LEUKAEMIA AND FOETAL HAEMOGLOBIN: A CASE STUDY

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The persistence of foetal haemoglobin formation after infancy has been taken to indicate an anaemic state dating from early life and is most commonly found in association with the hereditary haemoglobinopathies, though it may also occur to a less extent in congenital spherocytic anaemia (Lehmann, 1959). An extensive search has been made in many other types of anaemia, but only slight increases in foetal haemoglobin (Hb-F) have been found (White and Beaven, 1959). A considerable increase (10–20%) was, however, found by Beaven and White (1953) in a child aged 3 years with leukaemia.

We wish to record a similar finding in an adolescent girl with acute myeloid leukaemia because of the interesting possibilities which this observation raises in relation to the aetiology and diagnosis of the condition.

Case History

The patient, a schoolgirl aged 12 years, had no complaints of ill-health, but her mother had noticed the child's increasing pallor over the previous year, and eventually asked her practitioner for a "blood test." The girl was then referred to the medical unit for further investigation of anaemia. On admission she gave the additional history of unilateral epistaxis over the previous five years which had stopped after cautery six months before she was first seen by us. There was no complaint of lassitude, weakness, or dyspnoea, jaundice had not been noticed, and she had been able to continue her usual school activities. She had a normal appetite, and had gained weight and grown appreciably in the year before her admission to hospital. Her menarche was at 11 years and the menses were normal.

Past History.—A year before her present admission she had attended a hospital and had had a general anaesthetic for incision of an axillary boil.

Family History.—There was no family history of anaemia or jaundice; her parents were healthy and both were English, but one paternal grandparent was Swiss. A brother and a sister were well, but another brother had died in infancy of an unknown cause.

On examination she was seen to be a well-built girl, advanced for her age both physically and mentally. She was slightly jaundiced and moderately anaemic. There was no lymphadenopathy, the liver was not palpable, but the tip of the spleen could just be felt.

Haematological Investigations

Blood Picture.—Red cells, 2,400,000/c.mm.; haemoglobin, 7.8 g./100 ml.; P.C.V., 29%; M.C.H.C., 28%; M.C.V., 120 cubic microns; reticulocytes, 6.5%; nucleated red cells, 450/c.mm.; leucocytes, 6,100/c.mm. (neutrophils 47%, lymphocytes, 42%, monocytes 1%, leucoblasts 5%, myelocytes 2%, metamyelocytes 3%); platelets, 164,000/

c.mm. The stained film showed macrocytosis, marked anisocytosis, and polychromasia of the red cells, but there were no target cells, spherocytes, or elliptocytes. Her blood group was O CDe/cde.

Bone Marrow.—There was marked hyperplasia of the sternal marrow, particularly of the erythroid series, resulting in a reduction of the myeloid:erythroid ratio, and there was no excess of myeloblasts. Erythropoiesis appeared to be mainly normoblastic in type although some of the cells showed features suggestive of late megaloblasts. There were no features such as atypical erythroblasts to suggest erythraemic myelosis.

Other Findings.—The direct antiglobulin (Coombs) test gave a positive reaction which, however, was very weak and appeared to be of the non-gamma-globulin type. The osmotic fragility of the red cells was normal. The serum bilirubin concentration was 3.4 mg./100 ml.; serum protein 6.6 g./100 ml., with a normal electrophoretic pattern; thymol turbidity 5 units; serum alkaline phosphatase 8.1 K-A units; urobilinogen was present in excess in the urine, though no bile salts or pigments were present; serum iron 262 µg./100 ml. The serum vitamin B₁₂ was 867 µµg./ml. The significance of this figure, which is above the upper limit of normal for the method in this laboratory (Kilpatrick *et al.*, 1960), is uncertain because the patient had taken an oral preparation of vitamin B₁₂ before coming under our care.

From these results the patient seemed to have a haemolytic type of anaemia of unknown aetiology, and despite her nationality the possibility of a haemoglobinopathy was raised.

Estimation of the foetal haemoglobin (Hb-F) using the alkali-denaturation method of Singer *et al.* (1951) showed this to be increased to 19%, and its electrophoretic mobility was consistent with that of Hb-F, in that it could not be completely resolved from Hb-A. It was, however, possible to identify the abnormal haemoglobin as Hb-F immunologically by a precipitin reaction with an anti-Hb-F rabbit serum. There was no increase in Hb-A₂ and no other abnormal haemoglobins were found (Lehmann, personal communication). The normal physical development of the patient, however, made the diagnosis of a long-standing anaemia extremely unlikely. The haemoglobin of both parents showed a normal electrophoretic pattern and their Hb-F concentrations were less than 1%.

Radioactive Isotope Studies

A series of radioactive isotope studies were made during the third week of her admission in order to study red-cell survival and erythropoietic activity in this patient.

