Brief Communication

Cold Agglutinin Disease and Cryoglobulinemia

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Abstract

Cold agglutinin disease is a form of direct, extravascular, antiglobulin-positive hemolysis. In vivo, immunoglobulin (Ig) M fixes complement molecules to the red cell membrane. Successive passages through the mononuclear phagocyte system result in loss of red cell membrane. The resultant spherocytes lose resiliency and are ultimately lost from the circulation extravascularly. The high concentration of complement molecules on the red cell surfaces makes this syndrome resistant to the standard therapies for immune-mediated hemolysis. Rituximab has been reported to reduce the severity of hemolysis. Type II cryoglobulins are composed of a monoclonal IgM and a polyclonal IgG. These complexes have rheumatoid factor activity and can produce immune-complex vasculitis. The target organs are the skin, nerves, kidney, liver, and joints. More than 80% of patients have evidence of hepatitis C infection. Interferon and interferon plus ribavirin have been shown to produce serologic responses. When vasculitis is active, corticosteroids are often required to permit healing of ulcers in the skin or to treat the membranoproliferative glomerulonephritis that is seen, thereby preventing loss of renal function. Rituximab therapy has been found to be effective in mixed cryoglobulinemia, with decreases in cryoglobulin values and improvement in complement values.

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Cold Agglutinin Disease

The first report of cold agglutinin hemolysis caused by monoclonal antibodies appeared in 1957. These were the first monoclonal proteins shown to have antibody activity.[1] The cold agglutinin syndrome is a form of immune hemolytic anemia with a frequency of 1 per 100K persons. Its peak incidence occurs in the seventh decade of life.[2] Hemolytic anemia is caused by the immunoglobulin (Ig) M–mediated deposition of complement molecules on the red cell membrane. The most severe form is associated with the production of a monoclonal IgM κ protein by a clonal population of lymphoplasmacytic cells found in the bone marrow.[3] By definition, these patients have a low-grade lymphoproliferative disorder, and the production of the IgM κ monoclonal protein results in chronic sustained hemolysis. Bone marrow cytogenetics in patients with cold agglutinin disease demonstrates the presence of trisomy 3 and trisomy 12.[4,5]

In the majority of patients, hemolytic anemia is the sole manifestation of the disorder. The hemolysis is cold sensitive. The IgM autoantibody attaches to the red cell at temperatures that vary from patient to patient but may be close to 37°C. When patients are exposed to temperatures below that value, antigen antibody complexes form and complement fixation occurs. These patients may develop all the signs of intravascular hemolysis. Acrocyanosis and Raynaud's phenomenon are commonly seen. In vitro agglutination results in artifactual changes, such that automated particle counters record a false increase in the mean corpuscular volume and a false decrease in the red cell count. Indirect hyperbilirubinemia is seen when the hemolysis is moderate or severe. Decrease in serum haptoglobin and increase in plasma-free hemoglobin are seen when the hemolysis is severe.[6] The direct antibody test is always positive with anti-C3 and should be negative with anti-IgG. Immunoglobulin M alloantibodies do not bind to the red cell surface at room temperature and therefore usually are not detected in vitro.[7]

Low-titer and low-avidity cold agglutinins can be detected in healthy adults when specimens are tested at temperatures < 4°C. These are benign antibodies of low thermal amplitude with no in vivo activity. When agglutination occurs at 37°C, the autoantibody is virtually always clinically significant. In general, the titers exceed 1:1000 dilution.[8] Many patients have much higher titers. High titers and the monoclonal protein distinguish chronic cold agglutinin hemolysis from transient cold agglutinin hemolysis caused by mycoplasma and other

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organisms. Complement bound to the red cell membrane is recognized by C3b receptors in the mononuclear phagocyte system, with the majority of red cell destruction occurring in the liver and spleen. Spherocytes are found frequently in the peripheral blood film.

In general, the cold autoantibody shows specificity against the Ii blood group system: 90% against "I" and the remainder against "i." Rarely, a cold autoantibody is identified against the Pr antigen. This antigen is the same target seen in patients with paroxysmal cold hemoglobinuria,[9] which is caused by the Donath-Landsteiner antibody, a cold hemolysin of the IgG type.

