Erythroid Homograft Following Leukocyte Transfusion in a Patient with Acute Leukemia

I. Clinical Studies and Implications

By ROBERT H. LEVIN, JACQUELINE WHANG, PAUL P. CARBONE AND EMIL J. FREIREICH

GRANULOCYTOPENIA and infection occurring in patients with acute leukemia has been managed successfully in many instances by transfusion of peripheral blood leukocytes. Granulocyte replacement transfusion utilizing as donors patients with chronic myelocytic leukemia (CML) who have more than 100,000 granulocytes per cu. mm. in their venous blood (WBC) has resulted in cure of pseudomonas septicemia in patients with acute leukemia. Since the Philadelphia (Ph1) chromosome abnormality as well as the sex chromosome markers can be identified in cells taken from bone marrow and peripheral blood of the patients with CML who served as leukocyte donors, their cells are ideally suited for following the fate of transfused leukocytes from single or multiple donors. We have previously reported the occurrence of functioning myeloid bone marrow homografts in patients with acute leukemia, following leukocyte transfusion from donors with CML. The present report considers a patient with acute leukemia who developed an erythroid marrow homograft after transfusion of peripheral blood cells from a patient with CML, that functioned for at least 2 months.

METHODS

Leukocytes from the patients with CML were obtained by plasmapheresis of 2 to 4 units of whole blood at 1 donation. The white cell rich plasma was harvested by centrifugation of the blood at 50 times gravity for 30 minutes (International PR-2 centrifuge). The plasma was transfused within 4 hours of the donation. A total of 1-1.6 x 10^11 leukocytes were obtained from each donation. Cytogenetic studies of the bone marrow were performed by direct air-dry technic without prior in vitro culture. Only those cells in metaphase which could be accurately interpreted were scored in determining the percent of cells in any specimen which contained the Ph1 chromosome. Antibody determinations in response to the Tularemia vaccine were performed by reacting a purified cell wall preparation with bentonite particles and performing a flocculation test with dilu-
tions of the patients' serum. Serologic technics pertinent to red blood cell identification and isoagglutinins are described elsewhere in this journal.

HISTORY PRIOR TO LEUKOCYTE TRANSFUSION

A 29-year-old male was admitted to the National Cancer Institute for the second time on July 5, 1963 with a diagnosis of acute lymphocytic leukemia. During a previous admission from October 29 to December 20, 1962, the patient was treated with adrenocorticosteroids and transfusions of 85 units of fresh platelets and nine units of red blood cells. After enjoying a remission of his disease for seven months he was readmitted with WBC of 110,000 per cu. mm., hemoglobin (Hb) 9.3 Gm. per 100 ml., and platelets of 2,000 per cu. mm. Stained smear of his peripheral blood showed that 95 per cent of his leukocytes were abnormal blast forms. The liver was palpated three centimeters below the right costal margin. The spleen and lymph glands were not enlarged. Purpura and petechiae were prominent. The patient was immediately started on combined chemotherapy which consisted of 6-mercaptopurine 60 mg./m.2 daily, oral, amethopterin 25 mg./m.2 intravenously every fourth day; prednisone 40 mg./m.2 per day, oral, and vincristine 2 mg./m.2 intravenously, weekly. The WBC fell precipitously to as low as 3,000 per cu. mm. within 1 week. On July 12, the patient developed fever of 40.3 C. Antibiotic therapy was initiated. Pseudomonas Aeruginosa was cultured from the rectum and throat. On that day his WBC was 7400 but there were only 4 per cent granulocytes. For this reason granulocyte replacement was attempted by transfusion of 10¹¹ leukocytes obtained from a female donor with CML.

