Hematopoietic Transplantation by Means of Fetal (Cord) Blood

A New Method

MILTON ENDE, M.D.
Petersburg, Virginia
N. ENDE, M.D.
Newark, New Jersey

A series of eight transfusions consisting of 30 to 85 ml of umbilical cord blood was utilized to establish a hematopoietic transplant in a patient with acute lymphoblastic leukemia on conventional therapy.

THUS FAR, allogenic hematopoietic transplants have offered somewhat limited clinical usefulness. One of the few definitely successful applications of this form of therapy lies in the treatment of accidental lethal whole body irradiation. Less established, however, is evidence of any beneficial effect of marrow transplantation in those cases of hypoplastic marrow which are unaccompanied by irradiation.

The clinical value of hematopoietic transplantation therapy in patients with leukemia is generally considered debatable. Most objections to the use of marrow transplants in leukemia relate to those complications which result from massive chemotherapy and whole body irradiation.[5] Other objections cite the large amounts of marrow necessary for transplantation[4] as well as the relatively few recipients who have subsequently presented convincing evidence of successful grafts. An extensive review of 417 cases of attempted marrow transplantation revealed that only 10% of the recipients had shown acceptable evidence of successful allograft.[6] In a recently published compendium, which summarized 203 human cases of hematopoietic transplants, there were only 11 unequivocal allogenic chimeras.[2]

The case presented in this paper is that of a patient on conventional antileukemic therapy, who after being transfused with cord blood from multiple donors, apparently developed a successful temporary allograft from a relatively small number of fetal cells (45 ml of cord blood). So far as the authors can determine, the use of cord blood for transplantation has not been previously attempted. Although not necessarily related, this temporary allograft was associated with both clinical and hematological remission of the patient's acute lymphoblastic leukemia.

Material and Methods

Fetal blood was collected from the umbilical cord, after the latter's separation from the baby and prior to the removal of the placenta from the uterus, milking of the cord was occasionally necessary. The volume of recovered blood varied between 25 ml and 120 ml; this blood was collected in sterile recepticles containing 5 ml of heparin and stored under 6°C until administration to the recipient. At the same time, a pilot tube of clotted blood was obtained for phenotyping and crossmatch; the crossmatch was carried out in the usual way and the fetal blood was phenotyped in the usual fashion. Prior to transfusion, the blood was

transferred to a sterile vacuum container; transfusion was effected within 24 hours by means of a blood administration set.

A baseline phenotype was obtained on the recipient prior to his initial fetal blood transfusion; this was compared with his later phenotypings, which were performed at weekly intervals. Phenotypings were performed on representative samples of all donor fetal blood and on the two routine transfusions which were necessary to alleviate the patient's anemia. The disappearance or development of one or more blood groups in the recipient was taken as indication of a hematopoietic transplant.

Case

The patient was a 16-year-old Negro male who, three weeks prior to hospitalization became extremely weak, short of breath and had not been able to continue the usual procedures of a karate class. The patient had also noted some recent cervical lymphadenopathy. There was no history of any past major illness. The patient was admitted to the hospital February 23, 1970.

On physical examination, marked cervical and some axillary adenopathy was present. There was a 2+ mitral systolic murmur. The spleen was enlarged but the liver was within normal limits. Clinical impression was that the patient had acute leukemia. His initial laboratory workup (February 23, 1970) revealed a WBC of 189/000mm^3, a red cell count of 1,530,000/mm^3, hemoglobin of 6 gm/100 ml, hematocrit of 17, and a platelet count of 195,000/mm^3. The bone marrow aspiration was diagnosed as acute lymphoblastic leukemia. The urinalysis was normal. The VDRL and sickle cell tests were negative.

The patient was started on daily medication of 150 mg of 6-mercaptopurine and 60 mg of prednisone. The first blood administered to the patient was fetal blood, transfused on February 25, 1970, the third day of hospitalization (Fig. 1). Because the patient's hemoglobin dropped to less than 4 gm/100 ml, two routine blood transfusions were administered March 1 and March 6, 1970. During the nine day period following his first fetal transfusion, the patient was given four additional fetal blood transfusions. On March 7, 1970, he was transfused with 45 ml of baby M's fetal blood (sixth fetal donor). Baby M was born on March 6, 1970, and the fetal blood was given approximately 24 hours after collection. The white count, which had begun to fall immediately after initiation of treatment, dropped