Because a single clone of B lymphocytes produces the cold antibody in chronic cold agglutinin disease, a low-grade lymphoproliferative disorder is present by definition. In a study of 14 patients, the estimated prevalence was 1.4 in 100,000. All patients had chronic hemolytic anemia, cold agglutination on the peripheral blood film, a positive direct antibody test, and a titer of > 1:64. The mean patient age was 75 years, with equal numbers of men and women.[10] Bone marrow biopsies confirmed the presence of a low-grade malignant non-Hodgkin's lymphoma in 5 patients, and 1 additional patient had expansion of M-κ–positive cells, which is considered evidence of a lymphoproliferative disorder. Bone marrow lymphocytes ranged from 3% to 40%. If there is evidence on a bone marrow trephine biopsy of infiltration by lymphoplasmacytic lymphoma with a predominantly intratrabecular pattern, these patients are classifiable as having Waldenström's macroglobulinemia (WM).[11] However, in the absence of overt marrow lymphoma, this is referred to as an IgM-related disorder—cold agglutinin hemolysis.[12]

As a result of the high density of complement molecules on the red cell surface, the hemolysis might be severe, and treatment is unsatisfactory.[13] These patients usually have high thermal amplitude and are more susceptible to ongoing or severe hemolysis. In these patients, the temperature sensitivity may be more important than the number of complement molecules on the red cell surface. Folic acid replacement is warranted because of increased red cell turnover. Corticosteroids, the therapy of choice for warm hemolytic disorders, are beneficial for only those patients with low-titer or high thermal amplitude cold agglutinins. Splenectomy has been of marginal benefit as well, because the liver is a major source of red cell destruction. Plasma exchange can provide temporary improvement in severe hemolysis and has been used regularly for patients undergoing surgical procedures that result in cold exposure.[14,15] Because patients have a monoclonal IgM protein, suppression of antibody production has been attempted with oral chlorambucil, cyclophosphamide, and combination chemotherapy regimens that include cyclophosphamide, vincristine, and prednisone. Severe cold agglutinin disease has been reported to respond to danazol therapy.[16] Cladribine has been used to treat the low-grade lymphoproliferative disorder, and no response has been reported in 5 patients.[17] Avoidance of cold exposure is the mainstay of management. Blood transfusions when necessary must be given through a blood warmer, and intravenous fluids must be warmed to 37°C before administration.

Treatment with Rituximab

Five patients were treated with 4 weekly rituximab infusions; 4 had a partial response and 1 had a complete response (CR). In a literature review, 21 of 23 patients responded with 14 CRs.[18,19] As a result of the low toxicity of rituximab, this has become a firstline therapy for patients whose hemolysis warrants intervention. One patient's hemoglobin concentration increased from 8.5 g/dL to 12.5 g/dL despite a persistent positive result of the direct antibody test.[20]

Cryoglobulinemia

Wintrobe and Buell reported on cryoprecipitation in 1933.[21] The first patients with mixed cryoglobulinemia were patients with WM.[22] Macroglobulinemia might also develop later in the course of the disease.

The classification system dates back to 1974, when cryoglobulins were defined as type I, a monoclonal component only; type III, polyclonal IgM and polyclonal IgG; and (the focus of this article) type II or mixed cryoglobulins, a monoclonal IgM autoantibody with anti-IgG activity that defines it as a rheumatoid factor in which the titers typically are high.[23] This cryoprecipitable immunocomplex can be found in the complete absence of clinical symptoms, but can also deposit diffusely on endothelial surfaces, activate complement, and produce a systemic vasculitis syndrome. Samples should be collected and processed at 37°C to avoid loss of the soluble protein during the separation of serum from red cells.

