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 erythropoietic tissues. This view still has many adherents. Hirschfeld, 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. Hodgkin's disease, lymphosarcoma, reticulum-cell sarcoma, reticuloendotheliosis, histiocytic medullary reticulosis, follicular lymphoblastoma, and multiple myelomatosis.

With the development of reliable techniques, shortage of the life span of the red blood cells has been demonstrated to be a significant feature in patients suffering from the malignant reticuloses. Seaman et al., 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, to spherocytosis of erythrocytes with resultant increased fragility, to increased splenic activity, and to the haemolytic effect of metabolites generated by proliferating lymphadenomatous or reticulo-sarcomatous tissue. 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.

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 lesion could combine with tissue haptene. The specificity of such complexes of foreign protein and tissue hapten would be determined by the hapten.

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·% eosinophil polymorphonuclear cells, 9·5·% lymphocytes, and 3·5·% 'blast' cells. The red blood cells were normochromic, and showed anisocytosis and increased diffuse polychromasia. The reticulo-
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%, polymorphocytes 15:6% and lymphoblasts 3-5%).
The patient was under observation for 10 months before he
died. During this period he became severely anaemic and re-
quired repeated blood transfusions. The reticuloocyte count
fluctuated between 2:4 and 36:0%. Increased excretion of ur-
bilin was demonstrated in the urine, and the serum bilirbin
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-
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

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<th>TABLE I. THE QUANTITATIVE COOMBS REACTION (CASE 1)</th>
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<tr>
<td>Dilutions of antglobulin serum</td>
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<td>-+ denotes weak agglutination; +++ denotes strong agglutination.</td>
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and haemolysins and cold saline haemagglutins 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 leukemia 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. Lymph-

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<th>TABLE II. THERMAL AMPLITUDE OF ANTIBODY (CASE 1)</th>
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<td>(using ficinated cells; sensitization for 1 hour)</td>
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<td>Dilutions of the patient's serum</td>
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<td>1 in 1</td>
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<tr>
<td>Room temp.</td>
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<td>37°C</td>
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<td>± denotes weak macroscopic agglutination; (+) denotes moderate macroscopic agglutination.</td>
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**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 and the MCHC 34 %. There were 3,200 leucocytes per
c.mm., with 26% neutrophil polymorphonuclear cells, 18%
monocytes, 55% lymphocytes and 1% eosinophil polymorpho-
nuclear cells. The red blood cells were normochromic, and showed
anisocytosis, poikilocytosis and increased diffuse basophilia.
The platelets appeared to be reduced in numbers. The reticulo-
cyte count was 2-7%. Urine analysis showed an increased ex-
cretion of bile pigments, and the serum-bilirubin concentration
was 1-6 mg. per 100 ml., of which 0-2 mg. 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 quantita-
tive Coombs test gave a reaction of the 'cold' antibody type
(Table III). An abnormal anti-erythrocyte antibody in the patient's

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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).

**Summary:** A case of lymphosarcoma with a secondary immuno-
haemolytic anaemia of the 'warm' antibody type.
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 also was not determined.

**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 Meticoten 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 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 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. 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. 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.

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.

2. In some cases the haemolytic anaemia is the predominant feature, and the underlying disease may remain undetected until special studies such as sternal-marow 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.

I wish to express my gratitude to Prof. E. H. Cluver, Director of the South African Institute for Medical Research, and to Dr. A. Zoutendyk, head of the Blood Group Research and Transfusion Laboratories of this Institute, for the facilities put at my disposal to carry out these investigations.

**REFERENCES**

A CASE OF TRAUMATIC PERFORATION OF THE INTESTINE ASSOCIATED WITH INGUINAL HERNIA

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While the literature abounds with reports of ruptured bowel due to closed abdominal trauma, few cases are recorded of intestinal rupture caused by hyperextension or torsion of the trunk. There are still fewer reports of intestinal rupture due to muscular strain associated with inguinal hernia.

