (when the rate of hemolysis is brisk), may also be seen in the peripheral blood smear.

Serology
Serologic evaluation reveals that the hemolytic process is immune mediated. DATs using polyspecific reagents, including both anti-IgG and anti-C3 (C3d), are typically positive in patients with warm AIHA.33-35 IgG alone is demonstrated by DAT in 20–66% of warm AIHA,36,37 and IgG in combination with C3 coat the red cells in 24–64% of cases. Complement alone is found in only 7–14% of warm AIHA36,37 (Table 12.2). When the DAT demonstrates IgG, eluates of the antibody coating the red cells confirm the finding of IgG. Indirect antiglobulin tests, using either untreated or enzyme-treated red blood cells at 20 °C or 37 °C, typically demonstrate IgG antibody in the serum. Indirect antiglobulin tests may be negative if the antibody is adsorbed onto the patient’s red cells; when the amount of autoantibody exceeds the binding capacity of the patient’s red cells, the antibody will be detectable in the patient’s serum. Although 97–99% of patients with warm AIHA will have a positive DAT, 50–90% of patients will have a positive indirect antiglobulin test, depending on the testing methodology.3

The IgG eluted from the red cells frequently demonstrates panagglutination when tested against a panel of commercially available red cells. Likewise, the serum typically demonstrates panagglutination. Occasionally, the autoantibody may demonstrate apparent specificity; these specificities are more often and more clearly seen in the serum as compared to the eluate. Specificity is most frequently seen as broad reactivity with antigens of the Rh system. Less commonly, specificity is toward a single Rh antigen.

Table 12.2 Typical Serologic Features of Autoimmune Hemolytic Anemia

<table>
<thead>
<tr>
<th>Warmed Autoimmune Hemolytic Anemia</th>
<th>Cold Agglutinin Disease</th>
<th>Paroxysmal Cold Hemoglobinuria</th>
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<tbody>
<tr>
<td>Direct antiglobulin test</td>
<td>IgG only or IgG and C3; less commonly, C3 only</td>
<td>C3 only</td>
</tr>
<tr>
<td>Immunoglobulin class</td>
<td>IgG, rarely IgA</td>
<td>IgM</td>
</tr>
<tr>
<td>Eluate</td>
<td></td>
<td>Nonreactive</td>
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<tr>
<td>Serum</td>
<td>IgG agglutinating red cells at the antihuman globulin phase, panagglutination</td>
<td>IgM agglutinating antibody, often with titers &gt;1000, reacting at 30 °C in albumin</td>
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<tr>
<td>Antibody specificity</td>
<td>Rh</td>
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Alternatively, the autoantibody may demonstrate relative specificity for glycophorin A. On occasion, autoantibodies may demonstrate apparent specificity that appears consistent with an allotypant. In these cases, there is transient decreased expression of the corresponding antigen. Following resolution of the AIHA, expression of that antigen will increase, returning to normal levels. Subsequent testing of the patient’s convalescent red cells with retained serum will confirm the nature of the antibody as an autoantibody.

**DAT-negative warm AIHA**

In 1–3% of patients with warm AIHA, the DAT is negative indicating that the quantity of bound IgG is too low to be detected by a DAT, or that the bound autoantibody is not of the IgG immunoglobulin class. As few as 230 molecules of IgG3 can result in hemolysis, yet this number is insufficient to produce a positive DAT. Although IgG is the most common immunoglobulin class associated with warm AIHA, other immunoglobulin classes have been shown to cause AIHA, including IgA and IgM; these immunoglobulin classes are not detectable by routine antihuman globulin reagents. In 14% of warm AIHA cases, IgA was found coating the red cells. It should be noted that cases solely attributable to IgA autoantibody are rare (less than 1%) and often IgG and/or IgM are found in combination with IgA. Note that there are no anti-IgA or anti-IgM reagents that are currently licensed for serologic testing. In patients presenting with clinical features highly suggestive of warm AIHA, extended testing to identify DAT-negative cases should be performed, because DAT-negative cases often respond to typical therapy for warm AIHA.

**Differential diagnosis**

Other causes of anemia, such as hemorrhage or bone marrow failure, should also be considered. Other hemolytic disorders such as thrombotic thrombocytopenic purpura and the microangiopathic hemolytic anemias should be ruled out. Drug-induced immune hemolytic anemia produces a clinical syndrome with serology virtually indistinguishable from that of warm AIHA. The patient’s clinical history should be reviewed for all recent medications. Although there are extensive lists of drugs that have been associated with drug-induced IHA in hematology texts, a suggestive drug history should be evaluated and suspected drugs discontinued even if they have not been previously reported.

**Positive DAT results in patients without hemolytic anemia**

Confirmation of the immune-mediated cause of a suspected warm AIHA should be sought and serology performed to determine the type of autoantibody coating the red cells. However, it is worth bearing in mind that disproportionate weight should not be placed on the results of the DAT. Up to 0.1% of healthy donors and 8% of hospitalized patients were found to demonstrate a positive DAT in the absence of signs of hemolysis. Although a positive DAT can provide supportive evidence for warm AIHA in a patient with hemolysis, it is not independently indicative of the disease. The solitary finding of a positive DAT is not diagnostic of warm AIHA. Note that a negative DAT does not exclude the possibility of warm AIHA, because an IgA-mediated warm AIHA can present with a negative DAT and signs and symptoms of immune-mediated hemolysis.

**Treatment**

Treatment in warm AIHA is aimed at relieving clinical symptoms and transfusion dependence.

**Corticosteroids**

The primary initial treatment for warm AIHA is corticosteroids. A standard therapeutic approach for adults includes prednisone 1.0 to 1.5 mg/kg per day or 60 to 100 mg/day for 1–3 weeks. A clinical response may be evident within several days to one week. Response rates of up to 80% within 3 weeks of treatment have been achieved with this therapy. Most hematologists recommend continuation of higher corticosteroid doses for the first two weeks of therapy. Following initial response with improvement of hemoglobin and stabilization of hematologic parameters, the corticosteroid dose may be reduced. Sudden decreases in dosage or rapidly progressive tapers may lead to relapse. If clinical relapse does occur, the prednisone dose should be increased. Maintenance doses of prednisone greater than 15 mg per day to maintain a hematocrit of at least 30% are considered therapeutic failures.

The adverse effects of corticosteroid therapy are well established. Initial complications include insomnia, increased appetite, weight gain, and emotional lability. Diabetes and hypertension may worsen. Long-term corticosteroid use is associated with the development of a cushingoid habitus, osteoporosis, atherosclerotic disease, posterior subcapsular cataracts, and glaucoma. Steroid use is associated with an increased risk of infection. Because of these serious adverse effects, steroids must be used judiciously and doses should be tapered as quickly as possible. Giving steroids every other day may reduce the complication rate.

The efficacy of corticosteroid therapy is likely related to several factors. Steroids have been shown to have an early effect on tissue macrophages, leading to less efficient clearance of IgG- and C3-coated red blood cells within the first eight days of therapy. Steroids also affect antibody avidity and ultimately lead to a decrease in antibody production. Following corticosteroid therapy, permanent remission is uncommon; it is seen in only 20–35% of adult patients. When clinical response cannot be maintained despite high doses of corticosteroids, other therapies, such as splenectomy, rituximab, and cytotoxic therapy, are employed. Although splenectomy has traditionally been the therapeutic approach following corticosteroid failure, rituximab is being used as a second-line approach with greater frequency. If a patient does not respond to rituximab, the selection of the next most appropriate therapeutic intervention should be based on evaluation of the specific patient and the severity of hemolysis.

