Cold Hemolytic Syndrome

Morie A. Gertz

In most cases, immune-mediated hemolysis occurs extravascularly and is associated with IgG antibodies on the surface of red cells. Rare syndromes include IgG antibodies that cause direct intravascular hemolysis, such as paroxysmal cold hemoglobinuria. Also rare are extravascular hemolytic syndromes caused by IgM polyclonal or monoclonal antibodies that demonstrate red cell agglutination at 3°C, so-called cold antibodies. Because cold agglutinin disease has a high association with several lymphoproliferative disorders and IgM monoclonal gammopathies, its management differs significantly from that associated with warm autoimmune hemolytic anemia. This case-based presentation is designed to guide the reader to the diagnosis and to the initiation of prompt, effective therapy.

Paroxysmal Cold Hemoglobinuria

Paroxysmal cold hemoglobinuria was first recognized as a distinct clinical entity in 1872. Its association with syphilis followed, and Donath and Landsteiner described the hemolytic antibody in 1904. The Donath-Landsteiner antibody is an IgG antibody most commonly encountered in children after a viral illness or immunization. It generally is transient, but recurrent cases have been reported. The typical clinical presentation is that of a young child with a postviral syndrome who has “coca-cola” urine, a positive direct Coombs test, and a positive Donath-Landsteiner test. Supportive care and corticosteroid therapy generally lead to full recovery. The most sensitive way to perform a Donath-Landsteiner test is to use papainized pooled O-cells (papainization exposes more P-antigen sites on the cell membrane). Complement is added after the cells have been sensitized with Donath-Landsteiner antibody at 0°C and placed at 37°C. The detection of Donath-Landsteiner antibody is technically challenging. The direct Coombs test is positive but often weakly reactive and may be negative because the IgG that coats red blood cells elutes from the cells during their preparation. The Donath-Landsteiner test is positive only when the antibody titer in the serum is high. It is also important that complement be added to the serum to demonstrate the agglutination because complement may be depleted by fixation to the red cell membrane and by intravascular hemolysis. The causes of a false-negative Donath-Landsteiner test include 1) low level of the antibody from consumption during hemolysis, 2) low complement levels from consumption in the hemolytic process, and 3) the presence of globoside in the serum (even with the addition of complement); this can neutralize the Donath-Landsteiner antibody. The most sensitive way to perform the Donath-Landsteiner test is to use papainized pooled O-cells to expose more antigen sites on the cell membrane to the antibody. More complement is added only after the cells have been sensitized with the Donath-Landsteiner antibody at 0°C and then incubated at 37°C.

The antibody is always polyclonal and directed against the P-antigen on the red cell membrane. The antibody activates a complement cascade, causing true perforation of the red cell membrane and intravascular hemolysis. The antibody usually appears a week after the onset of an illness and can persist from 1 to 3 months. Hemoglobinuria, hemosiderinuria, and a decrease in serum haptoglobin are all seen. In children, the severity of the anemia can lead to sudden death. The Coombs test is positive when complement coats red cells. Therapy is usually expectant because the syndrome is self-limited. Corticosteroid therapy has been reported to be successful. In adults, the syndrome has been treated with cyclophosphamide. It is important to note that splenectomy has no role in treatment because the hemolysis does not occur extravascularly (Table 1).

Cold Agglutinin Hemolysis

Cold agglutinin hemolysis caused by monoclonal antibodies was first reported in 1957. These were the first monoclonal proteins shown to have antibody activity. Cold agglutinins frequently are found in low titer in the sera of normal adults. Christenson and colleagues showed that patients with high-titer cold agglutinins have a serum monoclonal band that can be removed by adsorbing the patients’ serum with red cells bearing the I or i antigen. Previous reports have indicated that immune hemolytic anemia with cold agglutinins occurs in 1:100,000 persons, with an age peak in the seventh decade of life, which corresponds to the decade with the highest prevalence of monoclonal proteins. Of the 34,633 patients at Mayo Clinic who had monoclonal gammopathies, 60 (0.17%) had cold agglutinin disease. However, these 60 patients represented 1.1% of the 5405 patients who had IgM monoclonal proteins.