CASE REPORT

Mr. B.J.M., aged 28 years, was admitted to the professorial surgical unit of the Johannesburg General Hospital on 13 October 1957 at 4.30 a.m. complaining of severe abdominal pain. He stated he had drunk a large quantity of beer on the previous afternoon and evening and, while 'wrestling' with a friend at about 11.30 p.m., suddenly twisted his body to the left and immediately experienced a severe 'stitch' in his right side. This pain soon spread over his whole abdomen, particularly the lower part. The patient was quite positive that he had not received a direct blow to the abdomen. He also stated that the cause of experiencing his 'stitch', his right inguinal hernia had suddenly become painful and increased in size. This hernia had been present for the past 3 years and had remained in his groin as a constant lump which he had never attempted to reduce. The rest of the history was non-contributory except for an appendicectomy performed in 1951.

The patient was a well-built adult male in obvious pain. The temperature was normal, the pulse rate 96 per minute and the blood pressure 120.75 mm. Hg. Except for the abdomen, the general examination was negative. The abdomen did not move on respiration and exhibited board-like rigidity down the right side. The left side was extremely tender and rebound tenderness was present. Auscultation revealed no bowel sounds and an old right inguinal hernia scar was noted. The left inguinal region was normal but the right side contained a large inguinoscrotal hernia with no cough impulse. The swelling was tense and exquisitely tender and no attempt was made to reduce it. A presumptive diagnosis was made of incarcerated or strangulated right inguinal hernia and a ruptured intra-abdominal viscus or possible partial reduction en masse of the hernia.

Operation

Under general anaesthesia, a right inguinal incision was made and, on division of the external oblique aponeurosis, a tense, inguinoscrotal sac was delivered and isolated. The sac contained turbid greenish fluid suggestive of small-bowel contents and a large piece of greater omentum attached by firm adhesions to the fundus of the sac. There was no bowel in the sac and the neck was found to barely admit the tip of the little finger beside the protruding omentum. The redundant omentum was excised and the proximal piece was ligated and returned into the abdomen. The sac was ligated and the excess amputated. A Bassini repair with catgut was performed and the wound closed around a gloverubber drain.

The abdomen was then opened through a right lower paramedian incision. The peritoneal cavity contained fluid identical to that found in the hernial sac and about 200 c.c. was sucked out. On examination of the bowel, a small perforation of the ileum, 15 cm. from the ileo-caecal junction on the anti-mesenteric border, was discovered. The perforation was round, the edges punched out, and there was no sign of surrounding bruising or reaction of any kind. The perforation was closed with two layers of chromic catgut sutures. Further exploration revealed an absent appendix. The caecum was noted to be quite mobile and the area of perforated ileum was thought to have been lying at the back of the right internal ring. The abdomen was closed with a stab drain to the recto-vesical pouch and a drain to the paramedian wound.

The patient made an uneventful recovery.

DISCUSSION

Aird1, 2 has drawn attention to the association of traumatic perforation of the intestine and inguinal hernia. Obviously, in many cases the abdominal trauma is sufficiently severe to cause intestinal rupture without incriminating the associated hernia but, when the trauma is trivial, the presence of the hernia may be a contributory factor. It is remarkable that in most previously reported cases the rupture has occurred in the lower ileum, usually on the anti-mesenteric border and, with one exception,1 the injured loop has been found lying in the abdominal cavity (usually near the internal inguinal ring) and not in the hernial sac. It is also interesting to note that the condition has not been observed in women, nor in association with any external hernia other than inguinal. The hernia has usually been long standing, though not necessarily irreducible, and usually right-sided.

An ingenious theory has been propounded by Bange (quoted by Aird1) to explain why the presence of a hernia should render the intestine more susceptible to violence of a trivial nature. He states that 'any sudden rise of intra-abdominal pressure is invariably associated with a rise in