# Cold agglutinin disease

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The first report of cold agglutinin hemolysis caused by monoclonal antibodies appeared in 1957.[1] These were actually the first monoclonal proteins shown to have antibody activity.[2] Cold agglutinins are frequently found in low titer in the sera of normal adults. Christenson and Dacie demonstrated that patients with high titer cold agglutinins have a serum monoclonal band what could be removed by adsorbing the patients' serum with red cells bearing the I or i antigen.[3] Previous reports have indicated that immune hemolytic anemia that cold agglutinins occur in 1:100,000 persons with a peak incidence in the seventh decade of life[4,5] corresponding to the decade with the highest prevalence of monoclonal proteins. At the Mayo Clinic, among 31,479 cases of monoclonal gammopathies seen, cold agglutinin disease was present in 46 patients, representing 0.1% of the cases seen. When the analysis was restricted just to those patients with IgM monoclonal proteins, cold agglutinin hemolytic anemia constituted 0.6% of cases.

The term *cold agglutinin* is somewhat misleading because it implies that the disease has a clear relationship with cold exposure. In fact, this terminology 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 antisera. Recognition requires incubation with the antiglobulin antibody at 37°C for two hours, thus the term *warm*. The use of the antiglobulin antisera (Coombs' reagent) is necessary since the electrostatic charge on the red cells causes them to mutually repel in solution, the so-called zeta potential. The bridging effect of the Coombs' antibody binding to IgG molecules on the red cell surface overcomes the electrostatic repulsive force and enables agglutination. Since cold agglutinin disease is mediated by an IgM molecule in 90% of patients and the IgM molecule has a molecular weight of nearly one million daltons (1000 kD), its size can span the intercellular distance between red cells; agglutination is seen at 4°C in the microtiter well without the use of any antiglobulin antisera, thus the term *cold* agglutination is used.

In warm immune hemolytic anemia, IgG molecules cover the red cell surface, and pieces of the red cell membrane are sequentially removed after multiple passages through the spleen. Removing bits of membrane decreases the surface area of the red cell, resulting in the classic spherocyte. In cold agglutinin disease, the IgM molecule fixes complement to the red cell surface, but 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, distinguishing cold agglutinin disease from paroxysmal cold hemoglobinuria in which the complement-fixing antibody (usually IgG) results in activation of the complement cascade and lysis of the red cell. In cold agglutinin disease, the complement affixed to the red cell surface undergoes processing in a manner similar to IgG antibody-coated red cells of warm hemolytic disease. The cells undergo successive removal of the red cell membrane within the mononuclear phagocyte system leading to their ultimate destruction. There is also a hemolysis-resistant population of $C3_d$ coated red cells, which can lead to an equilibrium of the hemoglobin level such that therapeutic intervention may not be required. Cold agglutinins are estimated to be the cause of antibody mediated hemolysis in 10% of patients.[6] Over a period of 32 years, Stone and colleagues, assayed sera from 172 patients with IgM monoclonal proteins.[7] Cold agglutinin activity was present in 10 of 117 or 8.5%. The anti-I titers ranged from 1:512 to 1:65,536.

IgM antibodies resulting in hemolytic disease can be polyclonal, post-infectious, 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, although may require transfusional support.[8,9] There are reports in the literature on the successful use of intravenous immunoglobulins for polyclonal cold agglutinins through inhibition of the hemolysis until spontaneous clearance of the IgM antibodies occurs.[10,11] These post-infectious cold agglutinins are most notably seen with mycoplasma pneumonia infection and infectious mononucleosis. Therapy of the underlying mycoplasma infection has been associated with more rapid resolution of the hemolytic process. Patients with polyclonal cold agglutinin are younger than those with chronic cold agglutinins disease. The hemolytic anemia associated with monoclonal IgM proteins is far more serious since it is chronic and sustained because the IgM monoclonal protein persists indefinitely. Cold agglutinin hemolysis has historically been more resistant to therapy, presumably because the density of complement molecules on the red cell surface is quite high, making it less responsive to the traditional therapies that have been used for warm antibody-mediated immune hemolytic anemia.

Cytogenetic studies have been performed in patients with cold hemagglutinin disease. Both trisomy 3 and trisomy 12 have been reported,[12] and t(8;22) has also been recorded,[13] reflecting the association with underlying lymphoproliferative disorders. The monoclonal IgM cold agglutinins that bind to the I/i carbohydrate antigens on the surface of the red cells all appear to have immunoglobulin heavy chains encoded by the V4.34 gene segment.[14] This mandatory use indicates that dis-

tinctive amino acid sequences may be involved in recognition. Critical amino acids exist in framework region 1 (FR1) of V4.34-encoded immunoglobulin, and these generate a specific idiotype determinant, which lies close to the I-binding site. I-binding by idiotype-expressing immunoglobulin can be modulated by sequences in complementarity determining region CDR(H)3.[15] The crystal structure of an anti-I cold agglutinin has revealed a hydrophobic patch in the FRI involving residue W7 on beta strand A and the AVY motif on beta strand B. Unlike most blood group antigen pairs, the I and i antigens are not produced by allelic pairs but are reciprocal.[16] The I antigen is formed by the action of an enzyme which adds branches onto 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 have a very wide tissue distribution.