FETAL BLOOD ANTIGENS

DONOR & DATE	C	D	E	c	e	M	N	S	s	K	k	Fy^a	Fy^b	JK^a	P_1	ABO
T 2/25/70	+	+	+	+	+	+	–	–	+	–	+	+	–	–	–	B
L 2/25/70	+	+	–	+	+	+	+	–	+	+	+	+	+	+	–	B
G 2/27/70	+	+	–	+	+	+	+	–	+	–	+	+	+	+	–	B
M 2/27/70	+	+	+	+	+	+	+	–	+	–	+	+	–	+	–	B
WB* 3/4/70	+	+	–	+	+	+	+	–	+	–	+	+	+	–	–	B
Q 3/3/70	–	+	–	+	+	+	–	–	+	–	+	–	+	–	–	B
WB* 3/6/70	+	+	+	+	+	+	+	–	+	–	+	–	–	–	+	B
M 3/7/70	+	+	–	+	+	+	+	–	+	–	+	–	–	–	–	B
H 3/12/70	+	+	–	+	+	+	+	–	+	–	+	+	+	–	+	B
A 3/16/70	+	+	–	+	+	+	+	–	+	–	+	–	–	–	–	B

* whole blood

Fig. 1. Blood antigens of cord blood administered to recipient.

from 189,000/mm^3 on admission to 119,000/mm^3 on 3/6; 62,000/mm^3 on 3/8 and 24,000/mm^3 on 3/11. Over a period of 17 days, this patient received a total of eight transfusions of fetal blood varying in volume from 30 ml to 85 ml per transfusion (total: 500 ml) (Fig. 1).

Typing of the patient's blood antigens on March 11, 1970, showed only a questionable change in the M antigen typing; however, on March 13, 1970, a definite change in the patient's blood type was first detected. At this time mixed field were noted in both the M & JK^a typing. On March 16, 1970, the patient's hemoglobin was 10 gm/100 ml and his white cell count was 12,700/mm^3 with a differential of 45 neutrophils and 55 lymphocytes.

The patient continued to improve and at the time of discharge from the hospital, March 21, 1970, he showed a hemoglobin of 11.1 gm/100 ml, red cell count of 4,180,000/mm^3, WBC 10,600/mm^3, with 55 neutrophils, 40

lymphocytes, 5 monocytes, with no abnormal cells present in the peripheral blood.

By April 14, 1970, the mixed field noted in the M typing had begun to decrease and by May 21, 1970, the patient's phenotype had returned to its original typing (Fig. 2).

RECIPIENTS ANTIGENS

DATE	C	D	E	c	e	M	N	S	s	K	k	Fy^a	Fy^b	JK^a	P_1	ABO
2/25/70	+	+	–	+	+	–	+	–	+	–	+	–	–	+	–	B
2/27/70	+	+	–	+	+	–	+	–	+	–	+	–	–	+	–	B
3/2/70	+	+	–	+	+	–	+	–	+	–	+	–	–	+	–	B
3/11/70	+	+	–	+	+	±	+	–	+	–	+	–	–	+	–	B
3/13/70	+	+	–	+	+	MF 50%	+	–	+	–	+	–	–	MF 75%	–	B
3/17/70	+	+	–	+	+	MF 50%	+	–	+	–	+	–	–	MF 75%	–	B
3/25/70	+	+	–	+	+	MF 75%	+	–	+	–	+	–	–	MF 75%	–	B
4/3/70	+	+	–	+	+	MF 75%	+	–	+	–	+	–	–	–	–	B
4/9/70	+	+	–	+	+	MF 75%	+	–	+	–	+	–	–	MF 75%	–	B
4/14/70	+	+	–	+	+	MF 50%	+	–	+	–	NT	–	–	MF 75%	–	B
4/23/70	+	+	–	+	+	MF 25%	+	–	+	–	NT	–	–	+	–	B
4/29/70	+	+	–	+	+	±	+	–	+	–	NT	–	–	+	–	B
5/8/70	+	+	–	+	+	±	+	–	+	–	NT	–	NT	+	–	B
5/13/70	+	+	–	+	+	–	+	–	+	–	NT	–	NT	MF 75%	–	B
5/21/70	+	+	–	+	+	–	+	–	+	–	+	–	–	+	–	B

NT = Not Tested MF = Mixed Field % of Cells Agglutinated

Fig. 2. Changes of antigens noted in the recipient following the administration of cord blood from baby "M".

The patient was maintained on a variable dose of 6-mercaptopurine and 60 mg of prednisone daily. Following the development of some lymph node enlargement, the 6-mercaptopurine was discontinued. The patient was then started on methotrexate (2.5 mg twice a day) for a period of 11 days (April 22-May 11); although the peripheral white count remained within normal limits during this period, the glands did not seem to respond. On May 11 the methotrexate was discontinued and the patient's medication was changed to vincristine (2mg weekly). Since then, the patient has been maintained on 2 mg vincristine per week.

