Auto-immune Haemolytic Anaemia and Paroxysmal Nocturnal Haemoglobinuria Red Cell Abnormality in the Same Patient

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SUMMARY

This report concerns a young Indian woman with idiopathic auto-immune haemolytic anaemia. The erythrocytes repeatedly gave positive tests for the paroxysmal nocturnal haemoglobinuria abnormality, as well as positive antiglobulin tests. The possible reasons for this unusual association are discussed.

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The association of a positive antiglobulin (Coombs) test and positive tests for paroxysmal nocturnal haemoglobinuria (PNH) on the red cells of the same patient appears to have been recorded twice. Hill *et al.*[1] described a White man who developed a positive acidified serum test during the course of a lymphoproliferative malignant disease as well as having a concomitant auto-immune haemolytic anaemia. The authors point out that the positive acidified serum test was not characteristic of PNH in that it was negative with the patient's own serum. Dameshek and Fudenberg[2] described a classical case of PNH with a transient positive Coombs test.

We report a patient whom we observed for 4 years, who presented with an acute auto-immune haemolytic anaemia. In addition to a persistently positive Coombs test, with specific red cell auto-antibodies, the acidified serum test and the sucrose haemolysis test were repeatedly positive.

CASE REPORT

A 24-year-old Indian woman was admitted to hospital in July 1969. Two months before admission, she first noticed shortness of breath on effort, palpitations and general tiredness. These symptoms became progressively more severe, and in addition, for a few days before admission, she experienced central chest pain related to effort. She was in good health before this illness and had been through two normal pregnancies, the second of which was in 1964.

On examination she was pale, jaundiced and pyrexial (38°C). There were no palpable lymph nodes. The extremities were warm, the pulse rate 126/min and the blood pressure 120/60 mmHg. The heart was normal in size, hyperdynamic, and an ejection systolic murmur was heard. The liver was enlarged to 4 cm and the spleen to 2 cm below the costal margin, and both were smooth and non-tender. The respiratory system and the nervous system were clinically normal. The optic fundi were normal in appearance.

The initial haematological findings were: haemoglobin 4 g/100 ml; packed cell volume 13%, mean corpuscular haemoglobin concentration 33%; reticulocytes 42%; and white blood cell count 16 000/mm³ (neutrophils 78%, lymphocytes 18%, monocytes 3%, myelocytes 1%). The peripheral blood film showed anisocytosis, increased polychromasia and numerous microspherocytes. The platelets were normal in number and morphology.

The serum bilirubin level was 4,0 mg/100 ml (all unconjugated), and the alkaline phosphatase 7 King-Armstrong units. The total serum protein level was 7,1 g/100 ml (albumin 3,7 g/100 ml, globulin 3,4 g/100 ml). Haemoglobin electrophoresis was normal and the sickling test negative. Fetal haemoglobin was within normal limits. Glucose-6-phosphate dehydrogenase activity was normal and the Donath-Lansteiner test was negative. The direct Coombs test was strongly positive and a warm antibody with Rh 'e' specificity was present in the serum and could be eluted from the red cells. The osmotic fragility of the red cells was markedly increased and the acidified serum (Ham) test was positive as was the sucrose haemolysis test. The lupus erythematosus phenomenon and the fluorescent test for antinuclear antibodies were negative. A diagnosis of idiopathic auto-immune haemolytic anaemia was made.

Treatment with prednisone resulted in a steady rise in the haemoglobin to 10,1 g/100 ml and a fall in the reticulocyte count to 1,4% over 2 months. The dose was gradually reduced and adequate maintenance was possible on 5 mg prednisone daily.

Towards the end of 1969 she developed dyspeptic symptoms although no ulcer could be demonstrated radiographically. The steroid therapy was discontinued and she was treated with antacids. Six months later it was necessary

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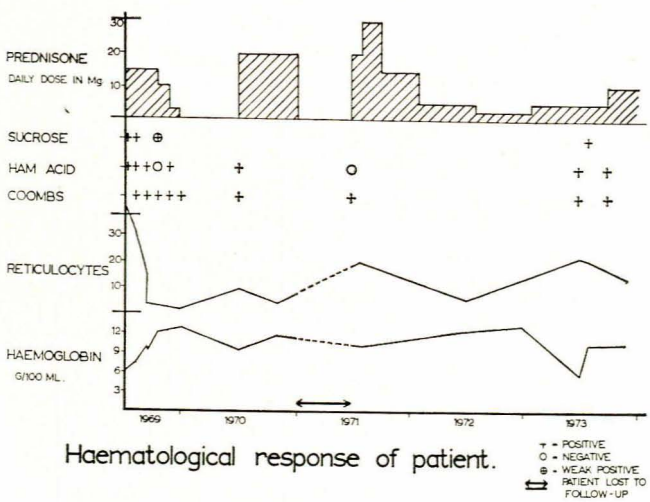
to reinstate prednisone therapy because of recurrence of severe anaemia. For the first 6 months of 1971 the patient did not attend follow-up clinic and stopped taking her treatment. When she was seen again, the haemoglobin had fallen and the reticulocyte count had risen. Treatment was restarted in June 1971 and has continued to the present time. The haemoglobin has remained between 10 and 13 g/100 ml on doses of prednisone ranging between 5 and 30 mg per day.

