HAEMOLYTIC ANAEMIA AND ERYTHROCYTE SENSITIZATION IN THE MALIGNANT RETICULOSES

WITH A REPORT OF THREE CASES

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Anaemia not infrequently occurs in patients suffering from various types of the so-called malignant reticuloses. The cause of the anaemia, when not due to extravascular blood loss, is problematical. Up till 50 years ago it was accepted as being the result of simple mechanical replacement of the erythropoeietic tissues. This view still has many adherents.[1-6] Hirschfeld,[7] in 1906, was the first to suggest that increased destruction of erythrocytes may play a part in the pathogenesis of the anaemia. During the past 20 years the haemolytic theory has become increasingly popular, mainly as the result of numerous reports of secondary haemolytic anaemia occurring in leukaemia,[8-17] Hodgkin's disease,[11, 18-23] lymphosarcoma,[14, 16, 21, 24] reticulum-cell sarcoma,[24] reticuloendotheliosis,[17] histiocytic medullary reticulosis,[24] giant follicular lymphoblastoma,[14] and multiple myelomatosis.[23] With the development of reliable techniques, shortening of the life span of the red blood cells has been demonstrated to be a significant feature in patients suffering from the malignant reticuloses.[25-28] Seaman *et al.*[29] in analysing 212 cases of chronic lymphatic leukaemia noted the presence of overt leukaemia in 52 patients. They concluded that the development of haemolytic anaemia appeared to be a random effect developing as a function of the duration of the leukaemia, with one case of haemolytic anaemia occurring for every 231 months of leukaemic life.

The cause of the increased rate of red blood-cell destruction has been the subject of many speculations and investigations. It has been attributed to phagocytosis of erythrocytes by proliferating histiocytes,[11] to spherocytosis of erythrocytes with resultant increased fragility,[14] to increased splenic activity,[25, 30] and to the haemolytic effect of metabolites generated by proliferating lymphadenomatous or reticulosarcomatous tissue.[30] With the development of immunohaematological techniques during the last decade it has become evident that in many instances the associated haemolytic anaemia has an immunological basis, as evidenced by erythrocyte sensitization (positive direct Coombs test) and, in some cases, the demonstration of circulating anti-erythrocyte antibodies.[15, 24, 26, 30-33]

Various suggestions have been made to explain the development of 'auto-antibodies' by an individual against components of his own tissues. Proof for any of these theories is still lacking, and as yet no single hypothesis has gained universal acceptance. The disease process may in some way alter the red blood cells, causing them to become auto-antigenic, and resulting in the formation of antibodies directed specifically against erythrocytes. It is also possible that the tissue proteins in the pathological lesion may be altered to become auto-antigenic, yet retaining sufficient immunological resemblance to the original unmodified tissue protein to enable cross reactions to occur between the antibody and the unmodified protein. The erythrocyte sensitization (and the haemolytic anaemia) would then be part of a generalized autoimmune process, affecting many of the body's tissues. Such a generalized immunological process could well be the explanation of the 'toxic' effect of certain diseases. It has also been suggested that 'foreign' protein generated in the pathological tissue could combine with tissue haptene. The specificity of such complexes of foreign protein and tissue haptene would be determined by the haptene.

CASE REPORTS

Case 1

The patient was a middle-aged European male, suffering from chronic lymphatic leukaemia. When he first came under observation the haemoglobin concentration was 10·0 g. per 100 ml. There were 229,500 leucocytes per c.mm., with 2·5% neutrophil polymorphonuclear cells, 0·5% basophil polymorphonuclear cells, 9·5% prolymphocytes, 84·0% lymphocytes and 3·5% 'blast' cells. The red blood cells were normochromic, and showed anisocytosis and increased diffuse polychromasia. The reticu-

locyte count was 2·6%. Examination of an aspirated sample of sternal marrow showed a total of 395,000 nucleated cells per c.mm., with a myeloid erythroid ratio of 4·8 : 1. There was a predominance of cells of the lymphocytic series (lymphocytes 62·0%, prolymphocytes 15·6% and lymphoblasts 3·2%).