The term “cold agglutinin” is misleading because it...
Table 1. Cold hemolytic syndromes.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Antibody</th>
<th>Coombs</th>
<th>Specificity</th>
<th>Course</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paroxysmal cold hemoglobinuria</td>
<td>Donath-Landsteiner IgG</td>
<td>±</td>
<td>BA P</td>
<td>Acute</td>
</tr>
<tr>
<td>Cold hemagglutinin disease</td>
<td>Monoclonal IgMk</td>
<td>+++</td>
<td>BA I</td>
<td>Chronic</td>
</tr>
<tr>
<td>Childhood cold agglutinin</td>
<td>Polyclonal IgM</td>
<td>+++</td>
<td>BA i</td>
<td>Self-limited</td>
</tr>
</tbody>
</table>

Abbreviations: BA, blood antigen

implies that the disease has a clear relation with cold exposure. In fact, the term is derived from the immunology of cold agglutinin disease. In warm antibody-mediated hemolytic anemia, agglutination is visible to the naked eye after the red cells are incubated with antihuman globulin antiserum. Recognition requires incubation with the antiglobulin antibody at 37°C for 2 hours; thus, the term “warm.” The use of the antiglobulin antiserum (Coombs reagent) is necessary because the electrostatic charge on the red cells causes them to be mutually repellant in solution, the so-called zeta potential. The bridging effect of the Coombs antibody binding to IgG molecules on the red cell surface leads to agglutination overcoming the electrostatic repulsive force. In 90% of patients, cold agglutinin disease is mediated by an IgM molecule, which has a molecular weight of nearly one million daltons (1000 kDa). With this size, the molecule can span the intercellular distance between red cells. Agglutination occurs at 4°C in the microrotter well without the use of any antiglobulin antiserum, thus the term “cold agglutination.”

In warm immune hemolytic anemia, IgG molecules cover the red cell surface, and pieces of the red cell membrane are removed sequentially after multiple passages through the spleen. With this sequential removal of membrane, the surface area of the red cell decreases, producing the classic spherocyte. In cold agglutinin disease, the IgM molecule fixes complement to the red cell surface; however, at core temperature, the IgM is not bound to the red cell surface. In spite of the presence of complement on the red cell surface, intravascular lysis is rare, differentiating cold agglutinin disease from paroxysmal cold hemoglobinuria in which the complement-fixing antibody (usually IgG) causes activation of the complement cascade and lysis of red cells. In cold agglutinin disease, the serum complement affixed to the red cell surface undergoes processing in a manner similar to the IgG antibody–coated red cells of warm hemolytic disease. The cell membrane of red cells is removed in successive passages through the mononuclear phagocyte system, leading to the cells’ ultimate destruction. A hemolysis-resistant population of C3d-coated red cells also results, which can lead to stabilization of the hemoglobin level that may not require therapeutic intervention. Cold agglutinins are estimated to cause antibody-mediated hemolysis in 10% of patients.9 Over 32 years, Stone and colleagues10 assayed sera from 172 patients with IgM monoclonal proteins. Cold agglutinin activity was present in 10 of 117 patients (8.5%). The anti-I titers ranged from 1:512 to 1:65,536.

IgM antibodies resulting in hemolytic disease can be polyclonal, postinfectious, or monoclonal, the classic cold hemagglutinin disease. The polyclonal disorder tends to be associated with viral infections (most commonly in children), is usually self-limited, and resolves spontaneously, but it may require transfusional support.11,12 The use of intravenous immunoglobulin for polyclonal cold agglutinins reportedly has been successful in inhibiting the hemolysis until spontaneous clearance of the IgM antibodies occurs.13,14 These postinfectious cold agglutinins are seen most notably with Mycoplasma pneumoniae infection and infectious mononucleosis. Treatment of the underlying mycoplasma infection has been associated with more rapid resolution of the hemolytic process. Patients with polyclonal cold agglutinin disease are younger than those with chronic cold agglutinin disease. The hemolytic anemia associated with monoclonal IgM proteins is more serious; it is chronic and sustained because the IgM monoclonal protein persists indefinitely. Historically, cold agglutinin hemolysis has been more resistant to therapy, presumably because the density of complement molecules on the surface of red cells is high, making the hemolysis less responsive to traditional therapies that have been used for warm antibody-mediated immune hemolytic anemia.