RESULTS OF LEUKOCYTE TRANSFUSION

Figure 1 summarizes the hematologic and cytogenetic data which resulted from observations of the recipient patient for the next 79 days. The days are numbered from the first leukocyte transfusion which is indicated by a vertical arrow under the leukocyte portion of the graph. Platelet and red blood cell transfusions are similarly indicated under their respective curves. The patient's temperature fell to normal within 24 hours after the granulocyte transfusion. Nine days after transfusion the bone marrow was still nearly replaced by lymphoblasts but 3 per cent of the marrow cells in metaphase were identified as female (XX) and contained the Ph² chromosome abnormality of the leukocyte donor. Twelve days after the first transfusion, fever recurred (39.8 C.) due to pneumonia. Since granulocytopenia was severe (fig. 1) a second transfusion of 1.4 x 10¹¹ leukocytes was given. Because the original donor was not available, a male donor with CML was used. There was a defervescence of fever within 12 hours. Six days later or 18 days after the first transfusion 2 donor populations were identified in the patient's bone marrow; 60 per cent of cells were female Ph¹ positive cells (1st donor) and 40 per cent were male Ph¹ positive cells (2nd donor). At the same time examinations of a Giemsa stained smear of the bone marrow showed a diminution in lymphoblasts with replacement by normal appearing erythroid and myeloid precursors. However, 10 days later, 28 days after transfusion, it was clear that the cells from the first donor had replaced the entire bone marrow, as 97 per cent of scoreable metaphases were female Ph¹ positive cells. Figure 1 illustrates that subsequent to this observation 80 to 100 per cent of marrow cells were from the female donor and they persisted for at least 66 days after the first transfusion. In addition the proportion of nucleated
cells in the bone marrow switched from a near normal myeloid:erythroid ratio on day 18 to a very marked erythroid hyperplasia by day 46, which accounted for over 90 per cent of the nucleated marrow cells.

**Homograft Function**

Evidence for function of the erythroid homograft was apparent by day 25 when 5 per cent of the patient's circulating red blood cells were found to be Group A, Rh positive. This was the same blood group as the female donor, whereas the patient and the male donor were both Group O, Rh positive. The direct Coombs test also became positive and remained so throughout. The proportion of Group A cells rose gradually to a maximum of 85 per cent. During this period the anti-A isoagglutinins remained fairly constant (1:16-1:32), however, the anti-B titer rose to a high of over 1:2,000. Detailed consideration is given to the observations concerning the production of red blood cells and isoagglutinins in the patient in an accompanying report. The rise in Group A erythrocytes paralleled a reticulocytosis that reached 16 per cent as well as a rise in hemoglobin to 15 Gm. per 100 ml. (fig. 1). The survival of autologous Cr<sup>51</sup>-labeled red blood cells was normal. A spontaneous rise in platelets to a level of 1,000,000 per cu. mm. was seen, and was accompanied by a marked megakaryocytosis in the bone marrow. In addition, granulocytes were maintained at near normal levels. These findings indicated that the homograft produced all 3 cellular blood elements.

However, by day 66, pseudomonas pneumonitis, septicemia, and granulocytopenia had recurred. The patient received 10<sup>11</sup> CML cells from the original female donor. This was followed by cure of the septicemia as manifested by prompt fall in temperature to normal and sterilization of the blood. Six days later the per cent of female Ph<sup>1</sup> positive cells in the marrow had increased from 79 to 90 per cent. However, return of lymphoblasts was noted in the marrow. The hemoglobin had fallen by this time (day 72) to 9.0 Gm. per 100 ml and reticulocytes decreased to less than 0.5 per cent. Cavitation of the pulmonary lesion led to recurrence of *Pseudomonas* septicemia once more. The patient received a fourth granulocyte transfusion on day 72 of 1.6 x 10<sup>11</sup> cells from a different male CML donor. This transfusion resulted in a temporary clinical response. Five days later 3 cell populations were noted in the patient's bone marrow: 74 per cent of scoreable cells in metaphase were female Ph<sup>1</sup> positive, 23 per cent were male Ph<sup>1</sup> positive, and three per cent were the patient's own male Ph<sup>1</sup> negative cells. The patient expired two days later; 79 days after the first leukocyte transfusion, from complications of *Pseudomonas* lung abscess and pneumonia.

**Immune Capacity**

Except for prednisone, combination chemotherapy was withheld from day 18 up to day 65, when it was re instituted in an attempt to prevent further loss of homograft function. During the period of no chemotherapy, (day 41), the patient was stimulated with a primary antigenic stimulus (Tularemia vaccine) to evaluate his immune response. The antibody titer rose from 0
ERYTHROID HOMOGRAFT AND ACUTE LEUKEMIA

591 to 1:512 in 14 days. At the same time, skin grafts from the male and female donors, and from another CML patient who was not a donor, were placed on the recipient’s forearm. Although chemotherapy was not reinstituted for 14 days, all 3 skin grafts were viable at the time the patient expired. Thus, the skin grafts survived at least 38 days.

