Paroxysmal Cold Hemoglobinuria: Report of a Case With an Exceptionally High Thermal Range Donath-Landsteiner Antibody

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A patient with idiopathic paroxysmal cold hemoglobinuria had severe hemolytic anemia despite minimal exposure to cold. The patient's autoantibody demonstrated all the characteristic features of Donath-Landsteiner antibodies, except that strong hemolysis occurred with simple cooling (monophasic hemolysis), as well as with traditional cold-warm incubation (biphasic hemolysis). Monophasic hemolysis was caused by a Donath-Landsteiner antibody of exceptionally high thermal range, which caused red cell sensitization and hemolysis up to 32°C. The patient's severe hemolytic anemia was probably a result of the high thermal range of the autoantibody, which would allow intravascular hemolysis with even minimal exposure to cold. Corticosteroids had a beneficial effect in controlling hemolysis; however, strict environmental control, with vigorous prophylaxis against even minimal cold, was the most important part of the therapeutic program.

Paroxysmal Cold Hemoglobinuria (PCH) is a rare form of autoimmune hemolytic anemia caused by a complement-dependent cold-acting autoantibody that produces intravascular hemolysis and hemoglobinuria after exposure to the cold in vivo, and gives rise to the classic Donath-Landsteiner (D-L) reaction in vitro. The D-L hemolysin is an IgG antibody that requires complement in both cold and warm phases for maximal lysis and has distinct blood group specificity. The specific thermal characteristics of a patient's D-L antibody are of great importance clinically because the maximum temperature of antibody binding, together with environmental temperature, determines the severity of hemolysis in vivo.
The maximal temperature at which the lytic antibody binds to erythrocytes in PCH has generally been considered to be less than 18–20°C. This report describes a patient who had idiopathic (nonsyphilitic) PCH and severe hemolytic anemia despite minimal exposure to cold. We attribute the severity of hemolysis to the exceptionally high thermal range of this patient's D-L antibody, which caused red cell sensitization and lysis at temperatures up to 32°C.

CASE REPORT

A 60-yr-old widow of Portuguese-Irish heritage, who resided in northern California, had been well except for mild labile hypertension and "hypothyroidism" until late July 1970, when she first had intermittent fevers, chills, and night sweats, followed by progressive weakness, fatigue, and malaise. She was hospitalized elsewhere in August 1970; she was anemic and had a low-grade fever. Blood tests revealed a hematocrit of 19%, hemoglobin of 6.5 g/100 ml, reticulocytes of 12.8% and normal white cell, differential, and platelet counts (a routine complete blood count in May 1970 had been entirely normal, with a hematocrit of 43% and hemoglobin of 14.9 g/100 ml). A direct antiglobulin test with commercial broad-spectrum antiserum was weakly positive, and a cold agglutinin titer was 1:16. Cultures of blood and urine were sterile; tests for febrile agglutinins and a PPD skin test were negative. The patient had been taking reserpine, chlorothiazide, thyroglobulin (Proloid), diethylpropion hydrochloride (Tenuate), and griseofulvin before admission to the hospital.

A provisional diagnosis of autoimmune hemolytic anemia was made. She received two units of packed red cells; administration of prednisone was begun and the dose was increased rapidly to 80 mg daily. All other medications were discontinued. During this initial hospitalization gross hemoglobinuria was first observed, occurring daily in urine voided at about 1 1 a.m. No exposure to cold could be identified, and the patient experienced no prodromal symptoms except mild, vague back discomfort which occurred 30 min before the hemoglobinuria. Daytime temperatures in the city where the patient was hospitalized averaged 32–38°C (90–100°F), and nighttime temperatures 18–24°C (65–75°F). A week after transfusion and initiation of corticosteroid therapy, her hematocrit was 38%, her hemoglobin was 13.8 g/100 ml, and she had 3.2% reticulocytes. She was afebrile, but continued to be awakened by drenching night sweats between 1 and 4 a.m., and continued intermittently to have hemoglobinuria at 11 a.m. She was discharged 1 mo after admission and instructed to continue taking 80 mg of prednisone daily.

Her low-grade fever, fatigue, and malaise gradually recurred, and her night sweats and hemoglobinuria increased. There was no apparent exposure to cold. She remained in her home most of the time, where the temperature was 24–30°C (75–85°F) during the day, and 18–24°C (65–75°F) at night. Eighteen days after discharge from the hospital, her hematocrit was 25%, her hemoglobin was 8.4 g/100 ml and she had 22% reticulocytes; her total bilirubin was 3.2 mg/100 ml (2.8 mg/100 ml indirect), and she had heavy hemoglobinuria. Prednisone was increased to 100 mg daily, and she was referred to the University of California, San Francisco, for further evaluation.