Cytogenetic studies in patients with cold hemagglutinin disease have reported both trisomy 3 and trisomy15 and t(8;22),16 a reflection of the association with underlying lymphoproliferative disorders. The monoclonal IgM cold agglutinins that bind to the Ia carbohydrate antigens on the surface of red cells all appear to have immunoglobulin heavy chains encoded by the V4.34 gene segment.17 This mandatory use indicates that distinctive amino acid sequences may be involved in recognition. Critical amino acids exist in framework region 1 of V4.34-encoded immunoglobulin, and these generate a specific idiotype determinant, which lies close to the I-binding site. B cells that use the VH4.34 gene segment represent approximately 10% of all mature B cells. These cells account for low-titer cold agglutinins found in the serum of normal subjects. I-binding by idiotype-expressing immunoglobulin can be modulated by sequences in complementarity-determining region CDR(H)3.18

Unlike most blood group antigen pairs, the I and i antigens are not produced by allelic pairs but are reciprocal.19 The I antigen is formed by the action of an enzyme that adds branches to the i antigen; thus, the I antigen is
formed at the expense of its precursor, the i antigen. These antigens are present on all blood cells and are widely distributed in tissue.

In most patients with cold agglutinin disease, hemolytic anemia is the sole manifestation. The basis for the common recommendation to avoid cold exposure is primarily anecdotal, and the benefits of thermal protection are not as clear as they are for type II cryoglobulinemia. The hemolysis tends to be extravascular. Because most patients with symptomatic cold agglutinin disease have antibodies that will bind to the red cell at temperatures above 25°C, it is presumed that the binding of the IgM molecule occurs when blood circulates to the core from the periphery and the IgM, which is bound only for a few seconds, activates the complement cascade to the stage of C3b, which adheres to the red cell after it reenters the central circulation. The C3b-coated red cells encounter receptor-specific macrophages, resulting in clearance of the red cells. This clearance occurs predominantly in the liver, which partly explains why splenectomy is not effective therapy. The high incidence of incidental cold agglutinins (polyclonal) in the adult population detected at crossmatch is a reflection of the benign nature of these antibodies, which have a low thermal amplitude and no activity above 20°C.

Clues to the diagnosis of cold agglutinin disease include the presence of acrocyanosis and Raynaud phenomenon. The in vitro phenomenon of agglutination results in artificial changes such that automated particle counters record a false increase in the mean corpuscular volume to levels as high as 140 fL and a false decrease in the red cell count.20 Typical laboratory features common to all forms of extravascular hemolysis include indirect hyperbilirubinemia and increased concentration of lactate dehydrogenase. In stable patients, hallmarks of intravascular hemolysis (a decrease in the serum level of haptoglobin and an increase in plasma-free hemoglobin) are not present. The direct antiglobulin test (Coombs) is always positive. A retrospective study from a single institution reported on 58 patients (median age, 59 years). The direct antiglobulin test revealed C3 in 74% of patients, C3 + IgG in 22.4%, and IgG alone in only 3.4%.21 Seventy-eight percent of patients with a cold agglutinin had an associated autoimmune disorder, an infection, or a lymphoproliferative disorder. As noted, low-titer, low-avidity cold agglutinins are frequently found in routine screens of donated red cells from otherwise healthy adults.22 These antibodies are of low thermal amplitude and are benign, with no in vivo activity. When the thermal amplitude of the protein is high, clinical hemolysis can occur with cold agglutinin titers as low as 1:64.23 Nonetheless, most patients who have cold agglutinin disease usually have titers in excess of 1:1000.

An occasional cold antibody is identified outside the I/i antigen system and is directed against the Pr antigen, the same target as in patients with paroxysmal cold hemoglobinuria caused by the Donath-Landsteiner antibody. Gene usage studies for anti-Pr cold agglutinins indicate a preference of gene usage for the light chain variable domain κ IV.17 The majority of patients with cold hemagglutinin disease have a monoclonal IgM protein; thus, by definition, there should be a detectable clone of lymphocytes responsible for the synthesis of this protein.24