Postmortem examination revealed slight focal leukemic infiltration of bone marrow and spleen. Lymphoid aplasia was noted, as was chronic inflammation of the hepatic parenchyma. Death was attributed to bilateral pneumonitis due to *Pseudomonas Aeruginosa, Aspergillus fumigatus* and *Candida albicans*.

**DISCUSSION**

This patient had the longest recorded survival of a hematopoietic homograft induced by cells circulating in the peripheral blood. Transfusions of peripheral blood cells have protected rodents against the effects of lethal irradiation, as a result of marrow and lymphoid cell repopulation. Such experiments provide evidence for the presence of stem cells of both myeloid and lymphoid cell lines in the peripheral blood.14-16 Whang et al. have shown that the Philadelphia chromosome abnormality found in patients with CML is present in granulocyte, erythrocyte and platelet producing cells, but not in lymphocytic cells. These findings indicate that the myeloid cells of the bone marrow probably derive from a different progenitor than the lymphoid cells.5 Moreover, the bone marrow homografts observed in recipients of peripheral blood leukocytes from CML donors, have shown differentiation primarily to granulocytic, erythrocytic and thrombocytic cells.6 These bone marrow homografts may result from a greater proliferative capacity of the abnormal cells in the peripheral blood of the donors with CML.17 However, it is likewise possible that the ability to harvest and transfuse a very large number of cells from patients with CML, contributes to the eventual “take” of the donor cells. Since there are fewer cells capable of establishing a homograft in the peripheral blood than in the bone marrow,14-16,18 it is understandable that at least 10 to 100 times as many peripheral blood cells as bone marrow cells must be transfused to obtain marrow homografts. This quantitative relationship has been observed in mice14,15 and in man. The patient presented, and the patients previously reported to have hematopoietic homografts following leukocyte transfusion, received at least 1011 white blood cells with each transfusion.8 On the other hand, 109 bone marrow cells have brought about marrow repopulation in the rare instances of homologous marrow grafts described by Beilby et al.,19 and Mathé and associates.20 The majority of marrow homografts observed after transfusion of leukocytes from CML donors, have consisted primarily of granulocytic cells.9 The bone marrow of the recipients was almost completely replaced with granulocytic leukocytes in all stages of development, and the peripheral blood showed a leukemoid response of WBC as high as 37,000 per cu. mm. with 80 per cent granulocytic cells. Since granulocytic hyperplasia is characteristic of CML, it was not surprising that a hematologic picture similar to the pattern of the donor would be seen in the recipients. The present case was unusual because
the erythroid cells constituted virtually 100 per cent of the activity of the homograft. An erythroid homograft was detected in a previous patient by change in red cell group. However, cytogenetic proof was lacking. In the present patient the erythroid homograft was shown to be of donor type both by cytogenetic techniques and change in blood group. Not only is erythroid hypoplasia common in patients with CML, but the female CML donor was anemic at the time she donated the leukocytes for transfusion (8.5 Gm. Hb. per 100 ml.) and had required periodic transfusion of red blood cells. Thus, although erythropoiesis was reduced and ineffective in the donor, when her peripheral blood cells were transfused and a marrow homograft developed in the recipient, these same cells differentiated almost entirely into the red blood cell series and established very effective erythropoiesis with spontaneous rise in hemoglobin to 15 Gm. per 100 ml. and normal red cell life span. It seems clear from this example that differentiation into one or the other cell series must depend in a large degree on environmental influence and not exclusively on genetic control.