The patient's past medical history and review of systems were unremarkable except for mild labile hypertension, intermittently treated with reserpine and thiazides, and "hypothyroidism" treated with thyroglobulin (Proloid). There was no past history of syphilis, and no symptoms suggestive of Raynaud's phenomenon or other vasomotor abnormality.

On physical examination, the patient was moderately obese and pale, but otherwise appeared relatively healthy. She was in no distress. Her temperature was 38.0°C, pulse was 100, respirations were 20 and blood pressure was 140/80. Examination was otherwise within normal limits; there was no lymphadenopathy or hepatosplenomegaly.

Blood tests revealed a hematocrit of 25%, hemoglobin of 8.1 g/100 ml, and reticulocytes of 15.8%; the white cell count was 10,600/cu mm (78% neutrophils, 18% lymphocytes...
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and 4% monocytes), and the platelet count was 312,000/cu mm. Blood smear showed moderate anisocytosis and polychromatophilia. The urine was dark reddish-brown and contained free hemoglobin and hemosiderin. Stool specimens contained no occult blood. Total serum bilirubin was 4.1 mg/100 ml (3.0 mg/100 ml indirect), lactic dehydrogenase was 141 IU/liter, serum glutamic oxaloacetic transaminase was 41 IU/liter, serum glutamic pyruvic transaminase was 49 IU/liter, alkaline phosphatase was 154 IU/liter, creatinine phosphokinase was 49 IU/liter, uric acid was 7.2 mg/100 ml and fasting blood glucose was 123 mg/100 ml. Serum electrolytes, calcium, phosphorus, and creatinine were normal. Serum total hemolytic complement and complement component C3 were normal; serum protein electrophoresis and immunoelectrophoresis appeared normal. Haptoglobins were absent. Tests for antinuclear antibody, thyroid antibody, rheumatoid factor, and heterophile antibody, and serologic tests for syphilis were all negative. Serum iron, total iron-binding capacity, and vitamin B12 and folate levels were normal. Red-cell glucose-6-phosphate dehydrogenase activity and osmotic fragility were normal. Thyroid function tests were normal. Routine direct antiglobulin test using commercial broad-spectrum antiserum was weakly positive; indirect antiglobulin test was negative. Cold hemagglutinin titer was 1:16; Ham’s test was negative.

Chest and abdominal X rays and electrocardiogram were normal. Cultures of blood, urine, sputum, and stool grew no pathogens, and PPD and fungal skin-sensitivity tests were negative. Bone marrow showed marked erythroid hyperplasia and diminished iron stores. Imaging of liver, spleen and bone marrow with 99Tc-sulfur colloid showed a normally sized liver and spleen, and peripheral extension of the active bone marrow.

The patient’s treatment was directed toward keeping her as warm as possible, with strict avoidance of even minimal exposure to cold. She was kept warmly dressed at all times, wore long cotton gloves and stockings, and was encouraged to rest, covered up, in bed frequently during the day. Her low-grade fever and hemoglobinuria disappeared and her anemia improved in this controlled environment, although intermittent mild night sweats persisted. Her dose of prednisone was gradually reduced to 20 mg daily without evidence of increased hemolysis. Three weeks after admission, while taking 20 mg of prednisone daily, her hematocrit was 35%, her hemoglobin was 11.4 g/100 ml, she had 4.4% reticulocytes, and her total bilirubin was 1.2 mg/100 ml (1.0 mg/100 ml indirect). She was discharged from the hospital on prednisone (reduced to 5 mg daily), iron, and folate, and was instructed to take the same precautions against cold as in the hospital.

She again had intermittent hemoglobinuria after returning home, and her night sweats increased in frequency and intensity. By this time the outdoor temperatures were gradually becoming cooler, with nighttime temperatures of 2–5°C (35–40°F). Although she remained warmly dressed and avoided cold, as instructed, she felt uncomfortably warm if the thermostat in her home was set higher than 18°C (65°F). Two weeks after discharge from the hospital, her hematocrit was down to 24%, her hemoglobin was 7.9 g/100 ml, and she had 15.3% reticulocytes. Prednisone was increased to 20 mg daily, and 2 wk later her hematocrit was 35%, her hemoglobin was 11.2 g/100 ml, and she had 6.3% reticulocytes. The hematocrit, hemoglobin, and reticulocytes have remained at these levels and there has been no further hemoglobinuria. The patient continues to take 20 mg of prednisone daily as maintenance therapy.