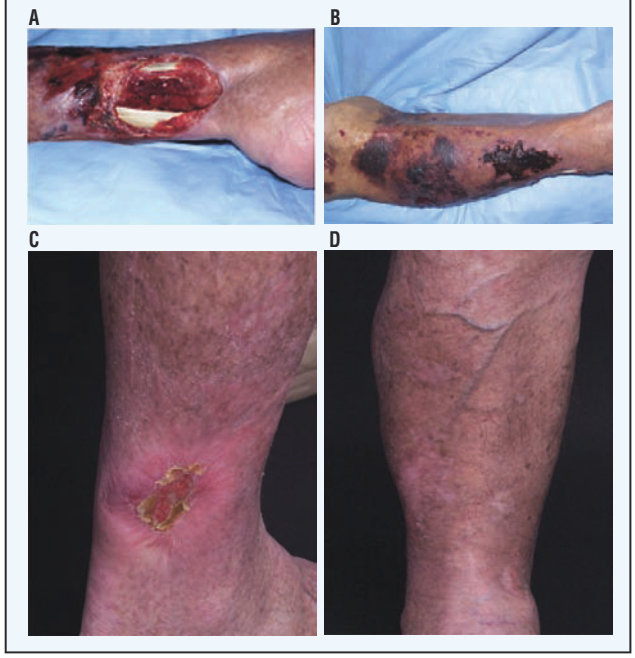
The presence of a monoclonal IgM implies that there is a clonal proliferation of lymphoplasmacytic cells in the bone marrow that can be an effective target for therapeutic interventions. Hepatitis C was recognized as an etiologic factor for type II cryoglobulinemia in the 1990s.[24,25] Patients in the past have been misdiagnosed as having rheumatoid vasculitis because of the positive rheumatoid factor in the absence of any other manifestation of a symmetric polyarthritis and the fact that vasculitis was well documented.

The most common target organs for cryoglobulins are the skin, nerves, kidney, and liver.[26] The cutaneous manifestations are most prevalent, with a characteristic gravitational purpura on the lower extremities representing cutaneous hemorrhage from the ankle to the top of the calf. Renal involvement is found in approximately one quarter of patients. These patients typically have significant degrees of proteinuria, active urinary sediment with red cells and casts, and hypertension.[27] The renal lesion is characterized as membranoproliferative glomerulonephritis, and electron-dense deposits, presumably the immunocomplexes, are found with intracapillary thrombi.

Frequently, increased concentration of transaminases is found. It is often difficult to distinguish whether this is a consequence of cryoglobulinemic vasculitis of the liver or a reaction to the hepatitis C infection found in > 80% of patients.

Patients with a symptomatic type II cryoglobulinemia who have only purpura frequently do not require therapy and respond to elastic support stockings and analgesic treatment of arthralgias. Avoidance of cold exposure is the backbone of management. For patients with hepatitis C, treatment with

Figure 1 Cryoglobulinemic Skin Ulceration Before (A, B) and After (C, D) Rituximab Therapy



interferon[28] or interferon plus ribavirin for 12 months[29] produces responses in more than half of patients, but these responses are sustained in only 28%. Interferon combined with ribavirin is safe and effective in patients with hepatitis C virus (HCV)–associated type II cryoglobulinemia. Eradication of the HCV results in improvement in cryoglobulinemic clinical manifestations, but durable responses are seen in the minority of cases. The only pretherapy variable that has been shown to predict a CR of cryoglobulinemia was the solitary anti-C22 (HCV core) antibody pattern, which is seen in 29% of patients.[30] Responses are accompanied by a decrease in viremia, anti-HCV antibody titers, and cryocrit.

When vasculitis[31] is manifested by cutaneous ulcers, active nephritis, or progressive neuropathy, high-dose corticosteroids interrupt the deposition of the immune complexes on the endothelial surface and improve symptoms.[32] Complete reversal of renal manifestations is commonly seen with high-dose corticosteroid therapy. In patients with severe vasculitis, corticosteroids are required because the response to interferon and ribavirin therapy can take 6-12 months to occur. An immediate reduction in circulating immunocomplexes can be achieved by total plasma exchange, and suppression of IgM antibody synthesis has been attempted with the use of oral chlorambucil and cyclophosphamide. In cryoglobulinemia affecting the kidney, interferon therapy has been found to be generally ineffective.