Mathé has described a syndrome developing in recipients of human homologous bone marrow two to three weeks after transfusion, which is believed to be a manifestation of immunological reaction of the graft against the host, similar to that described in primates. These patients suffered weight loss, severe diarrhea, nausea and vomiting, fever, maculopapular eruptions, hepatomegaly, and severe fungal or other infections. We did not observe such a syndrome in this patient, although he had a combination of three serious infections: Pseudomonas septicemia, pulmonary Aspergillosis, and disseminated Candidiasis. Such clinical infections might well occur as a result of immunologic incompetence or tolerance but they are also characteristic of the present spectrum of infection in patients dying with acute leukemia. Since lymphocytes are felt to be the immunologically competent cells of bone marrow or peripheral blood which cause graft versus host reaction and “secondary disease,” the characteristic paucity of lymphocytes in CML blood may account for the fact that “secondary disease” was not observed in this patient. Although it has been suggested that elimination of immunologically active cells (lymphocytes) from donor material is a goal of human marrow transplantation which would prevent secondary disease and allow grafts to flourish permanently, our failure to observe permanency in this patient may actually suggest the opposite to be true. Indeed, Mathé’s report of a human chimera of over 12 month’s duration illustrates that “secondary disease” can be treated with appropriate immunosuppressive, antibiotic, and supportive agents. Subsequent to this phase, Mathé’s patient recovered and developed normal hematologic and immunologic function. Thomas et al. have observed permanent chimerism in canine recipients of homologous marrow transfusions after successful treatment of secondary disease. Thus, a graft versus host reaction may be a manifestation of a period of immunologic accommodation between graft and host which is susceptible to treatment with immunosuppressive agents, as are other diseases of immune etiology. The end result of a successfully treated graft versus host reaction may then be permanent.
ERYTHROID HOMOGRAFT AND ACUTE LEUKEMIA

593

tolerance or chimerism. The fact that a phase of "secondary disease" was not seen in our patient may have led to the ultimate failure of the grafted cells. Although donor cells were present at least 66 days after the first transfusion, the graft had ceased functioning and permanent chimerism was not observed.

This patient was receiving combined chemotherapy for his leukemia at the time of leukocyte transfusion and for 18 days thereafter.11 Undoubtedly this combination of 6-MP, amethopterin, prednisone, and vincristine caused powerful immune suppression preventing initial rejection of the foreign homologous cells.13,29,30 Later, when these drugs were discontinued, the homograft continued to flourish as evidenced by reticulocytosis and spontaneous increase in hemoglobin due to the increasing production and release of Group A red cells from the marrow. On the other hand, the patient was able to respond normally to a primary exposure of a soluble antigen (Tularemia vaccine), and to produce a secondary antibody response of isoagglutinins. These findings indicate that he was not "immunologically incompetent." However, at about 60 days it became clear that the graft was no longer functioning as red cell production abruptly ceased. Chemotherapy was reinstituted in an attempt to prevent further loss of function, but without success. Since there is no adequate test for rejection of a hematopoietic homograft other than lack of function, we have to assume that either active rejection occurred or that the function of graft cells was inhibited by the overwhelming Pseudomonas infection.

The patient was the recipient of 2 leukocyte transfusions from unrelated donors of opposite sex. The female donor was of blood Group A, whereas the male donor and the recipient were both of Group 0. Although one might expect that individuals of the same sex and same major blood groups would have closer histocompatibility, the female Group A donor cells predominated in the graft, whereas the male Group O cells persisted only a little over a week. In dogs it has been shown that matching of donor and recipient animals on the basis of red cell groups did not increase the long-term survival of lethally irradiated dogs treated by homologous bone marrow transfusion.31 In man there is evidence that the Group A red cell antigen specifically does not play a role in homotransplantation, although "minor" red cell groups may.32 To support this view Terasaki et al. have shown that there is at least 50 times less A antigen on lymphocytes than on red blood cells.32 The anti-A isoagglutinins found in our patient, probably did not influence the course of the homograft.

Mathé et al. have utilized multiple donor bone marrow transfusion as a source of hematopoietic stem cells. In one dramatic success, the recipient's marrow was repopulated by only 1 of 6 donors.22 This technic provides both a considerably larger number of cells for transfusion and cells from individuals of varying histocompatibility with the recipient. The cells from the donor of closest compatibility have the best chance of survival. A 12-month survival of the homograft in the patient reported by Mathé's group attests to the attractiveness of this technic. The facts of our patient would confirm the observation that only one, of two or more donor cell populations persist in the recipient.

