PROLONGED SURVIVAL OF A BONE-MARROW GRAFT RESULTING IN A BLOOD-GROUP CHIMERA*

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Several reports of successful bone-marrow grafts in experimental animals have been published since the original report of Ford *et al.* (1956). Porter and Murray (1958) have recorded results in rabbits in which the grafts survived for several months. In all these cases the animals had been given lethal doses of irradiation before injection of the donor bone-marrow. Thomas *et al.* (1959a, 1959b) reported the results of treatment of cases of acute leukaemia with large doses of total body-irradiation followed by bone-marrow transfusions. In some cases there has been evidence of a "take," but in none has the donor marrow survived for more than two or three weeks. Mathé *et al.* (1959) have reported the successful treatment by bone-marrow transfusion of five patients who accidentally received a large dose of irradiation. They consider that the bone-marrow graft functioned and produced blood cells for approximately one month.

The purpose of this paper is to report a case in which a bone-marrow transfusion with a successful "take" resulted in the blood-group picture of a red-cell chimera. The donor graft still persists after nine months.

Case Report

The patient, a married woman aged 30 with two children, was first seen at the Middlesex Hospital in May, 1955. At that time she felt quite fit and had no abnormal signs apart from two firm, enlarged, adherent lymph nodes in the left supraclavicular region, and one firm mass behind the lower end of the right sternomastoid muscle. Her illness had started in June, 1953, when she noticed a swelling above the right clavicle. A biopsy in December, 1953, was reported to show changes suggestive, but not diagnostic, of Hodgkin's disease. In November, 1954, more swellings developed above the left clavicle, and a small swelling reappeared above the right clavicle. A biopsy in May, 1955, showed replacement of the lymph node by tissue characteristic of Hodgkin's disease. A course of radiotherapy was given between May 26 and June 27, 1955, using x-rays generated at 230 kV., half-value layer 2.4 mm. of copper. A skin-dose of 3,600 r to the supraclavicular regions and a tumour-dose of 4,730 r to the upper mediastinum were given. After this treatment the swellings disappeared. In April, 1956, an enlarged node became palpable in the left axilla. This was treated by a further course of radiotherapy, using x-rays with the same factors,

and to one direct axillary field she was given a total skin-dose of 2,000 r in two weeks.

The patient remained well until March, 1958, when night sweating and pruritus became exceedingly distressing and increased in severity until May, when in addition an enlarged lymph node became palpable in the left axilla. On May 15 treatment was begun with chlorambucil, 5 mg. twice daily, and on June 12 the patient reported that she was free from the night sweating and pruritus and that the axillary swelling had disappeared. The dose was reduced to 5 mg. once daily on June 26, and stopped on August 14; the blood count was not affected by the drug. The pruritus recurred five weeks later, and treatment was started with aminochlorambucil, 5 mg. b.d. By October 23 the irritation had cleared completely, and the dose was reduced to 7.5 mg. daily. On October 30 the blood count showed a marked fall in haemoglobin level, white cells, and platelets, and administration of the drug was stopped. The patient had no complaints, and no measures other than close observation were thought necessary. One week later she was admitted extremely ill, complaining of lassitude, malaise, and sore throat. She was pale and sweating, and dyspnoeic on even slight exertion; she had bruising of legs and arms and petechial haemorrhages of the skin, lips, and buccal mucosa.

A blood count on admission was as follows: Hb, 7.6 g./100 ml.; R.B.C., 2,400,000; P.C.V., 21.5%; W.B.C., total, 150/c.mm. (neutrophils 12%, lymphocytes 58%, monocytes 26%, eosinophils 4%); platelets, 17,000. Treatment had been started the day before with prednisone, 10 mg. t.d.s., and on admission penicillin, 500,000 units eight-hourly, was prescribed. Bone-marrow examination showed extreme hypoplasia. The total nucleated cell count numbered 1,200/c.mm. (early normoblasts, 0.5%; reticular cells, 12.5%; megalokaryocytes, none seen; myelocytes, 0.5%; metamyelocytes, 1.0%; polymorphonuclears, 1.0%; eosinophils, 2.0%; lymphocytes, 52%; monocytes, 16.5%; plasma cells, 14.0%).

During the next four days she was transfused with four pints (2.3 l.) of blood (two fresh and two bank), and the haemoglobin level rose to 11 g./100 ml.; but there was no improvement in the clinical picture, and the white-cell and platelet counts continued to fall. She remained extremely ill, and on November 12 the total white-cell count had fallen to 25 and the platelet count to 5,000. On the evening of that day a marrow transfusion was given.