In the majority of patients, hemolytic anemia is the sole manifestation. Although the literature is replete with recommendations to avoid cold exposure, this is primarily anecdotal, and the benefits of thermal protection are not as clear as in type II cryoglobulinemia. The hemolysis tends to be extravascular. Since most patients with symptomatic cold agglutinin disease have antibodies with high thermal amplitudes, it is presumed that the binding of the IgM molecule occurs when blood circulates away from the core to 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 re-enters 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 is in part why splenectomy is an ineffective therapy. The high incidence of incidental cold agglutinins (polyclonal) in the adult population detected at cross-matching is a reflection of the benign nature of these antibodies. These cold antibodies 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's phenomenon. The *in vitro* phenomenon of agglutination results in artifactual changes such that automated particle counters will record a false increase in the mean corpuscular volume to levels as high as 140 fL and a false reduction in the red cell count.[17] Typical laboratory features common to all forms of extravascular hemolysis include indirect hyperbilirubinemia and an elevation of lactate dehydrogenase. In stable patients, reduction in serum haptoglobin and elevated plasma-free hemoglobin, both hallmarks of intravascular hemolysis, are not present. The direct antiglobulin test (Coombs' test) is always positive. In a retrospective study from a single institution, 58 patients with a median age of 59 were described. The direct antiglobulin test revealed C3 in 74% of patients, C3+IgG in 22.4%, and IgG alone in only 3.4%.[18] Seventy-eight percent of patients with a cold agglutinin had an 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 in otherwise healthy adults.[19] 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.[20] Nonetheless, the majority of patients who have cold agglutinin disease usually have titers in excess of 1:1000.

An occasional cold antibody can be identified outside of the I/i antigen system and is directed against the Pr antigen, the same target seen 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.[14] Since the majority of patients with cold hemagglutinin disease have a monoclonal IgM protein, by definition, there should be a detectable clone of lymphocytes responsible for their synthesis.[21] In this issue of the Journal Berentsen and colleagues[22] report on 86 patients from Norway with cold agglutinin disease. Not unexpectedly, studies of the bone marrow 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. Disorders associated with the production of a monoclonal IgM protein included splenic marginal zone lymphoma, small lymphocytic lymphoma, and lymphoplasmacytic lymphoma. The monoclonal proteins reported in this series were quite modest in size, reflecting 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 report transfusion dependency at some point during the clinical course in approximately half of patients, frequently during febrile illnesses. The median cold agglutinin titer was 1:2048 (11 dilutions). A second published study of 14 patients demonstrated evidence by morphology alone of non-Hodgkin's lymphoma in five.[5] I have cared for one patient with a stable monoclonal IgM protein for 17 years before a falling hemoglobin led to a mistaken diagnosis of progressive Waldenström's macroglobulinemia. Further investigation revealed the development of cold-mediated immune hemolysis as the cause of his anemia without evidence of Waldenström's macroglobulinemia. When the criteria for Waldenström's macroglobulinemia are not fulfilled, <10% lymphoplasmacytic cells in the bone marrow, the current classification system defined at the Second International Workshop on Waldenström's Macroglobulinemia refers to this as an IgM-related disorder, a classification that includes cryoglobulinemia and peripheral neuropathy associated with monoclonal IgM proteins.[23]

Over the years, therapies have been directed at suppressing the synthesis of the IgM monoclonal protein and have included corticosteroids, alkylating agents, azathioprine, interferon, and purine nucleoside analogs. As reported in the current series, response rates to alkylating agents or steroids are far lower than in warm antibody-mediated disease, at <20%. Severe cold agglutinin disease has been reported to respond to danazol therapy. Cladribine has been used to treat the low-grade lymphoproliferative disorder, but in one report, none of five

patients responded,[24] and cladribine was reported to induce warm antibody-mediated hemolytic anemia in patients with low-grade lymphoproliferative disorders receiving the drug. Cryofiltration apheresis has been used for acute exacerbations and for surgical procedures requiring hypothermia such as coronary artery bypass surgery. Among five patients receiving cryofiltration apheresis, two responded favorably with a reduction in their titer.[25]

Recently, rituximab has been used in a number of centers to treat cold agglutinin disease.[26] The case series suggest higher response rates than have been achieved with alkylators, steroids, or purine nucleoside analogs. In a study of 27 patients receiving rituximab, 14 responded to a first course, six of ten responded to re-treatment, and combined responses were achieved after 20 of 37 courses for an overall response rate of 54%.[27] The median rise in hemoglobin in responders was 4 g/dL with a median time to response of 1.5 months. In a series of 20 patients, four doses of rituximab resulted in a response rate of 45%, with one complete response.[28] Eight of the nine responders relapsed, reflecting the difficulty in obtaining long-term responses. Rituximab has also been combined with oral cyclophosphamide with positive response.[29] In their paper, Berentsen *et al.* report a complete response rate of 5% and a partial response rate of 55% to rituximab as a single agent and a 25% complete response rate and 42% partial response rate when rituximab was combined with interferon or fludarabine.

In summary, cold agglutinin disease represents an extravascular 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 numbers to allow a firm diagnosis of non-Hodgkin's lymphoma or Waldenström's macroglobulinemia. The introduction of rituximab appears to be improving responses in this disorder.

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