**Splenectomy**

Splenectomy should be reserved for patients who show no initial response to steroids or who require large maintenance doses. It is nearly as clinically effective as steroids and approximately 60–70% respond within two weeks. In about 50% of patients who clinically respond to splenectomy, steroid therapy is still required, but at much lower doses than required preoperatively. Despite an initial response, late clinical relapses are still seen in some patients. These relapses are thought to be due to enhanced antibody production as well as increased clearance of antibody-coated red cells by the liver. It should be remembered that postsplenectomy patients are particularly vulnerable to infection by encapsulated bacteria. When these infections occur, patients can rapidly progress to bacteremic shock due to an inability to efficiently clear the bacteria by phagocytosis. Overwhelming postsplenectomy sepsis syndrome represents a medical emergency with a risk of 3.2% in postsplenectomy patients and a mortality rate of 1.4%. Pneumococcal and meningococcal vaccines are strongly recommended to prevent
infection and mortality in these patients. Some clinicians advocate the use of prophylactic antibiotic regimens; commonly, prophylactic penicillin (250 mg twice a day) is given. Others prefer regimens of amoxicillin or bactrim. Febrile illness in splenectomized patients should be treated promptly with expeditious administration of antibiotics.

**Rituximab and other monoclonal antibodies**

Rituximab is a genetically engineered monoclonal antibody against CD20. It targets B-cell precursors and mature B cells. Plasma cells are not directly targeted as they do not carry the CD20 antigen. Most commonly, it has been reserved for cases of warm AIHA requiring large maintenance doses of steroids, for patients who cannot tolerate prednisone, and for patients who cannot undergo splenectomy. More recently, rituximab is being used as a second-line therapeutic approach following corticosteroid therapy, instead of the traditional secondary approach of splenectomy. Typical dosing in warm AIHA is 375 mg/m² weekly for 2–4 weeks and possibly longer for some patients. Its use as a single-agent therapy has been shown by several case studies and retrospective reports to be efficacious in both adults and children with warm AIHA resistant to therapy, including some patients with Evans syndrome. The combination of rituximab plus glucocorticoids has been evaluated in two small studies with promising initial results. Patients demonstrated higher initial clinical response rates and relapse-free survival over a three-year time period, when compared to glucocorticoid therapy alone. However, rituximab is expensive and can cause infusion-related reactions. Therefore, studies with longer term follow-up are needed before rituximab can be routinely recommended.

Alemtuzumab, a monoclonal anti-CD52 antibody, has been used as a single-agent therapy and in combination with rituximab. Its use has only been studied in a handful of papers, with side effects including marked immunosuppression and infections.

**Immunosuppressive and cytotoxic therapy**

Treatment with azathioprine or cyclophosphamide has demonstrated efficacy in reducing autoantibody formation and increasing hemoglobin levels. Immunosuppressive agents should generally be reserved for cases of warm AIHA requiring large maintenance doses of steroids, or for patients who cannot tolerate prednisone and cannot undergo splenectomy. Azathioprine takes at least one month to demonstrate an effect, and treatment failure is considered when there is no response after four months. Cyclophosphamide may be more effective than azathioprine but has many side effects, including nephrotoxicity, that require careful monitoring. Although it has shown efficacy in treating warm AIHA in many trials, it has also failed to demonstrate efficacy in others. In select cases of refractory, severe warm AIHA, high-dose cyclophosphamide (50 mg/kg/day × 4 days) has been used with success as measured by induction of durable remission. This intensive therapy has also been successfully used in other autoimmune diseases, including aplastic anemia.

Cyclosporine in combination with mycophenolate mofetil has been used with good results in some patients who are resistant to standard therapy. This combination has also demonstrated efficacy in patients with Evans syndrome and resistant disease.

**Other therapies**

Danazol, an attenuated androgen, has been used in some cases of warm AIHA following the failure of steroid therapy either due to refractoriness or relapse. The mechanism of action is unknown, but it appears to be most effective when used in initial treatment courses in combination with glucocorticoids.

Intravenous immunoglobulin (IVIG) shows neither clear efficacy nor failure; only 40% of patients respond to therapy, and the response is typically only sustained if IVIG infusions are continued every three weeks.

Therapeutic plasma exchange may be used as a temporizing measure. However, it is neither effective nor practical for long-term treatment. Severe warm AIHA is considered a Category III indication for plasma exchange therapy by the American Society for Apheresis, suggesting that the optimal role of apheresis has not been established for this disease with only case reports and case series demonstrating variable efficacy.

**Transfusion management**

Support of patients with warm AIHA often requires red blood cell transfusion for severe and life-threatening anemia. Due to the broad reactivity of autoantibodies in this disease, serologic evaluation to select appropriate red cells for transfusion is often complex and time-consuming. Sometimes transfusion is clinically required before the serologic workup is complete. Frequently, even after completion of the serologic workup, red blood cells selected for transfusion are still incompatible with the patient’s plasma. This finding should not be surprising given that the antibody found in the patient’s serum is often broadly reactive with a variety of commercial red blood cells and it is actively attaching to the patient’s own red blood cells. Transfusion of blood to a patient in true clinical need should not be delayed due to inability to find compatible red blood cell units. Most patients with AIHA show no adverse response to transfusion of serologically incompatible blood, and the survival of transfused incompatible units is comparable to that expected for the patient’s own red blood cells.

When assessing the need for transfusion, it is helpful to recall that patients with chronic anemia have a long history of physiologic compensation. They may appear hemodynamically stable even with life-threatening anemia. The onset of confusion in a patient with worsening anemia and/or reticulocytopenia should warrant immediate transfusion. Even young adults and children with gradual onset of anemia should be transfused to maintain a hemoglobin level above 4 mg/dL; higher hemoglobin levels are needed for older patients and patients with cardiovascular disease.

**Selection of blood for transfusion**

ABO discrepancies and difficulty with Rh typing can arise in association with warm autoantibodies. When ABO discrepancy occurs, accurate ABO typing cannot proceed until the IgG autoantibody coating the red cells is removed. Rh typing may also be problematic, although use of low-protein, monoclonal reagents may improve results in the setting of immunoglobulin-coated red cells. When transfusion is urgent, recall that group O red cell components can be issued and administered, even if ABO typing is incomplete.

In many patients with autoantibodies, their history of transfusion or pregnancy allows for the possibility of an alloantibody in addition to their autoantibodies. Underlying alloantibodies have been seen in 32–40% of patients with AIHA. Thus, identification of alloantibodies that may be obscured by the presence of a panreactive autoantibody is of great importance. The use of adsorption techniques is required for the removal of panagglutinating autoantibody from the patient’s serum so that any underlying alloantibodies can be tested against commercially available red cells for identification.
Autologous adsorption, utilizing the patient’s own red cells to draw off autoantibody, is a useful technique in patients who have not been transfused in the last three months.89 If the patient has been recently transfused, allogeneic adsorptions may be required.89,90 Adsorption techniques, particularly allogeneic adsorptions, are complex, labor-intensive, and time consuming.86 Therefore, they may be incomplete when a transfusion is clinically necessary.

Identifying the patient’s phenotype aids in the provision of appropriate blood for transfusion by focusing the alloantibody evaluation only on those alloantibody specificities the patient is capable of making. Phenotypically matched blood should be safe for transfusion even when the alloantibody workup is unenlightening.87 In addition, provision of phenotype-matched blood may prevent the formation of alloantibodies.91,92

The practice of finding “least incompatible blood” is not suggested. This practice includes selection of red cell units based on crossmatch results having the lowest strength of agglutination. This practice is used by some when all crossmatches are incompatible. However, use of “least incompatible blood” has not been shown to improve safety of transfusion for recipients.93 In addition, this practice is expected to lead to increased delays in the provision of blood due to time spent performing multiple crossmatches to compare the strength of agglutination.