Marrow Transfusion

The donor was the patient's sister. Under a general anaesthetic 40 ml. of marrow was obtained by means of multiple punctures from both ilia and sternum as described by Humble and Newton (1958). The marrow was placed in an equal volume of A.C.D. solution. The material was then taken into a 20-ml. syringe, expelled through a fine needle of approximately 150 μ bore to break up the larger particles, and spun at 1,000 r.p.m. for ten minutes to bring the fatty material to the surface, this being then removed by aspiration. The remaining material—76 ml.—containing 1.1×10^9 nucleated cells, was injected intravenously into the median cubital vein of the patient.

By the next morning there was a marked change in the clinical picture. The patient looked and felt better, and her only complaint was of a tiresome but now bearable sore throat. From this day she improved steadily, and there was a gradual rise in the white-blood-cell and platelet count, which can be appreciated most clearly by examination of the Chart (Fig. 1). She was discharged on December 2, symptom-free, with the following blood count: Hb, 66%; W.B.C., 2,620/c.mm.; platelets, 170,000/c.mm. The blood count continued to improve, and 16 weeks after the marrow transfusion it was within normal limits; nine months later she was still quite well.

Blood-group Investigation

Positive evidence was sought for the presence of the marrow donor's cells in the recipient's circulation.

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FIG. 1.—Graphic representation of the course of the patient's haemoglobin and leucocytes before and after bone-marrow transplantation. Details of the patient's treatment are also shown.

The patient and donor had been grouped before the transfusion. Their groups were as follows:

Patient: Group O; Rh genotype cde/cde; M; S+; P₁++; Lu^a-; K-; Le(a-b+); Fy^a-.

Donor: Group O; Rh phenotype ccDEe; probable genotype cDE/cde. MN; S+; P₁+++; Lu^a-; K-; Le(a-b+); Fy^a-.

The anticipated anti-A and anti-B were present in the sera of both.

If the marrow-graft cells were proliferating in the patient they should continue to be demonstrable in her peripheral circulation by showing agglutination with anti-N, anti-D, and anti-E.

Four months after the transfusion, when all original cells from both the blood donor and the marrow donor should have disappeared, the patient's blood was tested with these antisera, first by qualitative methods, and, when these were found positive, by quantitative procedures.

Qualitative Tests

Direct agglutination, using an avid saline agglutinating anti-D serum, showed small solid agglutinates in a sea of free cells (see Fig. 2); a control test with Rh-negative cells showed no such agglutinates.

Tests with anti-N sera gave a similar picture, but direct tests with anti-E sera did not show this result.

However, the presence of E-positive cells was conclusively demonstrated, and that of D-positive cells confirmed, by

the mixed agglutination technique of Richardson Jones and Silver (1958).

The patient's cells were sensitized for two hours with an equal volume of a strong incomplete anti-E serum, and washed four times: these sensitized cells were then mixed with excess of anti-human-globulin serum, optimally diluted, and left for 10 minutes and again washed four times with a large excess of saline. These double-sensitized cells were put out on a tile for Coombs-testing: no agglutination was observed.

On the addition of D-positive cells heavily sensitized with anti-D, islands of agglutination appeared; this could only be due to the E-positive cell combining with the anti-E, which in turn combined with the anti-human globulin. The cells thus sensitized were too few to form agglutinates, but the free sites of the anti-human globulin subsequently combined with the D sensitized cells present in excess, and visible agglutinates resulted. Cells of group MN, cDE were thus shown to be surviving in the circulation of this patient.

Quantitative Tests

To estimate the number of D-positive cells in the patient's circulation, doubling dilutions were made of a suspension of the patient's cells in a suspension of D-negative cells of equal strength. These dilution mixtures were then sensitized with incomplete anti-D and an indirect Coombs test was done. The approximate proportion of D-positive cells could then be estimated, previous experiment having shown that macroscopic positive reactions are obtained when 6% (or more) D-positive cells are present, and microscopic positive results when over 2½% positive cells are present.

The proportions were estimated at irregular intervals during the next seven months; the results are shown in the accompanying Table. The last figure (24%) was confirmed by a differential agglutination technique, using the modified Ashby technique of Dacie and Mollison (1943).

The patient's serum has been tested regularly for the presence of anti-D and E, but none has been demonstrated.

FIG. 2.—Photomicrograph of the patient's cells treated with saline anti-D on April 17, 1959, showing numerous free cells interspersed with clumps of agglutinated D-positive cells.