Berentsen and colleagues25 reported on 86 patients from Norway with cold agglutinin disease, and not unexpectedly, bone marrow studies showed clonal light chain dominance in 90% of patients, evidence of a lymphoplasmacytic lymphoma in 50%, and lymphoma of any type in 76%. Only 24% of patients did not have a demonstrable clonal population in their bone marrow. The histologic types reported included all disorders associated with the production of a monoclonal IgM protein, including splenic marginal zone lymphoma, small lymphocytic lymphoma, and lymphoplasmacytic lymphoma. The monoclonal proteins reported in this series were quite modest in size, indicating that it is the qualitative binding of IgM to the red cell rather than the quantity of IgM that is critical for the development of hemolytic disease. The authors reported that approximately half of the patients had transfusion dependency at one time during the clinical course, frequently during a febrile illness. The median cold agglutinin titer was 1:2048 (11 dilutions).

Another study of 14 patients has reported evidence, by morphology alone, of non-Hodgkin lymphoma in 5 patients.8 I have cared for 1 patient who had a stable monoclonal IgM protein for 17 years before a decreasing hemoglobin value led to the mistaken diagnosis of progressive Waldenström macroglobulinemia. Further investigation showed the development of cold-mediated immune hemolysis as the cause of this patient’s anemia, without evidence of Waldenström macroglobulinemia. When the criterion for Waldenström macroglobulinemia is not fulfilled (i.e., <10% lymphoplasmacytic cells in the bone marrow), the current classification system defined at the Second International Workshop on Waldenström Macroglobulinemia refers to this as an “IgM-related disorder,” which includes cryoglobulinemia and peripheral neuropathy associated with monoclonal IgM proteins.26

Transfusion may be required for cold agglutinin syndrome. Similar to warm hemolytic syndromes, cold agglutinin antibodies complicate transfusion management. The agglutination of cells makes it difficult to determine the ABO type and makes it almost impossible to recognize alloantibodies that may be present from previous transfusions. Washing the patient’s red cells with warm normal saline to remove the IgM autoantibody can facilitate accurate determination of the ABO group. If ABO typing is unclear, group O red cells should be used for transfusion. Although the data are weak, the use of blood warmers has been suggested during transfusion.

Over the years, treatment has been directed at suppressing the synthesis of the IgM monoclonal protein and has included corticosteroids, alkylating agents, azathioprine, interferon, and purine nucleoside analogues. Reported re-
of 5 patients had a response. Cladribine has been reported to be associated with a grade lymphoproliferative disorder, but in one report, none of 5 patients had a response. Cladribine has been reported to induce the remission of warm antibody-mediated hemolytic anemia in patients with low-grade lymphoproliferative disorders. Cryofiltration apheresis has been used for acute exacerbations and for surgical procedures requiring hypothermia, such as coronary artery bypass. Of 5 patients who received cryofiltration apheresis, 2 had a favorable response with a reduction in titer.

Recently, several centers have reported using rituximab to treat cold agglutinin disease. The results of these case series suggest higher response rates than have been achieved with alkylators, corticosteroids, or purine nucleoside analogues. In a study of 27 patients who received rituximab, 14 had a response to the first course and 6 of 10 had a response to re-treatment; combined responses were achieved after 20 of 37 courses, for an overall response rate of 54%. The median increase in hemoglobin concentration in those who had a response was 4 g/dL, with a median time to response of 1.5 months. In a series of 20 patients, 4 doses of rituximab produced a response rate of 45% (9 patients), one complete response. Eight of the 9 patients had disease relapse, reflecting the difficulty with obtaining a long-term response. Rituximab has also been given in combination with oral cyclophosphamide, with positive responses. Berentsen et al reported a complete response in 5% of patients receiving treatment with rituximab as a single agent and a partial response in 55%. The complete response rate was 25% and the partial response rate was 42% when rituximab was given in combination with interferon or fludarabine.

In summary, cold agglutinin disease is an extracutaneous immune hemolytic anemia produced by an IgM monoclonal protein. The source of the IgM monoclonal protein is a population of cells typically found in the bone marrow, often in sufficient number to allow a firm diagnosis of non-Hodgkin lymphoma or Waldenström macroglobulinemia. The introduction of rituximab appears to be improving therapeutic responses in this disorder (Table 1).

### References

24. Berentsen S, Bo K, Shammas FV, Myking AO, Ulvestad E. Chronic cold agglutinin disease of the “idiopathic” type is a premalignant or low-grade malignant lymphoproliferative