**Prognosis**

Historically, the outcome of warm AIHA was poor, with a 38% mortality rate in the 1950s and 1960s.26 More recent data indicate 91% survival at 1 year and up to 73% survival at 10 years.94 The prognosis for children is excellent,95–97 with most experiencing self-limited disease.98,99

**Cold autoimmune hemolytic anemia**

The autoantibodies responsible for cold AIHA optimally react at 0°C.1 When they react at warmer temperatures, demonstrating broader thermal amplitude, clinically significant hemolysis may ensue. Cold AIHAs are further subdivided into the more common CAD and the less common PCH, based on serologic and clinical properties. CAD is typically due to IgM autoantibodies.

**Cold agglutinin disease**

**Epidemiology and risk factors**

CAD accounts for 15–25% of cases of AIHA.96,100 The classic patient is a female in her 70s.3 In the largest study of patients with CAD to date, 61% of patients were female, and the median age at diagnosis was 72 years.101 CAD following viral infections is more typically seen in children with transient disease.

Acute, transient CAD is most often due to *Mycoplasma pneumoniae* infection in adults and infectious mononucleosis in children. Chronic disease is typically associated with hematologic malignancies in older patients. In adults with chronic CAD and no obvious hematologic malignancy, flow cytometry can often detect an abnormal clone that may be responsible for the hemolysis and treatable by chemotherapy. Chronic disease does not usually occur in children.9

**Pathophysiology**

The red blood cells in CAD are typically coated with IgM. IgM binds to red cells at low temperatures, due to either the environmental cold or the relative decrease in temperature observed in the extremities. Once bound, IgM activates the classical pathway of complement, leading to the generation and binding of C3 to the red cell. If a critical amount of C3 is generated by complement activation, formation of the MAC may proceed, leading to red cell lysis and intravascular hemolysis. Hemolysis generally occurs if IgM is capable of remaining bound to the red cell and is immunologically active at both cool and warm temperatures, displaying broad thermal amplitude. If, on the other hand, the IgM has a narrow range of activity (or thermal amplitude), it has a smaller range of temperatures over which it can remain bound and activate complement. In this case, C3 will be formed and bound at cooler temperatures, and the amount of C3 generated may very well be below the amount needed to form the MAC.102,103 In that case, the C3-coated red cells circulate to the spleen and liver where macrophages recognize and bind C3, and they phagocytose the red cells via extravascular hemolysis. Simultaneously, complement regulatory proteins work to inactivate C3 bound to red cells. Those red cells whose bound complement has been inactivated will still circulate through the spleen and liver, although the spleen will not recognize the inactive bound complement. The liver will recognize the inactive bound complement and sequester these red cells, frequently allowing them to return to the circulation after a period of time. Thus, when hemolysis in CAD occurs by way of the reticuloendothelial system, it is generally at a decreased pace and lesser degree, as compared to intravascular hemolysis. Extravascular hemolysis is the more common pathway in CAD. Unlike extravascular hemolysis in warm AIHA, in CAD, it more commonly occurs in the liver.10

Although the pathways of intravascular and extravascular hemolysis in IgM-mediated CAD are discussed as discrete entities for the purpose of understanding, these pathways are often not discrete. The percentage of hemolysis accounted for by each mechanism will depend on the titer of IgM, thermal amplitude of reactivity, and resultant number of red cells with sufficient complement activation for MAC formation.

**Clinical features**

As with other types of AIHA, patients with CAD present with symptoms of progressive anemia.104 Given the pathophysiology, it is not surprising that there is an association between the thermal amplitude of the IgM autoantibody and severity of symptoms. Those patients having autoantibodies with a high thermal amplitude, immunologically reactive at higher temperatures, are more likely to have severe symptoms of hemolysis.105 Those with low thermal amplitude, the more common occurrence, typically demonstrate an indolent course with a slower hemolytic rate than warm AIHA.106 In either case, the anemia can be exacerbated by exposure to cold temperatures, such as during the winter or in a cold operating room, with acute hemolytic crises accompanied by frank hemoglobinuria. In addition, as with warm AIHA, the quantity of IgM, as measured by titration, is associated with the degree of hemolysis and observable symptoms. A higher titer autoantibody is more likely to be clinically significant.

On physical examination, these patients demonstrate pallor and jaundice, consistent with chronic anemia. Acrocyanosis of the tip of the nose, ears, fingers, and toes is common101 and is resolved with warming.104 Unlike, warm AIHA, hepatosplenomegaly is not a major feature.101 In patients with secondary CAD due to an underlying process, whether it be a hematopoietic malignancy or infection, the symptoms associated with the underlying disease may predominate.
Diagnosis

Laboratory features
The first noticeable laboratory finding may actually be observed at the time of collection of a blood sample. The blood sample will demonstrate progressive agglutination as it cools from body to room temperature. The agglutination is reversible by warming the sample. On the wards, this phenomenon can be observed by holding the sample in your hands for a period of time. If the specimen cools prior to receipt in the lab or throughout the process of automated testing, spurious results such as macrocytosis may be encountered.106 These spurious results frequently provide the first suggestion of this diagnosis. In order to avoid preanalytical testing problems, it is often necessary to maintain blood samples at 37 °C, most simply achieved by expeditiously collecting the blood sample and promptly placing it in a warm location (such as the transporter’s axilla) for transport to the laboratory. Routine hematologic evaluation will reveal anemia with decreased hemoglobin and hematocrit. The peripheral blood smear will show agglutination with formation of irregular aggregates or clumps of red cells.

Serology
In CAD, a cold reactive, IgM antibody will be demonstrated by serology. The DAT will be negative with monospecific IgG reagent, but will be positive with C3 reagent (Table 12.2). Eluate studies are typically not performed, as they are not indicated in the setting of negative DAT results with anti-IgG. Instead, the IgM antibodies are further investigated by performing cold agglutinin titters and thermal amplitude measurement. Thermal amplitude measurement is often done in the presence of 30% bovine albumin as this medium enhances agglutination. These tests are important because higher titters and thermal amplitude are more likely to be associated with clinically apparent disease. Clinical CAD is most commonly seen in patients with cold autoantibodies having a titer >1000 and/or serum studies using 30% bovine albumin showing reactivity at 30 °C.107 Because healthy individuals may often have low-titer cold autoantibodies, it is important to avoid misidentifying a patient with another cause for anemia as CAD based upon low-titer antibodies.

Cold panels, testing the patient’s serum against cord blood cells, which mainly demonstrate the i antigen, and adult red blood cells, which mainly demonstrate I antigen, can be used to determine if the autoantibody demonstrates specificity. The most frequent antibody specificities are anti-i or anti-I.108 The finding of anti-i is often associated with infectious mononucleosis, whereas anti-I is typically associated with Mycoplasma pneumoniae infections. Other antigen specificities such as Pr have also been demonstrated, albeit rarely.109

Differential diagnosis
As with warm AIHA, other etiologies of anemia should be considered and excluded. Nonimmune causes of hemolysis, such as microangiopathic hemolysis and mechanical hemolysis, should also be excluded. Once an immune-mediated process for the hemolysis has been established, alloimmune antibodies (especially in the recently transfused patient) must be ruled out. For an autoimmune process, serologic evaluation will determine the immunoglobulin class of the causative antibody, the temperature of optimal activity, and the thermal amplitude and titer in an effort to determine if the autoantibodies are capable of causing clinically significant disease.