Treatment with Rituximab

Rituximab therapy has been found to be effective in type II mixed cryoglobulinemia.[33] In a study of 15 consecutive patients with type II cryoglobulinemia, 12 of whom were hepatitis C–positive, 4 doses of rituximab were administered. All patients had active, poorly controlled disease, and all had been treated previously with corticosteroids. Rituximab was effective on skin vasculitis manifestations in all patients, on peripheral neuropathy in 7 of 7 patients, on arthralgias in 4 of 4, and on fever. One patient of 2 with nephritis went into remission. Rheumatoid factor titers and cryoglobulin values decreased, and complement values increased as objective evidence of the therapeutic effect.[34] No impact on HCV titers was seen. Figure 1 shows a patient whose severe leg ulcers responded to rituximab after corticosteroids and cyclophosphamide failed.

Conclusion

Type II cryoglobulinemia is an immune complex disorder in which the underlying pathogenesis includes HCV infection. The clinical organ manifestations are skin, liver, kidney, and nerve.[35] Treatment includes interferon, ribavirin, corticosteroids, immunosuppressive or chemotherapeutic agents, and rituximab.

References

1. Christenson WN, Dacie JV, Croucher BE, et al. Electrophoretic studies on sera containing high-titre cold haemagglutinins: identification of the antibody as the cause of abnormal gamma 1 peak. *Br J Haematol* 1957; 3:262-275.
2. Mack P, Freedman J. Autoimmune hemolytic anemia: a history. *Transfus Med Rev* 2000; 14:223-233.
3. Berentsen S, Bo K, Shammas FV, et al. Chronic cold agglutinin disease of the "idiopathic" type is a premalignant or low-grade malignant lymphoproliferative disease. *APMIS* 1997; 105:354-362.
4. Michaux L, Dierlamm J, Wlodarska I, et al. Trisomy 3 is a consistent chromosome change in malignant lymphoproliferative disorders preceded by cold agglutinin disease. *Br J Haematol* 1995; 91:421-424.
5. Gordon J, Silberstein L, Moreau L, et al. Trisomy 3 in cold agglutinin disease. *Cancer Genet Cytogenet* 1990; 46:89-92.
6. Zilow G, Kirschfink M, Roelcke D. Red cell destruction in cold agglutinin disease. *Infusionsther Transfusionsmed* 1994; 21:410-415.
7. Gehrs BC, Friedberg RC. Autoimmune hemolytic anemia. *Am J Hematol* 2002; 69:258-271.
8. Silverman GJ, Chen PP, Carson DA. Cold agglutinins: specificity, idiotypy and structural analysis. *Chem Immunol* 1990; 48:109-125.
9. Herron B, Roelcke D, Orson G, et al. Cold autoagglutinins with anti-Pr specificity associated with fresh varicella infection. *Vox Sang* 1993; 65:239-242.
10. Hadnagy C. Agewise distribution of idiopathic cold agglutinin disease. *Z Gerontol* 1993; 26:199-201.
11. Silberstein LE. B-cell origin of cold agglutinins. *Adv Exp Med Biol* 1994; 347:193-205.
12. Owen RG, Treon SP, Al-Katib A, et al. Clinicopathological definition of Waldenström's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenström's Macroglobulinemia. *Semin Oncol* 2003; 30:110-115.
13. Nydegger UE, Kazatchkine MD, Miescher PA. Immunopathologic and clinical features of hemolytic anemia due to cold agglutinins. *Semin Hematol* 1991; 28:66-77.
14. Andrzejewski C Jr, Gault E, Briggs M, et al. Benefit of a 37°C extracorporeal circuit in plasma exchange therapy for selected cases with cold agglutinin disease. *J Clin Apheresis* 1988; 4:13-17.
15. Bedrosian CL, Simel DL. Cold hemagglutinin disease in the operating room. *South Med J* 1987; 80:466-471.
16. Lugassy G, Reitblatt T, Ducach A, et al. Severe autoimmune hemolytic anemia with cold agglutinin and sclerodermic features: favorable response to danazol. *Ann Hematol* 1993; 67:143-144.
17. Berentsen S, Tjonnfjord GE, Shammas FV, et al. No response to cladribine in five patients with chronic cold agglutinin disease. *Eur J Haematol* 2000; 65:88-90.
18. Camou F, Viallard JF, Pellegrin JL. Rituximab in cold agglutinin disease