In July 1973, while the patient was taking 5 mg of prednisone daily, there was a recurrence of symptoms of anaemia, and she was again found to be pale and jaundiced. Examination of the abdomen revealed a 4-cm tender, enlarged liver and a 4-cm firm splenomegaly. The haemoglobin was 5,8 g/100 ml and the reticulocyte count 22%. The blood film contained many microspherocytes and polychromatic cells, as well as numerous macrocytes. The bone marrow showed erythroid hyperplasia with partial megaloblastic change. There were no features of hereditary erythroblastic multinuclearity with the positive acidified serum test (HEMPAS).[3]

The serum bilirubin was 3,6 mg/100 ml. The tests for haemolytic disorders were repeated and gave results essentially similar to those obtained on the first admission. In particular, the direct Coombs test, the Ham test and the sucrose haemolysis test were again positive. The red cell auto-antibodies were of the same specificity as those originally identified. Various additional tests were done at this stage. There was no evidence of intestinal malabsorption. Antibodies to intrinsic factor, gastric parietal cells, nuclei, thyroid and smooth muscle were not detected in the serum. The lupus erythematosus phenomenon was negative, as was the Wassermann reaction. A Schilling test demonstrated normal absorption of vitamin B_{12}. The red cell chromium half-life (^{51}Cr $T\frac{1}{2}$) was 6 days. External counting showed approximately equal sequestration of labelled red cells in liver and spleen. The serum complement level was normal. Immunoglobulin levels were: IgG 1 240, IgA 220 and IgM 100 mg/100 ml. The direct Coombs test was also carried out on the cells remaining unlysed at the end of the acidified serum test. It was found to be positive. Haemosiderin was not found in the urine.



Haematological response of patient.

Fig. 1. Haematological response of patient.

This recrudescence of haemolysis subsided spontaneously, without the need for an increase in prednisone dosage. The rise in haemoglobin may have been assisted by supplementary folate therapy.

Fig. 1 illustrates the course of the illness over $4\frac{1}{2}$ years. It will be seen that the Coombs test, Ham acidified serum test and sucrose haemolysis test were repeatedly positive.

DISCUSSION

The presenting features, response to treatment and course of this patient's illness, are compatible with the diagnosis of idiopathic auto-immune haemolytic anaemia. The laboratory findings in support of this diagnosis are clear-cut in that an auto-antibody of 'IgG' or 'warm' type, with Rh specificity for 'e' was found in the serum and in red cell eluates. No change has occurred in the antibody over 4 years, and the patient does not appear to have developed any of the other auto-antibodies which were sought. The associated finding of positive tests for the red cell abnormality of PNH, on a number of occasions, has not been described before. The presence of microspherocytes in the sample of red cells being tested may produce false positive acidified serum tests, since mere lowering of the pH may cause such cells to lyse. To exclude this possibility, the test should include the use of heat-inactivated acidified serum. This control was always included in tests on this patient's cells, and was consistently negative, showing that lysis was dependent upon pH reduction *and* the presence of complement.

There appear to be two possible explanations for the coincidence of the positive tests in this case. The first is that the antibody is damaging the red cell membrane and rendering the patient's cells susceptible to lysis under the same experimental conditions as those which lyse PNH erythrocytes. If so, it is surprising that the coincidence has not previously been reported. Large series of auto-immune haemolytic anaemia (AIHA), such as those of Dacie[4] and Pirofsky,[5] do not mention such cases. Morphological evidence of membrane damage in AIHA is provided by the frequent occurrence of microspherocytes. Biochemical evidence was provided by Sirchia *et al.*,[6] who found reduced cell acetylcholinesterase in AIHA, particularly when the sensitising antibody was an IgG. They state that, although the mechanism is not clear, auto-antibodies are able to modify red cell metabolism and that different types of antibodies do this in different ways. The variations may depend upon properties of individual antibodies or upon differences in density or configuration of antigens on the red cell surface.

The second possibility is that the patient has both diseases. The chance of auto-immune haemolytic anaemia and paroxysmal nocturnal haemoglobinuria occurring as separate diseases in the same patient would seem, on the basis of prevalence, very remote. There is however, a well-documented association between paroxysmal nocturnal haemoglobinuria and other haematological disorders,

such as aplastic anaemia and myeloblastic leukaemia.[7,8] It has been suggested that they may be pathogenetically linked on the basis of a somatic mutation at stem cell level. The same hypothesis could well explain the findings in this case.

In either event, it is somewhat surprising that the usual features of episodic or chronic intravascular haemolysis have not appeared. There has been no evidence of undue susceptibility to infection, despite long-term prednisone therapy, or of spontaneous thrombosis. However, it is well recognised that paroxysmal nocturnal haemoglobinuria may occur in an occult form.

The finding that the cells left unlysed in the acidified serum test still give a positive Coombs test does not help. This may mean that the cells which lyse in acidified serum are a separate, unsensitised population, or that they constitute part of the sensitised cell population. Had the reverse occurred, with only unsensitised cells being left in the acidified serum test, the first possibility, namely that the lysed cells were those with antibody-induced membrane damage, would have been supported. At present it does not seem possible to decide between these. The electron microscopic appearances of the cells may be of assistance, and we are undertaking this investigation.

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