Positive cold agglutinins in patients without hemolytic anemia
Cold reactive autoantibodies are commonly found in normal individuals. As with warm AIHA, the serologic findings in suspected CAD should always be correlated with clinical presentation, so as not to overstate their importance. Cold reactive autoantibodies in the absence of hemolysis are not diagnostic of CAD.

Treatment

Avoidance of cold
The mainstay of therapy for CAD is avoidance of the cold. Many patients with mild, chronic anemia are able to use this simple tactic to avoid transfusions for prolonged periods of time. Warming techniques during hypothermic surgeries should also be considered.110

Rituximab
Rituximab has been used with encouraging results in patients without an underlying hematologic malignancy.111 Response rates as high as 79–83% have been demonstrated, although response rates in warm AIHA are still better. The role of prednisone in conjunction with rituximab is unclear at this time.101,112–118

Cytotoxic agents
Chlorambucil has been used with some success, but is associated with the side effect of bone marrow suppression.68 Chemo- therapeutic agents may be useful even in idiopathic cases where a malignant clone can be identified by hematologic analyses.

Other therapies
Therapeutic plasma exchange may be used as a temporizing measure, such as prior to surgery or during severe hemolysis, but the body temperature must be maintained at 37 °C and the use of an inline blood warmer is recommended. The IgM antibodies in CAD have a larger intravascular volume and are more efficiently removed by apheresis than the IgG antibodies causing warm AIHA. Based predominantly on evidence from case reports, the American Society for Apheresis has deemed severe CAD as a category II indication for plasma exchange, indicating that apheresis is an acceptable second-line therapy.83

Other treatments such as IVIG,39 interferon alpha,119 erythropoietin, splenectomy, and glucocorticoids103 are rarely useful in patients with CAD.

Transfusion management
Supportive care of patients with CAD occasionally includes transfusion. Transfusion may be episodic for those with well-controlled, mild, chronic disease. It may be urgent for those presenting with an acute exacerbation due to cold exposure.

As with warm AIHA, the serology and therefore provision of appropriate blood products may be complex. ABO typing in patients with CAD can be difficult. This test relies on the appearance of red cell agglutination as a positive result. Because the blood from patients with this disease agglutinates as the temperature declines and sometimes even at room temperature, the cold agglutinins can interfere with interpretation of this test. Warm washing of the red cells to remove the IgM autoantibody may be utilized to facilitate accurate ABO typing. Group O red cells can be transfused if ABO typing cannot be determined. For patients with a positive antibody screen, the distinction between a cold agglutinin reactive at room temperature and possible underlying alloantibodies must be made.

Use of a prewarming technique, where the patient sample is warmed
before being tested against an antibody panel of commercially available red cells, may prove useful. One can attempt to determine the specificity of the autoantibody; often, the antibody has specificity against the I antigen. It is not considered necessary to transfuse blood that is negative for the I antigen. Transfusion with the use of a blood warmer is typically recommended, although not proven to be required.

**Prognosis**
The prognosis for CAD is better than for warm AIHA. The majority of patients have CAD as a transient problem following a viral infection and subsequently recover without recurrences. Other patients have a chronic, indolent course. There are rare reports of severe and even fatal CAD in the historic literature. Although these cases did demonstrate an IgM autoantibody, the autoantibodies in these cases had unusually high thermal amplitudes.

**Paroxysmal cold hemoglobinuria**
**Epidemiology and risk factors**
PCH is uncommon, accounting for 2% of the AIHAs. It is most commonly seen in children. When syphilis was more common, PCH was also seen in adults. Historically, PCH was seen in association with tertiary syphilis infection. As the incidence of syphilis declined, so too did the incidence of PCH. At present, PCH occurs most often in association with viral infections; the prototypical presentation is a child with a recent upper respiratory infection.

**Pathophysiology**
In PCH, the responsible autoantibody is an unusual IgG, called a biphasic hemolysin or Donath–Landsteiner antibody. This antibody binds to red cells at cool temperatures and in the process reversibly binds complement. At warmer temperatures, the antibody no longer stays bound. However, complement remains bound and becomes activated, leading to formation of the MAC resulting in intravascular hemolysis.

**Clinical features**
The typical presentation is a child with a recent history of upper respiratory tract infection. They may present with the dramatic appearance of hemoglobin in the urine. In addition to hemoglobinuria, jaundice, pallor, and fever may also be seen. Hepatosplenomegaly is not a prominent feature. A history of cold exposure is usually not obtained.

**Diagnosis**

**Laboratory findings**
A positive DAT and positive P test may be suggestive of PCH. The diagnosis is confirmed by the Donath–Landsteiner test (Table 12.2). A special test, called the Donath–Landsteiner test, must be undertaken to demonstrate the biphasic nature of this antibody. In this test, the patient’s serum is combined with normal serum as a fresh source of complement. Test red cells expressing the P antigen are then combined with the patient’s serum. A positive test is one in which the mixture is subjected to the cold and then subsequently warmed to 37°C. If hemolysis occurs, the test is positive, indicating the diagnosis of PCH.

**Differential diagnosis**
Given the rather dramatic presentation of intravascular hemolysis, the differential diagnosis should focus on excluding other causes of severe, acute intravascular hemolysis.

**Treatment**
Although the presentation is dramatic, the majority of cases of PCH are self-limited following a viral infection. Therefore, patients require supportive care during their acute illness, including blood transfusion. Corticosteroids are frequently used; however, their utility has not been established.

**Transfusion management**
Since the disease is typically self-limited, transfusion is commonly only required in the acute phase. Selection of blood appropriate for transfusion is aided by the fact that the causative biphasic autoantibody does not interfere with compatibility testing. The antibody itself does not bind red cells at temperatures above 4°C and is therefore unable to exert its effects during routine testing.

Because the autoantibody usually demonstrates specificity for the P antigen, some advocate the use of red cell components negative for this antigen. However, donors with the p phenotype are uncommon and, consequently, these units are typically only available through rare donor registries. The provision of units with p phenotype is unlikely under urgent circumstances. Furthermore, it is more important to replace blood expeditiously in these patients than to obtain P antigen–negative blood. We recommend the selective use of leukoreduced red cells to prevent febrile, nonhemolytic transfusion reactions, because these reactions would complicate an already complex clinical situation. It is unclear if the use of a blood warmer for transfusion is necessary, as the causative antibody is not reactive in compatibility testing greater than 4°C. However, their use may be reassuring, especially for clinicians dealing with life-threatening anemia in a child.

**Prognosis**
The prognosis is excellent, with the disease typically following a self-limited course over a few days to weeks. The greatest risk to patients is in the acute phase when they may present with severe anemia and require urgent transfusion and supportive treatment.

**Other types of autoimmune hemolytic anemias**

**Autoimmune hemolytic anemia due to warm IgM antibodies**

**Epidemiology and risk factors**
AIHA due to warm IgM antibodies is rare. Most reported cases occur in adults. However, a few cases in children have been reported.
Pathophysiology
The causative autoantibody is an IgM antibody capable of coating red cells at body temperature. IgM is efficient at activating complement and causing intravascular hemolysis, via formation of the MAC. In addition, IgM may simultaneously bind to several red cells, causing them to link. These agglutinates of red cells linked by antibody may cause sludging and reduce perfusion to organs, leading to end-organ ischemia and tissue necrosis.