The patient was under observation for 10 months before he died. During this period he became severely anaemic and required repeated blood transfusions. The reticulocyte count fluctuated between 2·4 and 36·0%. Increased excretion of urobilin was demonstrated in the urine, and the serum bilirubin concentration was 2·7 mg. per 100 ml., of which 0·3 mg. was direct-reacting. There had been no significant extravascular blood loss.

Immuno-haematological investigations. These were carried out for the first time approximately 1 month before the patient died. The direct Coombs test was positive, and the quantitative Coombs test gave a reaction of the 'warm' antibody type (Table I). Anti-

TABLE I. THE QUANTITATIVE COOMBS REACTION (CASE 1)

Dilutions of antiglobulin serum

1 *in* 4	1 *in* 16	1 *in* 64	1 *in* 256	*Saline control*
+	+++	+++	++	—

+ denotes weak agglutination; +++ denotes strong agglutination.

erythrocyte antibodies were demonstrable in the patient's serum against ficinated cells as well as with the indirect Coombs test, the reactions being stronger at 37°C than at room temperature (Table II). The antibodies did not agglutinate trypsinized cells

TABLE II. THERMAL AMPLITUDE OF ANTIBODY (CASE 1)

(using ficinated cells; sensitization for 1 hour)

Dilutions of the patient's serum

	1 *in* 1	1 *in* 2	1 *in* 4	1 *in* 8	1 *in* 16	1 *in* 32
Room temp.	++	+	+	(++)	(+)	—
37°C	++	++	+	+	(++)	(±)

++ denotes moderately strong macroscopic agglutination; (+) denotes weak microscopic agglutination; (±) denotes doubtful agglutination.

and haemolysins and cold saline haemagglutinins could not be demonstrated. An eluate prepared from the patient's sensitized cells showed a similar reaction, antibodies being demonstrable against ficinated cells and with the indirect Coombs test (but not against trypsinized cells), and showing greater activity at 37°C than at room temperature.

Summary: A case of chronic lymphatic laukaemia with a secondary immuno-haemolytic anaemia of the 'warm' antibody type.

Case 2

The patient, a European female aged 60 years, complained of lassitude and anorexia. These symptoms had started 3 months previously, and had become progressively more severe. The significant findings on clinical examination were cervical and axillary lymphadenopathy, splenomegaly and anaemia. Lymphosarcoma was diagnosed on histological examination of a cervical lymph node.

After the patient had been in hospital for 10 days an increase in the pallor of the conjunctivae and the oral mucous membranes was noted, although she had not suffered any extravascular blood loss. Haematological investigation showed the haemoglobin concentration to be 7·4 g. per 100 ml., the PCV 25·5% and the MCHC 29·0%. There were 13,200 leucocytes per c.mm., with 71·5% neutrophil polymorphonuclear cells, 10·5% monocytes, 16·5 lymphocytes and 1·5% eosinophil polymorphonuclear cells. The red blood cells were normochromic, and showed anisocytosis, poikilocytosis and increased diffuse basophilia. The platelets appeared to be reduced in numbers. The reticulocyte count was 2·7%. Urine analysis showed an increased excretion of bile pigments, and the serum-bilirubin concentration was 1·6 mg. per 100 ml., of which 0·2 was direct-reacting.

Immuno-haematological investigation. The specimen of blood showed spontaneous auto-agglutination at room temperature. The direct Coombs test was strongly positive, and the quantitative Coombs test gave a reaction of the 'cold' antibody type (Table III). An abnormal anti-erythrocyte antibody in the patient's

TABLE III. THE QUANTITATIVE COOMBS REACTION (CASE 2)

Dilutions of antiglobulin serum

1 *in* 4	1 *in* 16	1 *in* 64	1 *in* 256	*Saline control*
+++	++	+	±	—

+++ denotes strong agglutination; + denotes weak agglutination.

serum was demonstrable against red cells suspended in saline, as well as against enzyme-treated cells. The antibody reaction was stronger at room temperature than at 37°C (Table IV). The

TABLE IV. THERMAL AMPLITUDE OF ANTIBODY (CASE 2)

