CONCISE REPORT

Chronic Hemolytic Anemia Due to a Monoclonal IgG Cold Agglutinin With Anti-Pr Specificity

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Immunologic studies performed in a case of autoimmune chronic hemolytic anemia with low titer cold agglutinins (1/16) demonstrated that the cold agglutinins corresponded to monoclonal IgG with anti-Pr specificity. This antibody had a large thermal amplitude, being active at 37°C. These unusual characteristics may define a distinct subset of chronic cold agglutinin disease.

The chronic cold agglutinin disease (CCAD) is usually characterized by the presence in the serum of a monoclonal IgM antibody directed to the Li, Pr, Gd, or Sa red cell antigens. The monoclonal anti red cell antibody may occasionally belong to the IgA class and in a single case reported so far to the IgG class.

We report here immunologic studies performed in a patient with a chronic autoimmune hemolytic anemia and low titer cold agglutinins that were identified as monoclonal anti Pr IgG antibodies.

MATERIALS AND METHODS

Case Report

This 33-year-old woman was first seen in October 1973 for a rapidly progressive anemia. There was no significant past history and a clinical examination revealed only pallor, icterus, and a slight splenic enlargement. There was no Raynaud phenomenon. Red blood cell count was 1.1 x 10^6/cu mm with hemoglobin 5 g/dl. WBC was 11,400/cu mm with 85% neutrophils, platelets were 220,000/cu mm. Reticulocyte count was 13%. Serum bilirubin was 30 μmole/dl. Haptoglobin level was decreased (10 mg/dl). A positive Coombs test for complement trapping. With a nonconjugated fraction of 25 μmole/dl. Haptoglobin level was decreased (10 mg/dl). A positive Coombs test for complement trapping. WBC was 1.1 x 10^6/cu mm with 85% neutrophils. platelets were 220,000/cu mm, neutrophils 85%. Serum bilirubin was 30 μmole/dl. Haptoglobin level was decreased (10 mg/dl). A positive Coombs test for complement trapping.

Prednisone therapy (2 mg/kg/day during the first month) resulted in an increase of hemoglobin levels with persistent hemolysis. Splenectomy was therefore performed in May 1974 and was followed by a partial improvement. A relapse occurred 4 mo later with anemia and hemoglobinuria. At this time, Coombs tests were intermittently positive with the anticomplement reagent. During the next 2 yr, a trial of immunosuppressive therapy with cyclophosphamide was followed by a complete remission of the hemolytic anemia and by decrease of the IgG spike level. Sixteen months after the end of treatment, anemia and hemoglobinuria recurred. At that time, extensive evaluation was essentially negative without any evidence of malignant disease. The serum IgG spike and immunoelectrophoretic pattern were unchanged compared to the 1973 results.

Special Studies

Cold agglutinins titer and thermal amplitude were determined by mixing one drop of a 2% red cell suspension and one drop of serum dilutions followed by a 2-hr incubation at 4°C, 22°C, 32°C, or 37°C. Normal adult, cord and neuraminidase or bromelain treated O Rh negative red cells were used.

Reduction of 19 S antibodies was obtained by incubating serum samples with 0.2 M 2 mercaptoethanol for 1 hr at 22°C.

Isolation of the cold agglutinins from patient's or control serum was performed according to the following steps: the serum was decanted at 40°C and mixed with an equal volume of washed normal O Rh negative red cells; the mixture was chilled at 4°C for 30 min, centrigufed and the pellet washed with cold phosphate buffered saline (PBS). Antibodies were thereafter eluted in PBS at 37°C for 30 min. This procedure was repeated twice and the last eluate was concentrated by vacuum dialysis and studied for cold agglutinin titer and Ig classes.

Since the above experiments suggested that the IgG spike of the serum most probably contain the cold agglutinin activity, 1 ml of the patient's serum was completely absorbed on red cells stromas and the supernatant studied by immunoelectrophoresis.