Clinical features
The classic presentation of AIHA due to warm IgM antibodies is intravascular hemolysis accompanied by tissue ischemia and/or infarcts. Cutaneous infarcts are manifested by visible gangrene. Ischemic infarcts elsewhere are frequently discovered by radiologic studies. Ischemia can be widespread, involving the brain, heart, and kidneys. These severely ill patients can therefore develop multi-organ failure.

Diagnosis
Laboratory findings
Laboratory results are often spurious due to the agglutination of samples caused by in vitro red cell linkage. When laboratory evaluations are possible, findings include anemia, hyperbilirubinemia, elevated lactate dehydrogenase, hemoglobinemia, hemoglobinuria, and decreased haptoglobin.

Serology
Serologic studies demonstrate spontaneous agglutination that cannot be resolved with warming of the sample. Even repeated warm washes will not disperse the agglutination. The diagnostic finding is an IgM autoantibody with high thermal amplitude. Treatment with dithiothreitol (DTT) or 2-mercaptoethanol (2-ME) may be used to disrupt the IgM autoagglutinin in order to perform further serologic testing.

Treatment
The goal of therapy is to decrease in vivo red cell agglutination by the rapid reduction of antibody. Whole blood and plasma exchange have been used successfully as temporizing measures. Despite immunosuppressive drugs, cytotoxic agents, and exchange protocols, successful long-term treatment approaches have not been identified and the mortality rate is high.

Prognosis
Autoimmune hemolytic anemia due to IgM autoantibodies is virtually universally fatal.124

Drug-induced immune hemolytic anemia
Epidemiology and risk factors
Drug-induced immune hemolytic anemia can occur in any age group; it may occur in association with a wide variety of medications, including prescription drugs, over-the-counter medications, and toxin exposures. The most commonly implicated drug class in current practice is the cephalosporins.129 In the largest series of drug-induced immune hemolytic anemias, the odds ratio for developing immune hemolytic anemia was significantly increased with cotrimoxazole (trimethoprim/sulfamethoxazole), fludarabine, lorazepam, and diclofenac.130

Pathophysiology
Three different mechanisms have been proposed for drug-induced immune hemolytic anemia, including the drug adsorption mechanism, immune complex mechanism, and autoimmune induction mechanism.

In the drug adsorption mechanism, the drug attaches to the surface of the red blood cell. The resultant antibody formed is directed against the drug. The antibody binds to the drug, which remains bound to the red cell. The antibody–drug coated red cell is removed, typically via extravascular hemolysis. The prototypic drug associated with this mechanism is penicillin, particularly with very high doses.

In the immune complex mechanism, circulating drug stimulates the immune system to produce antibody directed against the drug. The antibody binds to the circulating drug, and the resulting immune complex can then bind to a red cell. It is unknown whether binding to the red cell is specific or nonspecific. The red cells, coated by antibody–drug complexes, are typically removed via brisk, intravascular hemolysis. The prototypic drug for this mechanism is quinidine.

In the autoimmune induction mechanism, the drug induces autoantibody formation. The resulting immune-mediated hemolytic anemia is serologically and clinically indistinguishable from warm AIHA. The autoantibody may persist even long after the offending drug has been discontinued. The prototypic drug demonstrating this mechanism of immune hemolytic anemia is o-methyldopa, a medication commonly used to treat hypertension in the past.

Although explanation of these mechanisms as discrete entities is helpful to understand the many ways in which drugs, antibodies, and red cells can interact, there is little laboratory evidence of these purported mechanisms. In addition, some patients have laboratory findings that overlap with more than one mechanism. A unifying theory was therefore proposed by Garratty, which argues that the antibodies against the drug itself, against the red cell membrane or components of both, could be present simultaneously (Figure 12.1). The specificity of the formed antibodies depends on the site of interaction between the drug and red cell. The unifying theory explains how patients may present with clinical evidence of multiple simultaneous pathophysiology of drug-induced immune hemolytic anemia.129,131

Clinical features
The clinical presentation of drug-induced immune hemolytic anemia can be broad, ranging from mild anemia to severe hemolysis with life-threatening anemia. There may be hemoglobinemia and hemoglobinuria as well as renal failure. As previously described, the hemolysis produced is frequently indistinguishable from warm AIHA.

Warm and cold, or mixed AIHA
In addition to the outlined distinct cases of warm versus cold AIHA, some patients present with elements of both types of AIHA. There may be a predominance of one type over another, but laboratory and clinical findings ultimately overlap. Mixed AIHA comprises approximately 7% of all cases of idiopathic AIHA.128 Of note, this entity typically responds to treatments appropriate for warm AIHA; these patients frequently have a rapid response to corticosteroid therapy. Consequently, it is of the utmost importance to identify the warm autoantibody component; misclassification of mixed AIHA as CAD could delay appropriate therapy.
should be kept in mind that the majority of these drugs are implicated in case reports. A convincing clinical picture should prompt discontinuation of the drug, even if it has not previously been associated with hemolytic anemia.

**Treatment**

Treatment is aimed mainly at discontinuing the causative drug. Corticosteroids are frequently given, although there are only empirical data to support their use.

**Transfusion management**

Transfusion support may be necessary for patients presenting with massive intravascular hemolysis. The hemolysis may be so great as to cause renal failure, requiring supportive therapies. This severe presentation is typically associated with patients demonstrating IgG, IgM, or a combination of the two and complement on their red cells, presumed secondary to the immune complex mechanism of drug-induced immune hemolytic anemia. In the case of hemolysis persisting even after discontinuation of the offending drug, as is presumed to be due to the autoimmune induction mechanism, a prolonged period of transfusion support may be required.9

**Prognosis**

The prognosis is excellent for this form of AIHA; full resolution of hemolysis is expected. The offending drug or toxin must be avoided indefinitely.

**Paroxysmal nocturnal hemoglobinuria**

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare type of hemolytic anemia that can present with thrombosis and bone marrow dysfunction.136 It is a clonal disorder of hematopoietic stem cells with its biochemical pathogenesis based in a somatic gene mutation.137 The somatic mutation ultimately decreases availability of an enzyme required for synthesis of the anchor molecule, glycosylphosphatidylinositol (GPI), responsible for binding numerous proteins to the external surface of red blood cells. Two of the affected proteins, which are responsible for the majority of symptoms seen in PNH, are the complement-regulatory proteins CD55 and CD59. Without GPI binding of CD55 and CD59 to the cell surface, the red cells lack their normal defenses against random complement activation and have increased sensitivity to complement-mediated lysis.

**Epidemiology and risk factors**

PNH is rare, occurring at a rate of approximately 1–10 cases per million people.138,139 It has been diagnosed in patients of all ages.140,141 The median age at diagnosis is early thirties.141–143 Pediatric cases account for only 10% of reported cases of PNH.144,145 No apparent gender predilection has been identified.142

Although PNH can occur de novo, virtually all patients demonstrate some degree of bone marrow failure.136 Likewise, red cells or granulocytes deficient in GPI-linked molecules, such as CD55 and CD59, are identified in a large number of patients with bone marrow disorders such as aplastic anemia and, to a lesser extent, low-risk myelodysplastic syndrome (MDS).142,146 The leading hypothesis for this close association is that there is a selective growth advantage for PNH clones in these disorders; however, the exact etiology of PNH clones in these patients remains unclear.146–149 Additionally, the finding of PNH clones may not
always be clinically significant. In aplastic anemia and MDS, PNH clones are so commonly found that screening for undetected clones is recommended in patients with these disorders. In most cases, the percentage of abnormal cells is so small that clinical symptoms are not observed and therapy for PNH is not indicated. Furthermore, the stem cell mutations underlying PNH can be found at a low frequency in healthy controls. Therefore, many questions remain unanswered, including what leads to multipotent stem cell mutations, why the clones sometimes expand to a high enough percentage to cause clinical disease, and why bone marrow failure exists in virtually all cases.