Dilutions of the patient's serum

		1 *in* 1	1 *in* 2	1 *in* 4	1 *in* 8	1 *in* 16	1 *in* 32	1 *in* 64
37°C	A	(+)	—	—	—	—	—	—
	B	+	(+)	(±)	—	—	—	—
	C	++	++	+	(++)	(+)	—	—
Room Temp.	A	(+)	(±)	—	—	—	—	—
	B	+	(++)	(+)	(±)	—	—	—
	C	++	++	++	+	(++)	(+)	—
4°C	A	+	(+)	—	—	—	—	—
	B	++	(++)	(+)	(+)	(+)	—	—
	C	+++	++	++	+	+	(+)	(+)

+++ denotes strong macroscopic agglutination; (+) denotes weak microscopic agglutination; (±) denotes doubtful microscopic agglutination.
A = Cells suspended in saline. B = Trypsinized cells. C = Ficinated cells.

activity of the antibody against enzyme-treated red cells as well as cells suspended in saline was not affected by inactivation of the complement in the patient's serum. Haemolysins could not be demonstrated, and the cold saline haemagglutinin titre was within normal limits.

Treatment and progress. The patient was transfused with 1,000 ml. of packed cells, without any untoward reaction, and treated with Meticorten (30 mg. per day). The haemoglobin concentration after the transfusion was 13·0 g. per 100 ml., and the reticulocyte count 7·2%. For 3 weeks the patient required a blood transfusion every 2-3 days, by means of which the haemoglobin concentration was maintained in the region of 10 g. per 100 ml. During this period the dose of the Meticorten was gradually increased. When the dose of 80 mg. per day was reached, there was a dramatic response with a marked diminution in the severity of the haemolytic process. At this stage X-ray therapy directed to the spleen was commenced. This resulted in a noticeable reduction in the size of the spleen and the lymph nodes. The intervals between transfusions became progressively longer, until after 3 weeks of this treatment the haemoglobin concentration remained between 10 and 11 g. per 100 ml. without any further blood transfusions. Despite the virtual cessation of the haemolytic process the patient's general condition deteriorated and she died about 3 months after admission to hospital. Two days before she died normoblasts appeared in her peripheral blood for the first time.

Summary. A case of lymphosarcoma with a secondary immuno-haemolytic anaemia of the 'cold' antibody type.

Case 3

The patient, a Bantu male aged 23 years, was admitted to hospital complaining of persistant headache and generalized body pains for 14 days. On clinical examination the significant findings were anaemia, enlargement of the liver and spleen, slight cervical lymph-node enlargement, and a temperature of 102°F. The haemoglobin concentration was 8·3 g. per 100 ml., the PCV was 24% and the MCHC 34%. There were 3,200 leucocytes per c.mm., with 26% neutrophil polymorphonuclear cells, 18% monocytes, 55% lymphocytes and 1% eosinophil polymorphonuclear cells. The red blood cells were normochromic, and showed anisocytosis and increased diffuse polychromasia. The reticulocyte count was 1·8%, and there were 2 normoblasts per 001 leucocytes. The bone marrow was hyperplastic, with a myeloid-

erythroid ratio of 0·82 : 1. The serum-bilirubin concentration was 0·2 mg. per 100 ml. Four weeks after admission to hospital Hodgkin's disease was diagnosed on histological examination of a cervical lymph node.

Immuno-haematological investigation. Shortly after admission to hospital the direct Coombs test was negative. During the first week in hospital a gradual fall in the haemoglobin level occurred; this was associated with increased excretion of bile pigments in the urine. There had been no extravascular blood loss, and the patient had not received any blood transfusions. The direct Coombs test was repeated, with a positive result. The quantitative Coombs test gave a reaction of the 'warm' antibody type (Table V). Anti-erythrocyte antibodies could not be demonstrated against enzyme-treated cells or with the indirect Coombs technique, and the cold saline haemagglutinin test was negative. Haemolysins could also not be demonstrated.

TABLE V. THE QUANTITATIVE COOMBS REACTION (CASE 3)

Dilutions of antiglobulin serum				
1 *in* 4	1 *in* 16	1 *in* 64	1 *in* 256	*Saline control*
+	++	—	—	—

+ denotes weak agglutination; ++ denotes moderately strong agglutination.