SPECIAL STUDIES

All blood samples were drawn into prewarmed syringes and immediately placed in a 37°C water bath. Serum was separated by centrifugation at 37°C, and was stored at −20°C if not tested immediately.

Direct and indirect antiglobulin tests were performed using broad-spectrum and specific antisera (to IgG, IgA, and IgM, and to complement components C3 and C4) prepared in the authors’ laboratories. Direct antiglobulin tests were done with serial dilutions of the antisera and suspensions of the patient’s red cells. Titers were expressed as the greatest dilution that produced definite agglutination.
Serial dilutions of the patient's serum were investigated for antibody activity by testing for agglutination and hemolysis of normal group O pooled red cells (Selectogen I and II, Ortho Diagnostic Division, Raritan, N.J.), and by the indirect antiglobulin test. Agglutination and monophasic lysis were determined after incubation of serum with normal red cells for 60 min at 0, 5, 10, 15, 20, 25, 30, 32, 35, and 37°C; biphasic lysis and the indirect antiglobulin test (antiserum to C3 and IgG) were determined after rewarming the incubation mixtures to 37°C for 30 min. Papain-treated red cells were also tested, and the effects of serum acidification (final pH 6.5) and addition of fresh compatible serum (complement) before incubation were determined.

Blood group specificity of the antibody was determined by testing for agglutination and lysis against a panel of 10 group O red cell samples representing most genotypes (Spectra Biologicals, Oxnard, Calif.), fetal red cells (group Oi cord), and two samples of red cells of the rare type Pk and one of the type pp.

Serum proteins were separated by starch-block electrophoresis followed by gel filtration on a Sephadex G-200 column, using standard techniques. The purity of the IgG and IgM fractions was confirmed by immunoelectrophoresis and ultracentrifugation; the two fractions were dissolved in normal saline at physiologic concentrations and tested for hemolytic and agglutinating activity.

Results

The direct antiglobulin test was strongly positive (titer 1:128). Testing with specific antisera showed that this was due to the presence of complement component C3 on the red cells; no IgG, IgA, IgM, or C4 was detected.

The results of initial testing of the patient's serum at 0, 20, and 37°C are shown in Table 1. Hemagglutination was strongest at 0°C, moderate at 20°C, and undetectable at 37°C. Hemolysis was observed only at 20°C. The indirect antiglobulin test could not be interpreted at 0°C because of the strong cold agglutination. It was strongly positive with antiserum to C3 and weakly positive with antiserum to IgG at 20°C; it was negative with both at 37°C. Hemolysis was slightly enhanced by the addition of fresh compatible serum (complement), but not by acidification (adjusted to pH 6.5). Papain-treated red cells were slightly more susceptible to both agglutination and hemolysis than untreated cells.

Standard direct and indirect D-L tests were strongly positive to a titer of 1:64. Cold hemagglutination titers were 1:64 at 0°C and 1:8 at 20°C. When

| Table 1.—Results of Initial Testing of Patient’s Serum for Antibody Activity |
|-----------------|-----------------|-----------------|-----------------|
|                | 0°C             | 20°C            | 37°C            |
|                | S               | AS              | S+C            | AS+AC          | S               | AS              | S+C            | AS+AC          |
| Untreated red cells |                |                 |                 |                 |                |                 |                 |                 |
| Agglutination    | 4+ 4+ 4+ 4+     | 2+ 2+ 2+ 2+     | 2+ 2+ 2+ 2+     | 2+ 2+ 2+ 2+     | 0 0 0 0        | 0 0 0 0        | 0 0 0 0        | 0 0 0 0        |
| Lysis           | 0 0 0 0         | 2+ 2+ 3+ 3+     | 0 0 0 0         | 0 0 0 0         | 0 0 0 0        | 0 0 0 0        | 0 0 0 0        | 0 0 0 0        |
| Indirect antiglobulin test* |                |                 |                 |                 |                |                 |                 |                 |
| Antiserum to IgG | 1+ 1+ 1+ 1+     | 1+ 1+ 1+ 1+     | 0 0 0 0         | 0 0 0 0         | 0 0 0 0        | 0 0 0 0        | 0 0 0 0        | 0 0 0 0        |
| Antiserum to C3  | 4+ 4+ 4+ 4+     | 4+ 4+ 4+ 4+     | 0 0 0 0         | 0 0 0 0         | 0 0 0 0        | 0 0 0 0        | 0 0 0 0        | 0 0 0 0        |
| Papain-treated red cells |                |                 |                 |                 |                |                 |                 |                 |
| Agglutination    | 4+ 4+ 4+ 4+     | 4+ 4+ 4+ 4+     | 4+ 4+ 4+ 4+     | 4+ 4+ 4+ 4+     | 0 0 0 0        | 0 0 0 0        | 0 0 0 0        | 0 0 0 0        |
| Lysis           | 0 0 0 0         | 3+ 3+ 4+ 4+     | 0 0 0 0         | 0 0 0 0         | 0 0 0 0        | 0 0 0 0        | 0 0 0 0        | 0 0 0 0        |