Although paroxysms of hemolysis may occur without preceding risk factors, it is generally agreed that factors leading to complement activation can trigger hemolytic episodes. Infection, trauma, pregnancy, and surgery are examples of known triggers of complement activation that may form the background upon which a PNH paroxysm occurs.

Pathophysiology

PNH is a clonal disorder of multipotent hematopoietic stem cells that ultimately causes increased sensitivity to complement in its progeny cells. In virtually all cases, a somatic mutation in the X-linked phosphatidylinositol glycan class A (PIG-A) gene underlies the disorder. The PIG-A gene encodes for the PIG-A enzyme, which is needed in the first step of biosynthesis of the GPI anchor molecule. GPI anchor molecules are needed to bind numerous proteins to the external surface of hematopoietic cells. The majority of symptoms seen in PNH are due to the lack of binding of two of the affected proteins, CD55 and CD59. These complement-regulatory proteins are normally needed on the external surface in order to neutralize complement activation. CD55 accelerates the destruction of C3 convertase, reducing the activation of complement component C3 and its effect of leading to extravascular hemolysis by macrophages in the spleen and liver. CD59 prevents the MAC from completing its action of pore production in the lipid bilayer of the cell and its effect of cell lysis through hypertonic cell swelling. In brief, the PIG-A mutation leads to decreased or absent GPI molecules, which, in turn, results in decreased or absent binding of proteins such as CD55 and CD59 to the cell surface and, ultimately, increased cell sensitivity to complement.

Two conditions must be met in order for the mutation to cause clinical symptoms: it must occur in a multipotent hematopoietic stem cell, and the stem cell carrying the mutation must undergo clonal expansion. Evidence for the former requirement is that rare, circulating blood cells with PNH mutations can be seen in healthy blood donors and low-frequency PNH clones in MDS rarely, if ever, lead to clinical disease. Mutations in these cases appear to arise in hematopoietic cells without self-renewal capacity. If the mutation occurs in a multipotent hematopoietic stem cell, it will result in hematopoietic progeny (red cells, platelets, and white blood cells) that also harbor the mutation. Patients with aplastic anemia, unlike those with MDS, more commonly develop PNH because mutations in these patients arise from multipotent hematopoietic stem cells. Yet it is not enough that the mutations occur in a cell capable of creating progeny in all hematopoietic lineages. Low-frequency mutations do not cause clinical disease. On the contrary, patients with greater than 20–25% of their neutrophils and greater than 3–5% of their red cells lacking CD55 and CD59 surface proteins are more likely to demonstrate clinical signs of hemolysis and require specific treatment than those with lower values. Likewise, patients with a higher proportion of PNH neutrophils (and monocytes) are more likely to demonstrate thrombosis, with neutrophil clone sizes greater than 50% being highly predictive of thrombotic risk. Although there are several purported hypotheses, the mechanism by which PNH cells can achieve clonal expansion remains unknown.

The ultimate clinical effect of having a sizeable PNH cell population depends on the type of surface protein and cell affected. Affected red blood cells show decreased or absent CD59 and, as a result, are exquisitely sensitive to intravascular hemolysis from random complement activation. They also show decreased or absent CD55, causing them to be incapable of reducing C3 activation and making them vulnerable to extravascular hemolysis in the spleen and liver. On the other hand, the increased sensitivity to complement resulting from lack of CD55 and CD59 in platelets results in their inappropriate activation and an increased risk of thrombosis. In affected leukocytes, absence of a receptor for urokinase-type plasminogen activator reduces the potential of these cells to convert plasminogen to plasmin. The decreased formation of plasmin, which has a significant role in fibrinolysis, may also lead to increased risk of thrombosis. Although all patients with PNH are at risk for thrombosis, there is increased risk with larger PNH clone size. When thrombosis occurs in PNH patients, it often does so in atypical locations such as the portal circulation, although the reason is unclear.

Secondary effects of sizeable PNH clonal populations are also seen. Increased intravascular hemolysis leads to large amounts of free hemoglobin, which overwhelm the normal mechanisms of clearance, such as binding by haptoglobin. The circulating free hemoglobin in plasma then scavenges and reacts with nitric oxide. The depletion of nitric oxide is thought by some to contribute to arterial constriction, decreased blood flow to organs, kidney damage, and pulmonary hypertension. However, others believe that chronic kidney disease is due to tubular damage caused by microvascular thrombosis and iron deposition from hemolysis. Microthrombi may also contribute to pulmonary hypertension. Decreased nitric oxide may also lead to smooth muscle dystonia manifested as chronic dysphagia, abdominal pain, and erectile dysfunction.

In patients with PNH clones and clinical hemolysis, the hemolysis typically occurs at a chronic baseline rate punctuated by episodic periods of an increased hemolytic rate. The pathophysiology of these episodic exacerbations of hemolysis is thought to be due to enhanced complement activation in certain situations; infection, trauma, pregnancy, surgery, strenuous physical activity, and alcohol use are examples of known triggers. Additionally, repletion of iron in a deficient patient is a known trigger of PNH exacerbations; it is thought to cause increased hemolysis by increasing the number of PNH clones. Both enhanced complement activation and increased PNH clone size may form the background upon which a PNH paroxysm occurs.

Finally, although the PIG-A mutation described is prototypical for and present in the vast majority of PNH cases, other mutations have been described.

Clinical features

PNH classically presents with the triad of hemolytic anemia, thrombosis, and bone marrow dysfunction. Because the clinical
findings are variable, three clinical categories of PNH have been described. In classical PNH, patients frequently have >50% PNH granulocytes, and episodic hemolytic anemia accounts for the majority of the clinical picture. These patients may present with fatigue, jaundice, and a history of dark urine, consistent with hemoglobinuria. The hemoglobinuria may be nocturnal, as implied by the disease’s moniker; however, it may also occur at other times. These patients are also the most likely to develop thrombosis.\textsuperscript{136}

The second clinical category of PNH is clinically apparent disease occurring in the context of bone marrow disorders, most frequently aplastic anemia. In the context of an underlying bone marrow disorder, patients with PNH also present with symptoms of anemia, which is more likely due to bone marrow failure; they are less likely to demonstrate symptoms of hemolysis (jaundice, dark urine). They often have low platelet counts and a lower risk of thrombosis. The third category is subclinical PNH. By definition, these patients are asymptomatic,\textsuperscript{136} and typically have less than 10% PNH red blood cells or granulocytes in their peripheral blood.\textsuperscript{136,150} The discrete definition of this disease into three clinical categories may be useful for understanding the variable clinical presentations, but it is not perfect. A particular point of confusion not addressed by these categories is that some element of bone marrow failure underlies virtually all PNH cases.

As mentioned above, patients with PNH clones and clinical hemolysis experience a chronic baseline rate of hemolysis punctuated by episodes of increased hemolysis. Infection, trauma, pregnancy, surgery, strenuous physical activity, alcohol use, and iron replacement in deficient patients are known triggers\textsuperscript{154,176} of hemolytic paroxysms in PNH.