Results of Investigations

Date	Patient's Cells + Saline Anti-D	Titre of Patient's Cells + D-neg. Cells	Other Investigations on Patient's Cells	D-pos. Cells Present
12/11/58	Negative	Negative (all dilutions)	—	Nil
17/11/58	"	Weak positive 1:1 macroscopically	—	6%
21/11/58	"	Weak positive 1:1 macroscopically, 1:4 microscopically	—	6%
28/11/58	"	Weak positive 1:1 macroscopically, 1:4 microscopically	—	6%
2/12/58	"	Weak positive 1:1 microscopically	—	24%
11/12/58	"	Negative	—	Nil
30/1/59	Positive microscopically	Positive 1:1 macroscopically; mixed field agglutination	—	6%
5/3/59	"	Positive 1:1	Antigens N and E detected	
17/4/59	Positive macroscopically	Positive	—	
15/5/59	" "	Positive 1:4 macroscopically, 1:8 microscopically	—	24%

Skin grafts were undertaken on the following scheme: patient to patient, donor to donor, donor to patient, and patient to donor; only the skin grafts between patient and patient and donor and donor survived.

Discussion

The development of new blood-group antigens in a patient who has been irradiated, and transfused both with blood and with fresh marrow, is much more likely, on the analogy of animal experiments, to be due to proliferation of the transfused marrow than to any other cause, but consideration must be given to the possibility that the antigens may result from somatic mutations induced by the irradiation (Atwood, 1958); the fact that the new antigens developing in the present case were exactly those which were present in the marrow donor but not in the recipient is therefore important, because it leaves no reasonable doubt that they were derived from that donor.

It is believed that this case is the first example of prolonged survival of a marrow graft in a human being. We have to try to account for the fact that this graft has remained active for more than nine months, whereas the active life of all other known marrow grafts in human beings has been only a few weeks. The longest recorded survival, quoted by Kretschmer (1958), was that of an irradiated leukaemic patient who received a marrow graft from her mother, and in whom "donor-type cells were still present several months later." This patient "eventually improved to some extent" after several transfusions, but no further details are given.

Not only is the present case unique, so far as is known, in the prolonged survival of a graft, but the combination of manifestations in the haemopoietic and antibody-producing systems is most remarkable and difficult to explain. We must try to account, on the one hand, for the temporary marrow aplasia and the continued tolerance of several blood-group antigens, including the potent D antigen, and, on the other hand, for the persistence of the normal anti-A and anti-B in the serum and the absence, except perhaps for the sore throat, of any signs of intercurrent infection.

Three factors may be regarded as having possibly contributed to the development of the aplasia and immune tolerance: firstly, Hodgkin's disease is known to have anergic effects; secondly, the two courses of

irradiation; and, thirdly, the administration of large doses of chlorambucil and aminochlorambucil. All this treatment must have impaired her antibody-producing mechanism. The persistence of anti-A and anti-B and the relatively slight signs of infection show, however, that the ability to produce antibodies was not completely abolished.

It is thus difficult to account for the long-continued tolerance of marrow and red blood cells carrying three known antigens not present in the patient's own cells, and possibly others. Two main factors must be considered here: the precise relative timing of the disease and of the therapy, and the close relationship between the patient and the donor.

The bone-marrow was administered within an hour of being removed from the donor, and with a very small amount of treatment *in vitro*. We believe this to be an important point, as it results in a minimum of trauma to vulnerable cells.

The timing of the marrow transfusion may also be of critical significance. Our patient received the infusion on the eighth day after the start of agranulocytic symptoms, and presumably, therefore, about 15 days after the marrow ceased to function. This delay, though unintentional in our case, may be necessary in order to allow "tissue" antibodies which may be present in the patient's plasma to decline, and to permit the patient to become receptive to the graft.

It can hardly be doubted that the close relationship between donor and recipient was an important factor in allowing tolerance to develop, but the precise mechanism is not easy to discover.

More resemblance between the known blood-group antigens cannot be the whole explanation, for there must be many cases of graft-failure where the differences in this respect were no greater. "Tissue" antigens are, however, known to be important for the tolerance or intolerance of grafts in general, and are likely to affect bone-marrow even if not the peripheral red cells. There is likely to have been a considerable resemblance between patient and donor in this respect.

It is well known that marrow and other grafts are completely interchangeable within closely inbred lines of mice and other animals, and Donnall Thomas *et al.* (1959a, 1959b) have shown that there is a higher incidence of "takes" when beagle dogs receive marrow grafts from their litter-mates than from unrelated dogs.

The fact that in the present case the patient did not tolerate a skin-graft from the marrow-donor shows that the degree of immune tolerance developed is less than in the case of the natural twin blood-group chimeras (Woodruff, 1957), who each tolerated a skin-graft from the other. The range of antigens present in the haemopoietic tissue must be narrower than that present in skin. It is not known whether in the present case leucopoietic tissues have survived, as they did in the twins.