S, Untreated patient's serum; AS, patient's serum + 1/10 vol N/5 HCl (final pH 6.5); C, fresh compatible serum (complement); AC, fresh compatible serum + 1/10 vol N/5 HCl (final pH 6.5). * The indirect antiglobulin test could not be interpreted at 0°C because of strong cold agglutination.
serial dilutions of the patient’s serum were incubated with group O pooled red cells for 60 min at various temperatures between 0 and 37°C, agglutination was inversely related to the temperature of incubation (Fig. 1). Monophasic hemolysis (hemolysis on cold incubation only) did not occur below 10°C, was maximal between 15 and 20°C (titers 1:16), and gradually declined above 20°C, although weak hemolysis persisted up to 32°C. When serum dilutions were tested for biphasic hemolysis with a modified D-L test (cold incubation at various temperatures for 60 min, followed by warm incubation at 37°C for an additional 30 min), maximal hemolysis occurred after the initial incubation was performed at 0–5°C (titer 1:64), with titers gradually falling as initial incubation temperatures were increased. The curve for red cell sensitization by complement after biphasic incubation, as measured by the indirect antiglobulin test using antiserum to C3, closely resembled the curve for biphasic hemolysis. Below 20°C, biphasic hemolysis was more marked (titer 1:64) than monophasic hemolysis (titer 1:16), but the two curves became identical at 20°C and above. Both biphasic and monophasic hemolysis occurred up to 32°C.

At 20°C, the patient’s serum caused agglutination and lysis of the entire panel of group O erythrocytes, regardless of Rh genotype, or whether they were type P1 or P2. Fetal red cells (group Oi cord) agglutinated and lysed to the same extent as adult cells (Oi). No agglutination or hemolysis occurred when the patient’s serum was tested at 20°C with the rare types Pk and pp red cells, and the indirect D-L test was negative with these cells. Thus, the specificity of the cold antibody was shown to be directed against P, with both agglutination and hemolysis mediated by the same antibody.

When the patient’s serum proteins were separated by starch-block electrophoresis and gel filtration, the hemolytic and agglutinating activity was found to reside in the fraction identified as IgG by immunoelectrophoresis and ultracentrifugation. No antibody activity against red cells could be identified in the IgM fraction.
DISCUSSION

Although this patient’s paroxysmal cold hemoglobinuria had certain features resembling high-titer cold hemagglutinin disease, accurate differentiation was readily accomplished in the laboratory. Complement fixation, with spontaneous elution of the antibody after warming (resulting in a “complement only” direct antiglobulin test), occurs in both syndromes; however, complement is fixed by an IgG antibody in PCH and by an IgM antibody in cold hemagglutinin disease.2,4,12 The D-L antibodies of PCH are characteristically potent hemolysins, even at low titers, and only weak hemagglutinins; the antibodies of cold hemagglutinin disease are strong agglutinins and weak hemolysins, producing hemolysis only in the presence of very high agglutination titers.2,13 Hemolysis is enhanced by acidification of the serum in hemagglutinin disease, but not in PCH.4 Finally, the two syndromes have distinct blood group specificity: the antibody of PCH is directed against P, while the antibodies of cold hemagglutinin disease show specificity within the Ii system.2,8 The present case satisfied all these criteria for distinguishing it from cold hemagglutinin disease.

The finding of monophasic hemolysis, however, is not typical for PCH. Classically, the autohemolysin of PCH is described as biphasic, since it requires a cold phase for antibody binding and initial activation of complement, and a warm phase during which complement-mediated lysis occurs.4,6 In contrast, hemolysis in cold hemagglutinin disease is classically described as being monophasic, occurring if the red cell suspension is simply allowed to stand at 15–20°C.12 Schubothe12 and van Loghem and associates14 have emphasized these two characteristic thermal patterns to differentiate PCH from cold hemagglutinin disease. Dacie, however, points out that monophasic hemolysis can also occur in PCH if the thermal range of antibody binding is high enough.1,2 Our case dramatically illustrates this point.