Treatment and progress. After the diagnosis of Hodgkin's disease had been established, nitrogen mustard therapy and X-ray therapy directed to the spleen were started. The patient also received 90 mg. of Meticorten daily. Despite the maintenance of the haemoglobin concentration at approximately 8 g. per 100 ml. without blood-transfusion therapy, the patient died 7 weeks after admission to hospital.

Summary. A case of Hodgkin's disease with a secondary immuno-haemolytic anaemia of the warm antibody type.

DISCUSSION

It is interesting to note that the immuno-haematological pattern was not similar in these 3 cases. In case 2 the antibody appeared to be of the cold variety, whereas the antibody in the other 2 cases was of the warm variety, as evidenced by the prozone phenomenon exhibited by the quantitative Coombs test. Dacie[30] also found different immuno-haematological patterns in the 2 cases he investigated—one of chronic lymphatic leukaemia with an associated immuno-haemolytic anaemia of the warm antibody type, and one of reticulosarcoma with an associated immuno-haemolytic anaemia of the cold antibody type.

It should be pointed out that in case 1 the patient was only referred for immuno-haematological investigation after having received numerous blood transfusions. The immuno-haematological findings may therefore not be a true reflection of the pre-transfusion picture.

Erythrocyte Sensitization

Zoutendyk and Gear[34] carried out direct Coombs tests on the red cells of patients suffering from various diseases, including 1 case of lymphatic leukaemia and 3 of myeloid leukaemia; in 1 of the latter the direct Coombs test was positive. Rosenthal *et al.*[33] found a significant incidence of erythrocyte sensitization in patients suffering from chronic lymphatic leukaemia.

The results of direct Coombs tests carried out in 30 patients suffering from various forms of malignant reticulosis are detailed in Table VI. Although the number of cases of each of these disease entities is too small to furnish reliable conclusions, the results demonstrate a significant total incidence of erythrocyte sensitization (12 out of 30).

The significance of erythrocyte sensitization in these cases is not yet clear. Rosenthal *et al.*[33] consider a positive direct Coombs test to be indicative of a potential haemolytic anaemia. By the periodic use of this test in cases of chronic lymphatic leukaemia they consider it possible to uncover cases in which the haemolytic mechanism has already become established, but in which the haemolysis has not yet become clinically evident. In the series they studied they were able to predict, correctly, the development of haemolytic anaemia in 3 cases.

TABLE VI. ERYTHROCYTE SENSITIZATION IN VARIOUS TYPES OF MALIGNANT RETICULOSES

Disease	*Number Investigated*	*Direct Coombs Test positive*
Chronic lymphatic leukaemia ..	9	5
Chronic myeloid leukaemia ..	5	0
Acute myeloid leukaemia ..	2	1
Multiple myelomatosis	3	2
Hodgkin's disease	7	3
Lymphosarcoma	2	1
Unclassified malignant reticulosis	2	0
Total	30	12

It would appear that in these cases erythrocyte sensitization is indicative of an immunologic process, of which frank haemolytic anaemia is an extreme expression.

CONCLUSIONS

1. Frank haemolytic anaemia, very often of the immunologic type, may occur in patients suffering from various forms of malignant reticulosis. This is usually of serious import, heralding a terminal phase. The haemolytic process may be severe, requiring repeated blood transfusions. It is important to determine whether the haemolytic anaemia is of the immunologic type, for such an anaemia is usually controllable by adequate corticosteroid therapy.[35]

2. In some cases the haemolytic anaemia is the predominant feature, and the underlying disease may remain undetected until special studies such as sternal-marrow aspiration or lymph-node biopsy are undertaken. Haemolytic anaemia cannot be termed 'idiopathic' until the possibility of an underlying malignant reticulosis has been excluded.

3. As erythrocyte sensitization in a patient suffering from a malignant reticulosis may well be indicative of a potential haemolytic anaemia, it may be worth while carrying out direct Coombs tests in these patients as a routine procedure.

4. The immuno-haematological findings in cases of immuno-haemolytic anaemia complicating the various types of malignant reticulosis do not appear to follow a uniform pattern.

SUMMARY

Three cases of immuno-haemolytic anaemia complicating various forms of malignant reticulosis are presented. The significance of erythrocyte sensitization in cases not complicated by overt haemolytic anaemia is discussed.

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