[French]. *Rev Med Interne* 2003; 24:501-504.
19. Bauduer F. Rituximab: a very efficient therapy in cold agglutinins and refractory autoimmune haemolytic anaemia associated with CD20-positive, low-grade non-Hodgkin's lymphoma. *Br J Haematol* 2001; 112:1085-1086.
20. Ahrens N, Kingreen D, Seltsam A, et al. Treatment of refractory autoimmune haemolytic anaemia with anti-CD20 (rituximab). *Br J Haematol* 2001; 114:244-245.
21. Rieu V, Cohen P, Andre MH, et al. Characteristics and outcome of 49 patients with symptomatic cryoglobulinaemia. *Rheumatol (Oxford)* 2002; 41:290-300.
22. Grey HM, Kohler PF, Terry WD, et al. Human monoclonal gamma G-cryoglobulins with anti-gamma-globulin activity. *J Clin Invest* 1968; 47:1875-1884.
23. Trejo O, Ramos-Casals M, Garcia-Carrasco M, et al. Cryoglobulinemia: study of etiologic factors and clinical and immunologic features in 443 patients from a single center. *Medicine (Baltimore)* 2001; 80:252-262.
24. Cacoub P, Musset L, Lunel Fabiani F, et al. Hepatitis C virus and essential mixed cryoglobulinaemia. *Br J Rheumatol* 1993; 32:689-692.
25. Merlini G, Zorzoli I, Anesi E, et al. Immunochemical characterization of the cryoglobulins: pathophysiologic implications. *Clin Exp Rheumatol* 1995; 13(suppl 13):S71-S73.
26. Monti G, Galli M, Invernizzi F, et al, and the GISC (Italian Group for the Study of Cryoglobulinaemias). Cryoglobulinaemias: a multi-centre study of the early clinical and laboratory manifestations of primary and secondary disease. *QJM* 1995; 88:115-126.
27. Beddhu S, Bastacky S, Johnson JP. The clinical and morphologic spectrum of renal cryoglobulinemia. *Medicine (Baltimore)* 2002; 81:398-409.
28. Dammacco F, Sansonno D, Han JH, et al. Natural interferon-alpha versus its combination with 6-methyl-prednisolone in the therapy of type II mixed cryoglobulinemia: a long-term, randomized, controlled study. *Blood* 1994; 84:3336-3343.
29. Calleja JL, Albillos A, Moreno-Otero R, et al. Sustained response to interferon-alpha or to interferon-alpha plus ribavirin in hepatitis C virus-associated symptomatic mixed cryoglobulinaemia. *Aliment Pharmacol Ther* 1999; 13:1179-1186.
30. Casato M, Agnello V, Pucillo LP, et al. Predictors of long-term response to high-dose interferon therapy in type II cryoglobulinemia associated with hepatitis C virus infection. *Blood* 1997; 90:3865-3873.
31. Cacoub P, Costedoat-Chalumeau N, Lidove O, et al. Cryoglobulinemia vasculitis. *Curr Opin Rheumatol* 2002; 14:29-35.
32. Ferri C, Zignego AL, Pileri SA. Cryoglobulins. *J Clin Pathol* 2002; 55:4-13.
33. Dispenzieri A. Symptomatic cryoglobulinemia. *Curr Treat Options Oncol* 2000; 1:105-118.
34. Zaja F, De Vita S, Mazzaro C, et al. Efficacy and safety of rituximab in type II mixed cryoglobulinemia. *Blood* 2003; 101:3827-3834.
35. Miescher PA, Huang YP, Izui S. Type II cryoglobulinemia. *Semin Hematol* 1995; 32:80-85.