MacKenzie found the maximal temperatures for antibody binding in PCH to be 10–12°C,15 temperatures at which hemolytic complement would not be expected to be very active. Schubothe9 and Dacie1 considered 20°C to be the highest temperature allowing antibody binding in PCH, with the exception of one case reported by Dacie that had a thermal range up to 25°C. Antibodies with these higher thermal ranges would be expected to produce some degree of monophasic hemolysis, since complement would be active within the thermal range of antibody binding. Our patient’s D-L antibody was capable of binding to red cells at temperatures as high as 32°C, thereby producing vigorous monophasic hemolysis.

Dacie has emphasized that PCH is a chronic, relatively benign disease.1,2 Chronic hemolytic anemia without symptoms may occur, although paroxysmal attacks associated with constitutional symptoms are most typical. The severity of our patient’s hemolytic anemia, particularly in the absence of significant exposure to cold, is distinctly unusual, and we propose that it is a direct result of the unique high thermal range of her autohemolysin. Barcroft and Edholm have shown that the temperature of skin and subcutaneous tissues of the human forearm can drop to 25°C with even minimal exposure to environ-
mental cold.\textsuperscript{16} Even during the hot summer months, our patient was exposed to nighttime temperatures between 18 and 24°C (65 and 75°F)—well within the range of activity of her hemolysin. It is of interest that the patient's most dramatic constitutional symptom, the drenching night sweats that occurred with unusual regularity between 1 and 4 a.m., occurred at a time when both her body temperature and the environmental temperature would be expected to be at their lowest during a 24-hr period. The delay between presumed exposure to cold (1-4 a.m.) and hemoglobinuria (11 a.m.) is consistent with the 4-hr to 10-hr interval between exposure to cold and hemoglobinuria observed by Becker\textsuperscript{17} and others.

The treatment of PCH has received relatively little recent attention. The rarity of the disease makes controlled therapeutic trials with adequate numbers of patients difficult. In addition, prophylactic avoidance of cold is sufficient to control paroxysmal attacks and correct anemia in most cases of idiopathic PCH.\textsuperscript{1,2} PCH associated with syphilis generally responds to penicillin.\textsuperscript{1} Corticosteroids have not generally appeared to be of value, and Dacie has suggested that they not be used in chronic cases of the idiopathic type.\textsuperscript{1,2} Fisher\textsuperscript{5} recently reported an inconclusive trial of combined immunosuppressive therapy (steroids and azathioprine) in a patient with nonsyphilitic PCH. Splenectomy is not usually of value in PCH,\textsuperscript{1,3} although occasional long-term remissions have been reported.\textsuperscript{5,18}

Steroids did have a beneficial effect on hemolysis in our patient (Fig. 2), although not to the same extent as successful environmental control. During our patient's initial hospitalization, her anemia appeared to improve remarkably with high doses of steroids, but subsequent events suggest that environmental control during hospitalization may have been more important. Marked hemolysis recurred when she returned home, despite maintenance therapy of 80 mg of prednisone daily. During her subsequent hospitalization at this institution, strict prophylaxis against cold was emphasized therapeutically, and

\begin{figure}
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\includegraphics[width=\textwidth]{fig2}
\caption{Course of the patient's hemolytic anemia. Periods of hospitalization (double horizontal arrow) and dosage of prednisone (hatched area) are shown. Arrow pointing downward, transfusion with two units of packed red blood cells; asterisk, dates when Donath-Landsteiner antibody titers were measured. D-L antibody titers were 1:64 at each determination.}
\end{figure}
her anemia gradually improved, despite a simultaneous reduction to 20 mg of prednisone daily. When she again returned home and her prednisone was further reduced to 5 mg daily, hemolysis increased abruptly, but decreased when prednisone was increased to 20 mg daily, even through she was not re-hospitalized. Careful questioning of the patient elicited no other apparent explanation for the increased severity of the hemolysis when she was at home, except that she felt uncomfortable when her house was warmer than 18° C (65°F). Steroids appeared to produce their beneficial effect in our patient by interfering with sensitization of red cells, with destruction of red cells or with combination of these events in vivo, since there was no demonstrable reduction in D-L antibody titers.

Based on the response of our patient, we recommend that the mainstay of therapy in patients with PCH be the creation of a stable, warm environment, with strict attention to the avoidance of even minimal cold; steroids should be reserved for those patients with severe hemolytic anemia who cannot or will not maintain strict environmental control.

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