It is possible that the patient's mother, who is dead, possessed those antigens which are present in the donor but not in the patient, and that the patient therefore developed some degree of tolerance or potential tolerance to them *in utero*. The evidence for persisting tolerance of this kind in man is somewhat confusing (see Race and Sanger, 1958).

In animal experiments an important cause of death after marrow transfusion has been the development of

antibodies to the recipient's tissue cells by the marrow graft. That this has not been found in the present case may be due to the close relationship, and the fact that the donor is known to possess all the blood-group antigens found in the patient.

Summary

A patient is described who suffered from acute bone-marrow failure due to chemotherapy for Hodgkin's disease. She was treated with a bone-marrow transfusion from her sister. Evidence is presented to show that the bone-marrow graft survived for more than six months, responsible for the production of an increasing proportion (now 24%) of the patient's erythrocytes. A skin graft was undertaken between the donor of the marrow and the patient, but it was not successful.

[ADDENDUM.—We repeated the haematological investigation on this patient on September 26, 1959. Rh-positive cells are still present in her circulation. Titration studies indicate that approximately 40% of her circulating erythrocytes are Rh-positive. Woodruff and Lennox have recently published (*Lancet*, 1959, 2, 476) further details of the results of the skin grafts in blood-group chimeras.]

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In his Annual Report for 1958 Dr. I. GORDON, medical officer of health of Ilford, comments on the difficulty of assessing radiation dangers. Medical officers of health, he writes, are worried that they and their staffs are not competently trained to evaluate radiation hazards, and feel that the Ministry of Health is showing unnecessary reluctance in providing this training. He continues: "We hope that the Ministry will shortly make it possible for local authorities to take an active part in controlling and evaluating this potential hazard, but the experience of the Essex County Council with regard to their own scheme does not give rise to optimism. It is true that if a hazard is suspected the medical officer of health can obtain a trained expert from the Ministry who will visit, inspect, and advise, but how can one even suspect a hazard which is only demonstrable with special instruments that one doesn't possess, or how can suspicion be aroused by industrial or medical use of radioactive materials when information with regard to supply of these materials is withheld?"

MAINTENANCE TREATMENT OF PERNICIOUS ANAEMIA BY MASSIVE PARENTERAL DOSES OF VITAMIN B₁₂ AT INTERVALS OF TWELVE WEEKS

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Parenteral vitamin B₁₂ is now generally recognized as the treatment of choice in pernicious anaemia. Once full clinical and haematological remission has been obtained, various regimes have been recommended to provide adequate maintenance therapy (Mollin and Dacie, 1950; Blackburn *et al.*, 1952; Conley *et al.*, 1952; Davis and Brown, 1953; Hemsted and Mills, 1958). The dosage advised has been of the order of 20 to 100 µg. every two to four weeks.

If massive doses of vitamin B₁₂ of the order of 1,000 µg. or more could be given every three months for the maintenance treatment of pernicious anaemia, this would be a distinct advantage to the patient if it were to prove entirely safe.

The cost of the larger dose would be slightly more, and, as most of the injected vitamin B₁₂ is excreted in the urine within a short time of administration (Conley *et al.*, 1951; Mollin and Ross, 1953; Reisner and Weiner, 1953), there would be greater wastage. However, ample compensation would be obtained by the reduction in the number of injections required and the consequent saving of both the patient's and the general practitioner's time.

The effectiveness of large doses of vitamin B₁₂ parenterally at intervals of over six weeks in the maintenance treatment of pernicious anaemia has been studied in only a small number of patients (Conley *et al.*, 1952; Walker and Hunter, 1952; Reisner and Weiner, 1953).

The purpose of this paper is to report the results of a trial in which 112 treated cases of pernicious anaemia were changed from orthodox maintenance treatment to maintenance treatment with 1,000 µg. of vitamin B₁₂ parenterally every 12 weeks, most of them being kept on this regime for a period of two years.

Material and Methods

The diagnostic criteria observed for inclusion of patients in the trial were a macrocytic anaemia, megaloblastic bone-marrow, histamine-fast achlorhydria, and a clinical and haematological response to the administration of vitamin B₁₂ or liver extract.

Of 155 patients with pernicious anaemia who had been fully treated and were attending hospital regularly for supervision, 112 were considered suitable for inclusion in the trial. Of the remaining 43, 30 were regarded as unsuitable because of other complicating disease: 9 had early subacute combined degeneration, 6 had incapacitating cardiovascular disease, 8 were elderly arthritic patients, 2 had cirrhosis of the liver, 2 had polycythaemia vera, and 3 had other illnesses. Eight patients were unable to attend because of their employment, and five were partaking in another research trial.

The 112 patients selected consisted of 85 women and 27 men; their ages ranged from 31 to 85 years, the