RESULTS AND DISCUSSION

Our patient has a chronic autoimmune hemolytic anemia that was documented by isotopic studies; the Coombs test, however, was only intermittently positive with the anticomplement reagent only. There was no increase of the cold agglutinin titer (1/16) although there was a prominent autoagglutination of red cells soon after blood collection in the absence of cryoglobulin. The latter finding prompted us to perform detailed immunochemical studies of the patient's cold agglu-
tinins, especially as a small IgG spike (0.3 g/dl) was detectable in the serum.

Indeed, the cold agglutinins had several unusual characteristics. Their specificity was directed against the Pr red cell antigen as shown by the agglutination of both adult and cord red cells, whereas bromeline or neuraminidase treated erythrocytes were not agglutinated. The titer of the cold agglutinin was not reduced by mercaptoethanol treatment of the serum and its thermal amplitude was abnormally large: 1/16 at 4°C, 1/8 at 22°C, 1/4 at 32°C, and 1/1 at 37°C. These results suggested that we were dealing with IgG antibodies with specificity against Pr antigen. In order to test the possibility that the serum monoclonal IgG kappa carried the anti red cell antibody activity, the following experiments were performed. The absorption of all cold agglutinin activity of the patient’s serum with red cells stromas resulted in the disappearance of the monoclonal IgG as shown by comparative immunoelectrophoretic analysis of the supernant and the original serum at similar concentrations (Fig. 1). The cold agglutinins purified by absorption elution on red cells were studied by Ouchterlony immunodiffusion tests and immunoelectrophoresis. As expected, the cold agglutinins from normal serum were polyclonal IgM with anti I specificity. By contrast, cold agglutinins from the patient’s serum corresponded only to monoclonal IgG kappa molecules that had the same electrophoretic mobility as the serum monoclonal IgG (Fig. 2). The eluate (0.02 g/dl) did agglutinate red cells at a titer of 1/4. Moreover, a positive Coombs test for IgG was found when normal red cells had been sensitized in cold with subagglutinating doses of the purified cold agglutinin. Taken together, these results provided firm evidence that the chronic hemolytic anemia of our patient was related to low

Fig. 1. Immunoelectrophoresis of patient’s serum before (a) and after (b) absorption on red cell stromas. (c): anti IgG serum. Arrow indicates monoclonal IgG.

Fig. 2. Immunoelectrophoresis of patient’s serum (a) and purified cold agglutinin (b) with anti IgG serum (c). Arrow indicates monoclonal IgG.
titer monoclonal IgG cold agglutinins with anti-Pr activity. Monoclonal cold agglutinins of the IgG class with anti-I specificity have been reported in a single patient with chronic hemolytic anemia.\(^3\) IgG with anti-Pr\(\alpha\) antibody activity have been found so far only transiently in patients with viral infection.\(^4\) Our patient’s hemolytic anemia bears great similarity to the subset of CCAD, featured by low titer IgM cold agglutinin.\(^5\) In such cases, the most striking finding is a large thermal amplitude of the antibody that is active up to 37°C as in our case. The low titer of cold agglutinin is in sharp contrast to the finding of a monoclonal serum IgG (0.3 g/dl). Such a lack of correlation between the monoclonal IgM level and the cold agglutinin titer has previously been observed in some patients with IgM cold agglutinin disease.\(^6\)

It should be stressed that in the absence of a marked increase of the cold agglutinin titer, detailed immunologic studies were needed to reach the correct diagnosis of CCAD in our patient who otherwise would have been labeled as a case of extracorpuscular hemolytic anemia without detectable antired cell antibodies. The 7-yr history of hemolytic anemia is reminiscent of the evolution of classic CCAD with failure of both steroid therapy and splenectomy. The complete but transient remission obtained under cytoxan therapy is therefore of interest and has been already observed in CCAD.\(^7,8\)

REFERENCES

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