The patient was readmitted to the hospital June 19, 1970, with complaints of feeling tired, back pain, headache and a slight temperature elevation. He stated that he had resumed regular semiweekly karate class attendance immediately after his discharge from the hospital, but that shortly thereafter he developed some back discomfort which had become persistent. At the time of this admission the patient's laboratory findings were: direct bilirubin 3.2 mg/100ml, total bilirubin 7.6 mg/100 ml, urinalysis normal, WBC 4,500/mm^3, hemoglobin 12.7 gm/100 ml, a normal differential with 62 neutrophils, 28 lymphocytes, with 10 mononuclear cells present. X-rays of the lumbosacral region were normal. Total protein was 7.7 gm/100 ml with albumin 4.4 gm/100 ml, alkaline phosphatase 11.3 units, SGOT 609 units. The Coombs' test and the patient's blood cultures were negative. The reticulocyte cell count was 3.6%. After an unsuccessful aspiration, a core biopsy of the marrow was reported as hypoplastic marrow without evidence of leukemia.

At the time of his second discharge from the hospital, June 27, 1970, the patient's direct serum bilirubin had dropped to 2.0 mg/100 ml with a total bilirubin of 3.4 mg/100 ml. His hematological work-up was essentially normal. The patient's appetite had returned and he has continued to feel well; his weight has returned to the level prior to his illness. To the date of this writing, the patient's white count has remained within normal limits. The cause of the episode of jaundice was not clearly established; however, it was believed to be due to the effects of the chemotherapy employed.

Discussion

Hematopoietic transplants have been accomplished by means of aspirated bone marrow cells, fetal liver suspension and peripheral leukocytes.[7,12] The use of 45 ml of fetal blood which represented approximately one billion nucleated cells,[11] successfully accomplished a temporary viable hematopoietic transplant which functioned for over five weeks in the recipient. Confirmation of this hematopoietic transplantation is provided by the altered red blood cell phenotype of the recipient, which phenotype corresponded with that of only one (baby "M") of the fetal blood donors. Four days after tranfusing the recipient with baby "M" 's blood, his M antigen factor was still essentially negative, only some questionable

(±) agglutination existed which could not be confirmed on repeated typing. The first definite agglutination in the M typing occurred in the recipient six days after receiving the suspected donor's blood and at this time the JK^a typing was also a mixed field. The agglutination of the M antigen continued to increase in strength and on April 3, 27 days after transfusion of baby "M" 's blood, the recipient's typing by anti-M revealed over 75% agglutinated red cells and the JK^a typing was negative for any agglutination. At this time, the antigen typing of the recipient corresponded to the antigen typing of the suspected donor and baby "M" was the only donor with this exact phenotyping.

Two routine 500 cc blood transfusions given during treatment were also phenotyped; neither of these units corresponded with the phenotype which later developed in the recipient. Although the red cells of these two routine tranfusions supplied M+ cells, that they were insufficient to alter the recipient's type was demonstrated by the patient's unaltered typing of March 11, 1970, five days after the second routine transfusion was given.

Neither fetal hemoglobin nor A_2 hemoglobin was detected in the recipient at any time. Although A_2 hemoglobin has been demonstrated in hematopoietic allografts utilizing fetal liver cell suspension, fetal hemoglobin in the host was not demonstrated in those cases.[3]

It is of considerable interest that an allograft has been produced by the approximately one billion nucleated cells[11] of the 45 ml of cord blood administered to this patient. Review of the literature offers frequent citation of the necessity for large quantities of marrow for successful allograft. The quantity of bone marrow required for a syngenic marrow transplant in humans has been estimated at 50 ml of aspirated marrow (0.8×10^9 cells for a 70 kg man).[8] Pegg has suggested that the minimal effective dose of hematopoietic cells for an allogenic bone marrow graft is 9×10^9 cells for a 70 kg man or 500 ml of aspirated marrow.[9] It should be noted, in this connection, that Mathe's most successful transplant in a leukemic patient utilized 2,000 ml of marrow which was obtained from six members of the patient's family.[4]