Methods.—The red cells were labelled with approximately 75 µ of C⁵¹Cr as sodium chromate by a modification of the established method (Wetherley-Mein *et al.*, 1958), and assessment of red-cell destruction was expressed as "percentage chromium loss per day" as described by these authors. With a tracer dose of ⁵⁹Fe (approximately 10 µc.) assessment of erythropoiesis was based on surface-counting patterns and iron utilization in the week following the injection of the isotope (Wetherley-Mein *et al.*, 1956).

Because measurements were carried out almost simultaneously a double isotope-counting technique was used (Wetherley-Mein *et al.*, 1958).

Results.—In the first study the patient's own red cells were labelled with ⁵¹Cr. There was a 4.6% ⁵¹Cr loss per day and the ⁵⁹Fe surface-counting study showed considerable deviation of the isotope to the liver and spleen. The plasma half-clearance time for iron was normal (105 minutes), but the iron utilization was only 25% on the sixth day. In the second study the patient's red cells were tagged with ⁵¹Cr and injected into a recipient—a man with a bronchogenic carcinoma who died before the cell-survival study could be completed. A 5.4% ⁵¹Cr loss per day was demonstrated over a five-day period. At a later date red cells of appropriate

blood group from a normal healthy donor were tagged with ^{51}Cr and injected into the patient. In this instance a ^{51}Cr loss per day of only 1.3% was found.

Progress and Treatment

Folic-acid deficiency may occur in haemolytic anaemias (Chanarin *et al.*, 1959), and because there were cells in the marrow which resembled late megaloblasts folic acid was given for 10 days. There was no reticulocyte response (see

Chart showing progress and treatment of case.

Chart) and a second marrow preparation at the end of this period showed no change. The M.C.H.C. rose from 28% to normal (33%) after one week of parenteral iron.

During the next month the haemoglobin fell to 5 g./100 ml, and the platelet count to 65,000/c.mm., while the leucocyte count had risen to 15,600/c.mm. (neutrophil polymorphs 26%, lymphocytes 16%, monocytes 17%, neutrophil metamyelocytes 6%, neutrophil myelocytes 8%, premyelocytes 4%, leucoblasts 13%, normoblasts 8%, unclassified 2%). The leucocyte alkaline phosphatase was equivalent to 3.7 mg. of phenol liberated/10¹⁰ W.B.C./quarter-hour incubation as measured by the method of Valentine and Beck (1951) modified by Withey (personal communication). This low result is consistent with a diagnosis of leukaemia. These findings demonstrate that the haemolytic anaemia with which she had presented was probably symptomatic of leukaemia. The direct antiglobulin reaction had become more strongly positive by this time, but an antibody could not be eluted from the erythrocytes.

Cortisone was then given (200 mg./day) in the hope that it might slow down the rate of red-cell destruction, but it can be seen from the Chart that there was no striking improvement as measured by the rate of fall of haemoglobin, and that there was a marked rise in the leucocyte count due

mainly to primitive cells, the blast cells rising to 33% of the total. This deterioration may possibly have been coincidental, but it would seem to bear out the suggestion (Wintrobe, 1956) that cortisone may have an adverse effect on the course of an acute myeloid leukaemia. It can also be seen from the Chart that the foetal haemoglobin concentration remained unchanged during the first weeks after cortisone therapy. The subsequent decrease in Hb-F concentration was presumably due to destruction of red cells with little or no further red-cell formation and to dilution by transfused blood.

Treatment with busulphan ("myleran") 4 mg./day was started, and this was followed by a marked reduction in the number of circulating leucocytes during the next month. At this time the patient developed an acute arthritis affecting the right shoulder and left elbow which responded rapidly to colchicine. Although association of gout with rapid leucocyte destruction during remissions in leukaemia is recognized (Hickling, 1953), her serum uric acid was only 3.1 mg./100 ml. at this time. A second remission was induced by busulphan, but, at the time of writing, 10 months after she was first seen, the total leucocyte count in the peripheral blood was 38,000, of which 30% were blast cells.

Discussion

Although it is probable that the haemolytic process had been present in our patient for the best part of a year, there was no evidence when she was first seen that this was secondary to leukaemia. The leucoblasts in the peripheral blood were considered to be in keeping with a haemolytic process in a young person and the marrow appearances were consistent with this view. The diagnosis was further confused by the finding of 19% Hb-F in the blood, which suggested that the anaemia was congenital, and a diagnosis of a haemoglobinopathy such as thalassaemia was considered until the leukaemic nature of the disease became apparent.