Thrombosis is rarely the presenting symptom of PNH,\textsuperscript{183} but it is common over the course of the disease and occurs in up to 40% of patients.\textsuperscript{179} Additionally, thrombi are the most common life-threatening complication in PNH.\textsuperscript{159,177} The thromboses in PNH patients are often in atypical locations, most commonly affecting abdominal and cerebral vasculature, with veins more often affected than arteries. Commonly affected abdominal veins include the hepatic, portal, mesenteric, and splenic veins. The sagittal and cavernous sinuses are the most frequently affected cerebral vasculature. Overall, the most common site of thrombosis in these patients is the hepatic veins;\textsuperscript{136} thrombi in this location cause a decrease in the normal flow of blood out of the liver, a process termed Budd-Chiari syndrome.\textsuperscript{184,185} Despite frequently presenting in these atypical locations, thrombosis may occur in any site, and deep venous thrombosis, pulmonary emboli, and dermal thrombi are relatively common.\textsuperscript{136} When thrombosis does occur, it tends to be progressive despite anticoagulant therapy.\textsuperscript{159,177}

Patients with PNH may also present with symptoms of increased smooth muscle tone, manifested by chronic dysphagia, abdominal pain, and erectile dysfunction.\textsuperscript{178} Over time, these patients may develop renal insufficiency and pulmonary hypertension. There is greater than a sixfold increase in risk of developing chronic kidney disease in PNH patients.\textsuperscript{175} Although clinically significant pulmonary hypertension in PNH is rare, testing for terminal pro-brain natriuretic peptide\textsuperscript{186} and transthoracic Doppler echocardiography\textsuperscript{187} indicates that pulmonary hypertension is present in approximately half of these patients.

Most patients have a chronic illness that persists without therapy; interestingly, a small subset of patients may achieve spontaneous, long-term remission. A recent study found that 15% of patients had a spontaneous, sustained remission.\textsuperscript{140}

### Diagnosis

#### Laboratory findings

With the variable clinical presentation of PNH, the associated laboratory findings vary as well. In classical PNH, which includes patients with intravascular hemolysis and the highest risk of thrombosis, one can expect to find anemia, reticulocytosis, elevated lactate dehydrogenase,\textsuperscript{137} elevated bilirubin, and decreased haptoglobin. Bone marrow evaluation demonstrates an overall hypercellular marrow with erythroid hyperplasia but no karyotypic abnormalities.\textsuperscript{150,188} Patients with PNH in the context of an underlying bone marrow disorder also have anemia, but their laboratory results are predominated by the effects of bone marrow failure more so than intravascular hemolysis. These patients have severe thrombocytopenia, normal or only mildly elevated lactate dehydrogenase,\textsuperscript{136} normal or only mildly elevated bilirubin,\textsuperscript{189} and lower levels of reticulocytes than patients with classical PNH.\textsuperscript{136} Patients with subclinical PNH have normal or only modestly aberrant blood counts.\textsuperscript{136}

Peripheral blood smear findings are variable and include a broad array of anisocytosis and poikilocytosis.\textsuperscript{190}

#### Serology

In PNH, the DAT is classically negative.\textsuperscript{183} Patients undergoing eculizumab therapy may have a positive DAT; this therapy prevents formation of the MAC and intravascular lysis, but it does not prevent C3 coating of the red cells.\textsuperscript{191–193}

#### Confirmatory tests

**Acidified serum test (HAM test)**

The earliest confirmatory test for PNH was the acidified serum test, or HAM test (named after its developer, Thomas Ham).\textsuperscript{194} In this assay, red cells are combined with acidified serum. PNH red cells are more sensitive to complement and more likely to undergo lysis in the presence of acid. Spectrophotometry is utilized to quantify the presence of free hemoglobin liberated from hemolysis. This test is easy to perform, inexpensive, and reliable, but it cannot quantify the number of PNH cells present.\textsuperscript{195} A positive test is not specific for PNH and may be seen in other disorders leading to enhanced sensitivity to complement (such as congenital dyserythropoietic anemia type II [HEMPAS] where hemolysis is enacted by an anti-HEMPAS antibody that binds complemet).

#### Flow cytometry

Because of the sensitivity and specificity of flow cytometry for detection of PNH cells, it has become the gold standard. Classically, flow cytometry of the peripheral blood is aimed at detecting the marked reduction or absence of the GPI-anchored proteins, CD55 and CD59, on red cell or granulocyte surfaces. Monoclonal antibodies that bind each of these GPI-anchored proteins are tagged with a fluorescent label and combined with a patient sample, allowing the detection of cells capable of binding the antibody. This analysis provides useful information regarding the quantity of cells lacking or deficient in CD55 and CD59 as well as the strength of expression of these proteins on the cell surface.\textsuperscript{195} In normal patients, all red cells and granulocytes demonstrate strong expression of CD55 and CD59. In patients with PNH, a variable proportion of red cells and/or granulocytes lacking CD55 and CD59 will be demonstrated in addition to a population of cells with strong expression of these surface proteins.\textsuperscript{195} Flow cytometry of monocytes and granulocytes may be more accurate, as their numbers are
not affected by recent red blood cell transfusions or hemolysis. Lymphocytes show variable expression of GPI-anchored proteins and are therefore undesirable for use in PNH diagnosis. Ideally, PNH should be confirmed with findings of decreased or absent GPI-anchor proteins in two or more cell lines.

The newest flow cytometry test, called fluorescent aerolysin (FLAER), is also aimed at detecting a marked reduction or absence of GPI-anchored proteins. The concept underlying FLAER is that GPI-anchored proteins bind a bacterial toxin called aerolysin. PNH cells lack GPI-anchored proteins and therefore lack the ability to bind the aerolysin toxin. By tagging the aerolysin toxin with a fluorescent label, the amount of cells capable of binding the toxin and the strength of binding can be quantified. The sensitivity of FLAER is much higher than that achieved with a CD55- and CD59-based assay. The detection rate is further improved when the FLAER reagent is combined with fluorescent labeled antibodies for GPI-anchored proteins on granulocytes and monocytes. Red cells are not commonly included in analysis with a FLAER assay as they express glycophorin, which is capable to weakly bind aerolysin and thus affecting assay accuracy. Peripheral blood flow cytometric analysis is preferred to that of bone marrow for routine analysis; it shows no diagnostic disadvantage and represents an analysis of cells that are more homogeneous with respect to stage of maturation and expression of GPI-anchor proteins.

**Differential diagnosis**
The differential diagnosis includes other causes of anemia. If hemoglobinuria or other signs of intravascular hemolysis are present, other causes of hemolytic anemia must be ruled out, such as G6PD deficiency and PCH.

**Treatment**
In treating PNH, the choice of therapy (or therapies) depends on the type of symptoms the patient is experiencing (hemolysis versus thrombosis) as well as the degree of anemia. Indeed, therapy does not need to be initiated in an asymptomatic patient found to have PNH clones; these patients can be actively monitored for development of symptoms.

**Anticoagulants and thrombolytic agents**
Historically, anticoagulants represented one of the mainstays of therapy in PNH. Use of prophylactic anticoagulants in patients with PNH neutrophil clones greater than 50%, in the absence of contraindications, has been recommended. However, this recommendation has been called into question, particularly in the current era of eculizumab therapy. Problems with anticoagulant therapy include their lack of complete efficacy in preventing thrombotic events; in fact, the risk of thromboemboli remains high even with prophylaxis. Additionally, anticoagulants increase the risk of bleeding, which may be compounded when patients also have thrombocytopenia. Some experts recommend against routine prophylactic use of anticoagulants; they are currently used in only 25% of patients with no history of thrombosis. On the contrary, patients with other underlying risks for thrombosis such as pregnant patients or patients undergoing a recent surgery should still be considered for prophylactic anticoagulation. Even in patients with a history of thrombosis, anticoagulants have lacked success. In patients receiving eculizumab, thrombotic event rates decline independent of prior or concurrent anticoagulant therapy, making eculizumab the recommended therapy for the majority of patients with PNH and thrombosis. Despite these data, the discontinuation of anticoagulants in patients undergoing eculizumab is controversial, and anticoagulants are still used in 70% of patients with PNH and a history of thrombotic event.