Potentially several advantages should acrue from the use of multiple donors of fetal hematopoietic cells. Firstly, multiple bone marrow donors are believed to be desirable since it would appear that the host spontaneously selects that marrow which is offered by the most compatible donor. Further, the use of multiple donors has also been found helpful in preventing the occurrence of secondary (graft versus host) syndrome. Additionally, when used in transplantation, fetal hematopoietic tissue is believed to be more tolerant of the host than are those grafts obtained from adult marrow.[13,1] Although in this case it is uncertain as to whether the recipient had a transplant of the lymphoid elements, no evidence of a graft vs. host reaction was noted in the recipient during the brief period in which the hematopoietic graft was believed to be viable. Finally, any active obstetrical service offers the potential of providing many donors of cord blood over a relatively short period of time. In addition to this ready availability, it should be noted that we have detected no adverse reaction following the administration of fetal blood. Utilization of this technique could result in a simple method to study hematopoietic transplants.

Although much discussed, the effect of bone marrow transplantation on leukemia is difficult to evaluate because the reported marrow transplantations have been simultaneously associated with other forms of therapy,[10] as is also true in the case presented here. It must be noted, however, that, except for some cervical lymphoadenopathy, a clinical and hematological remission of over nine months has been obtained in this patient.

Summary

Fetal blood was successfully utilized in establishing a hematopoietic transplant in a leukemic patient. This method has not been previously attempted. Only a relatively small number of donor cells was necessary to estab-

lish the temporary allograft. Potentially, this method of utilizing cord blood could greatly reduce those problems which are related to the obtaining of an adequate number of donor cells. Further, by making many donors readily available to the recipient, enhanced opportunity is rendered for the host to select the most compatible donor. The utilization of cord blood could establish an easy technique for the study of hematopoietic transplants.

Acknowledgements: The authors would like to express appreciation for the assistance given by Dr. Richard Oakley, Mrs. Rosemary McGrail Fly, Mrs. Gail Robertson and Miss Linda Hart.

Support in full by the "Abraham S. Ende Research Foundation".

REFERENCES

1. Barnes, D. W. H., Ilberg, P. L. T., and Loutit, J. F.: Nature 181:488, 1958.
2. Bortin, M. M.: Transplantation 9:571, 1970.
3. Bridges, J. M., Neill, D. W., Lehmann, H.: Brit. Med. J., 1:1349, 1961.
4. Mathe, G., Amiel, J., Schwarzenberg, L., Gattan, A., Schneider, M.: Prog. Clin. Cancer 3:309, 1967.
5. Pegg, D. E.: In Bone Marrow Transplantation. Lloyd-Luke (Medical Books) LTD, England, p. 87, 1966.
6. Pegg, D. E.: In Bone Marrow Transplantation. Lloyd-Luke (Medical Books) LTD, England, p. 96, 1966.
7. Pegg, D. E.: In Bone Marrow Transplantation. Lloyd-Luke (Medical Books) LTD, England, p. 38, 1966.
8. Pegg, D. E.: In Bone Marrow Transplantation. Lloyd-Luke (Medical Books) LTD, England, p. 102, 1966.
9. Pegg, D. E.: In Bone Marrow Transplantation. Lloyd-Luke (Medical Books) LTD, England, p. 77, 1966.
10. Pegg, D. E.: In Bone Marrow Transplantation. Lloyd-Luke (Medical Books) LTD, England, p. 86, 1966.
11. Smith, C. A.: In Physiology of the Newborn Infant. Charles C. Thomas, Springfield, Ill., p. 162, 1959.
12. Storb, R., Epstein, R. B., Ragde, H., Bryant, J., Thomas, E. D.: Blood 30:805, 1967.
13. Uphoff, D. E.: J. Nat. Cancer Inst. 20:629, 1958.

---

121 South Market Street
Petersburg, Virginia 23803

Clinical Study of Reticulum Cell Sarcoma

The cooperation of physicians is requested in the referral of patients with reticulum cell sarcoma for studies being conducted by the National Cancer Institute's Medicine Branch at the Clinical Center, National Institutes of Health, Bethesda, Maryland.

Patients having previously untreated reticulum cell sarcoma are needed to participate in clinical trials. However, patients with all stages of the disease are suitable for study. Those in the early stages of disease are being studied in collaboration with the Radiation Branch.

Upon completion of their studies, patients will be returned to the care of the referring physician who will receive a summary of findings.

Physicians interested in having their patients considered for admission to this study may write or telephone: Phillip Schein, M.D., Clinical Center, Room 4-B-13, National Institutes of Health, Bethesda, Maryland 20014. Phone: 301-496-2031 or Vincent T. DeVita, M.D., Clinical Center, Room 12-N-226, National Institutes of Health, Bethesda, Maryland 20014. Phone: 301-496-4916.