The patient's red cells had a diminished survival, as measured at 24-hourly intervals after injection of ^{51}Cr -tagged cells both in her own circulation and when transfused to a recipient. An intracellular defect of the red cells is therefore postulated rather than an antibody-antigen mechanism in which one would expect the red-cell survival to be substantially normal in a normal recipient after an initial increased rate of destruction during the first five hours after injection (Mollison, 1959). This interpretation is supported by the finding of a normal survival rate when cells from a healthy donor were injected into the patient, though the possibility of a blood-group-specific antibody acting on the patient's cells cannot be excluded. The intracellular defect in the red cells could be part of the leukaemic process, but it is possible that, after infancy, red cells containing Hb-F have a shorter life-span than cells containing adult haemoglobin.

The chief point of interest in this case is undoubtedly the presence of large quantities of foetal haemoglobin. Production of foetal haemoglobin is under a different genetic control from Hb-A, and its presence in our patient must have been due either to a genetic abnormality recently acquired by her marrow cells or to a congenital genetic abnormality with persistence of Hb-F since birth. The frequency with which chromosome abnormalities were found in acute leukaemia led Baikie *et al.* (1959) to the hypothesis that this disease is related to changes in the genetic material in the marrow cells. We suggest that the presence of Hb-F in our patient is likewise a reflection of a

chromosome abnormality. The presence of Hb-F could have been caused by an alteration in the genetic pattern of the marrow cells occurring either at the time of onset of the leukaemia or subsequently. There is, however, the other possibility that a pre-leukaemic chromosomal abnormality which caused persistence of Hb-F had been present from early life.

It will be interesting to discover whether this association of Hb-F and leukaemia is fortuitous or whether it will prove to be an important observation from an aetiological as well as from a diagnostic point of view.

Summary

The case of a girl with leukaemia presenting as haemolytic anaemia associated with 19% foetal haemoglobin is described. Evidence suggestive of a defect in her red cells was found.

The possible significance of these findings is discussed in relation to the genetic hypothesis for the aetiology of leukaemia.

We wish to acknowledge our indebtedness to Professor H. Scarborough and Professor J. Gough for their advice and encouragement, to Dr. H. Lehmann for measurement of Hb A₂, and to Mr. R. G. Wood for his help with the isotope studies.

ADDENDUM.—Marrow and peripheral blood have now been cultured from this patient during a second remission induced by busulphan. There were no morphological abnormalities in the chromosomes of cells cultured from the peripheral blood, and the chromosome number was 46. The chromosomes of the few intact marrow cells which could be examined in detail were morphologically normal. This investigation was done by Dr. C. M. Clarke and Mr. G. Breckon, of the M.R.C. Microbiological Research Unit at Harwell, to whom we are greatly indebted.

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This year a home-printer's course for instructing spastics in the printing of Christmas cards was organized with the help of a retired master printer. It was designed to enable those who were too severely handicapped to be placed either in open industry or sheltered employment to earn money by working at home. As most of those who attended the course were too handicapped to handle small type a system was devised whereby all printing could be done by means of printers' blocks. The course was sponsored by the National Spastics Society's employment department. Christmas cards printed by spastics can be obtained from Sundew Cards, 32 The Mall, Ealing, London W.5.

DYSPHAGIA LUSORIA PRESENTING AS A SUPERIOR MEDIASTINAL SYNDROME

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In 1787 Dr. David Bayford carried out a necropsy on a woman who died of starvation, after having suffered from dysphagia from the age of 13 to 60. At first he could find no obvious cause for her symptoms, but ultimately his dissection led him to the discovery of an aberrant subclavian artery, which arose from the left side of the aortic arch and coursed between the oesophagus and the trachea to supply the right arm. He concluded that this anomalous vessel had pressed upon the oesophagus, causing dysphagia. He applied to this disorder the name of dysphagia lusoria, *lusus naturae*, meaning a freak of nature (Asherson, 1952).

Dysphagia lusoria in infancy signifies oesophageal compression by any congenital arterial abnormality, such as right aortic arch, any aberrant great vessel, vascular ring, double aortic arch, and right aortic arch with an aberrant left subclavian artery. In adults the term usually denotes oesophageal compression due to an aberrant right subclavian artery, this usually being the only abnormality.

Incidence

In the adult type of disorder the right subclavian artery arises from the left side of the aortic arch beyond the origin of the left subclavian artery. This vessel then courses to the right in one of three ways. It may run behind the oesophagus (80%), between the oesophagus and the trachea (15%), or in front of the trachea and oesophagus (5%) (Neuhauser, 1946). It will be seen from this that classical dysphagia lusoria as described by Bayford is probably extremely rare. Aberrant subclavian artery has been estimated to occur in about 1% of otherwise normal people. Some anatomical studies place this figure as high as 2%, but radiological studies obtain about 0.18% of positives (Palmer, 1955). The abnormality therefore is common, but the production of symptoms is very rare. I was able to find only seven references to dysphagia lusoria in the English language over the past ten years.

FIG. 1.—A. The caudal portion of the right aortic arch normally disappears. B. The cephalic portion disappears and the caudal part persists, forming the aberrant right subclavian artery.