Particular attention should be paid to clone size and symptoms of abdominal or chest pain. The risk of thrombotic events has been shown to correspond with clone size. In addition, patients with abdominal or chest pain have a greater risk of thrombosis. Thrombolytic therapy with tissue plasminogen activator may be life-saving in severe thrombus formation, such as Budd-Chiari syndrome.

**Eculizumab**
Eculizumab is a monoclonal IgG antibody that binds to the C5 complement protein, thereby inhibiting formation of the MAC and reducing intravascular hemolysis of red blood cells in patients with PNH. It was approved by the FDA for use in reduction of hemolysis in PNH in 2007. It has been shown to be efficacious in reducing red cell transfusions, lactate dehydrogenase levels, nitric oxide depletion, abdominal pain, pulmonary hypertension, and surgery-triggered hemolysis. Eculizumab also leads to improvement in time-dependent renal function and quality-of-life scores. Survival rates are significantly increased both in the short term and long term. The reduction of lactate dehydrogenase level is sustained over the course of treatment. The drug is not curative, and therapy must be continued for life. Specific guidelines for use are still being determined. Some suggest eculizumab for patients with symptoms of hemolysis that are not managed by transfusion alone; others use eculizumab in patients with fatigue that affects quality of life, transfusion dependence, thrombosis, frequent symptoms associated with smooth muscle dystonia (dysphagia, abdominal pain, or erectile dysfunction), renal insufficiency, or end-organ disease complications. Its use may be particularly indicated in the perioperative period or pregnant patient. See the “Special Clinical Situations” section.

Despite its successes, not all patients respond to eculizumab therapy, and some have persistent symptoms and chronic need for red cell transfusion. Some patients who fail to respond were found to have specific mutations of C5. Other patients experience continued extravascular hemolysis, due to continued accumulation of C3 on the surface of red blood cells. Further support of this mechanism is the observation of positive DATs with anti-C3 reagent in patients undergoing eculizumab treatment. Eculizumab does not treat underlying bone marrow failure.

Overall, eculizumab is well tolerated, with the occurrence of adverse events decreasing over time. There is no evidence for cumulative toxicity. Its use is associated with an increased risk of infection, particularly serious meningococcal infections. Meningococcal vaccine must be administered two weeks prior to treatment, patients’ vaccination status should be kept up to date per current medical guidelines, and they should be followed for signs of infection to expedite prompt antibiotic treatment. The most detrimental side effect of eculizumab is its extreme cost, averaging over $400,000 per year of treatment.
Hematopoietic cell transplantation

The only potentially curative therapy for PNH is hematopoietic cell transplantation. However, transplantation using either bone marrow or peripheral blood stem cells as the graft source,221 is associated with substantial morbidity and mortality,222–224 including high rates of rejection, side effects from the preparative regimen, and graft–versus-host disease.221 Hematopoietic cell transplantation is considered for patients with severe clinical symptoms who are unresponsive to eculizumab therapy or for whom eculizumab therapy is not an option due to cost or lack of availability.136 Using bone marrow as a source of stem cells may lead to a lower incidence of graft–versus-host disease, as compared to using peripheral blood.224 Nonmyeloablative conditioning regimens show promise for improved outcomes and reduced morbidity and mortality.221,225–228

Hematopoietic cell transplantation may also be indicated for treatment of the patient’s underlying bone marrow disorder, independent of PNH.

Immunosuppressive therapy

Patients with underlying bone marrow disorders, such as aplastic anemia, are more likely to receive immunosuppressive therapy with cyclosporine and/or antithymocyte globulin142 than other PNH patients. Immunosuppressive, but not bone marrow–suppressive, therapy is likely the most effective therapy in patients with underlying bone marrow disorders. Corticosteroids may reduce hemolysis and improve anemia in some patients, but they carry an added risk of long-term toxicity.188 In addition, others have reported little success with corticosteroid use in treating patients with underlying bone marrow disorders.190 and their use is therefore becoming less common.

Other therapies

Given that eculizumab is successful in most PNH patients but that some continue to have extravascular hemolysis due to C3 deposition, novel therapies targeting C3 and other components of the complement activation pathway are being tested in vitro and in animal models, with promising results.229–232

In patients with marked hemolysis, replacement of iron (for iron-deficient patients), B12, and folate to support production of new red blood cells may be indicated.

Special clinical situations

Complement activation is triggered by infection, trauma, pregnancy, and surgery.154,210 Therefore, the use of eculizumab may be particularly indicated in the perioperative period210,213 or for pregnant patients.214–217

Pregnancy

As previously discussed, pregnant patients with PNH frequently experience thrombosis. The risk of thrombosis in the setting of PNH is exacerbated by the hypercoagulability of pregnancy. Anticoagulant therapy may be an appropriate strategy to reduce morbidity and mortality during pregnancy. Eculizumab may also be useful and safe in pregnancy. The molecule is a hybrid IgG2 and IgG4, and IgG2 does not cross the placenta well.235 Only a handful of case reports on the use of eculizumab in pregnant PNH patients exist, and there is great heterogeneity in the use of therapy prior to pregnancy, time of discontinuation (either during or following pregnancy), and concomitant use of anticoagulant therapy.

Teratogenic effects of eculizumab have not been observed.214–216 Fetal outcomes to date have been good.214–217

Elective surgery

Few reports of perioperative eculizumab use suggest it may be helpful in preventing hemolytic paroxysms. One group reported successful cardiopulmonary bypass surgery in a PNH patient on maintenance therapy,213 whereas another reported prevention of hemolysis using perioperative induction with eculizumab for distal gastrectomy.210

Underlying bone marrow disorders

Asymptomatic patients with PNH clones do not need to be treated for PNH. Many of these patients have underlying bone marrow disorders, such as aplastic anemia or MDS, and therapy should be targeted toward those conditions instead of PNH.188,214

Children

PNH in children is rare; reports on their treatment are therefore also rare. Studies on the use of eculizumab in children have suggested its use is safe and effective.234

Transfusion management

Historically, blood transfusions have been a mainstay of therapy for PNH patients with moderate to severe hemolysis and anemia. They are still recommended for patients with severe, symptomatic anemia. Unlike with the warm and cold AIHAs, special testing and selection of specific red cells for transfusion are not required in PNH unless the patients develop a positive antibody screen. The red cell components do not typically need special modifications, such as washing or irradiation. However, leukoreduced red cell components should be selected to prevent febrile nonhemolytic transfusion reactions and alloimmunization, as would be indicated for any patient receiving chronic transfusions. Previous reports of the need to wash red cells have been discredited, because the hemolysis reported with unwashed cells was most likely due to minor incompatibilities. Platelet transfusion may also be given for a variety of clinical indications, including treatment of sequelae in patients with thrombocytopenia undergoing anticoagulant and/or thrombolytic therapy. Although not required based on the sole indication of PNH, blood product modifications may be necessary because of the patient’s associated conditions or therapy, such as hematopoietic cell transplantation.

Prognosis

The 10-year survival rate for PNH patients between 1940 and 1970 was 50%.140,143,178 More recently, the 10-year survival rate for PNH was 75%.235 A small subset of patients may achieve long-term remission. A recent study found that 15% of patients had a spontaneous, sustained remission.240

Key references

A full reference list for this chapter is available at: http://www.wiley.com/go/simon/