An important consideration is whether the homologous transfused leukocytes
exerted an antileukemic effect during the period of homograft function. Barnes and Loutit among many investigators have demonstrated that homologous bone marrow transfusions in leukemic mice after lethal irradiation might destroy persistent leukemic cells by an immunologic graft versus host or host-tumor reactions. Prolonged survival has been observed in mice treated by this technic.\textsuperscript{33,34} In this case, and in other patients with acute leukemia who developed a functioning homograft after leukocyte transfusion, the marrow was replaced by leukemic cells prior to transfusion. These leukemic cells had persisted despite exposure to maximum chemotherapy with antileukemic drugs. However, shortly after transfusion the morphologic picture in the bone marrow abruptly changed with disappearance of malignant cells and replacement by donor hematopoietic cells. Although there was an indication of beginning relapse in the last bone marrow aspiration before death, the only evidence of leukemia noted at autopsy was scattered foci of leukemic cells in the spleen and bone marrow. Such information encourages the investigator to pursue this approach to the treatment of leukemia. Indeed, if lymphocytes were present in the transfusate one might expect some immunologic reaction against the leukemic cells as well as other host cells; and if the donor's lymphocytes were sensitized against the recipient's tumor cells even more pronounced rejection of leukemic cells might occur.

Since leukocytes are a readily available source of hematopoietic stem cells, investigation and development of equipment and technics for the separation and collection of white cells from normal persons are in progress.\textsuperscript{35} Such technics may make it possible to obtain stem cells, granulocytes, and lymphocytes for treatment of infection and for transplantation of hematopoietic cells repeatedly from the same individual.

**Summary**

A patient with acute lymphocytic leukemia developed a functioning erythroid homograft after transfusion of leukocytes from donors with chronic myelocytic leukemia. Two donor populations were observed cytogenetically, by examination of the recipient's marrow for sex and Philadelphia chromosomes. The cells from a female donor of different blood group eventually repopulated the entire bone marrow and were exclusively responsible for the function of the homograft. Although the donor was anemic and required periodic transfusions, her transplanted cells caused normal erythropoiesis in the recipient for a prolonged period. During this time the recipient enjoyed remission of acute leukemia but expired as a result of overwhelming infection. It is proposed that multiple transfusions of leukocytes is a useful technic for treatment of infection, for increasing knowledge of factors influencing hematopoiesis, and for studying approaches to effective immunotherapy of leukemia.

**Summario in Interlingua**

Un patiente con acute leucemia lymphocytic disveloppava un functionante homograffo erythroide post le transfusion de leucocytes ab donatores con chronic leucemia myelocytic. Duo populationes de donatores esseva observe
ERYTHROID HOMOGRAFT AND ACUTE LEUKEMIA

595
cytogeneticamente per le examine del medulla del recipiente relative a
chromosomas de sexo e chromosomas Philadelphia. Le cellulas ab un donator
feminin de un differente gruppo de sanguine repopulava in le curso del
tempore le integre medulla ossee e esseva exclusivemente responsabile pro
le functionamento del homograffo. Ben que le donator esseva anemic e re-
quireva transfusiones periodic, su transplantate cellulas causava in le recipiente
un erythropoiese normal durante un prolongate periodo de tempore. Durante
iste intervallo le recipiente se trovava in remission de su leucemia acute, sed
ille moriva como resultato de un infection superante. Es proponite le theses
que multiple transfusiones de leucocytos representa un technica utile in le
tratamento de infection, in le effortio de augmentar nostre cognoscentias
defactores de influentia in le hematopoiese, e in le studio de possibile
methodologias pro le efficace immunotherapia de leucocemia.

REFERENCES

marrow homografts following homologous leukocyte transfusion in

2. Freireich, E. J., Morse, E. E., Bronson, W. R., and Carbone, P. P.: Trans-
fusion of granulocytes from donors with chronic myelocytic leukemia to

3. ---, Levin, R. H., Whang, J., Carbone, P. P., Bronson, W., and Morse, E. E.: The
function and fate of transfused leukocytes from donors with chronic
myelocytic leukemia in leukopenic recipients. J. Clin Invest. 13:

control of infection in patients with acute leukemia. Cancer
Res. In press.

distribution of the Philadelphia chromsome in patients with chronic

E. J.: Persistent mitosis of transfused homologous leukocytes in children

plasmapheresis of blood donors as a source of platelets. Blood 18:303–309,
1961.

8. Tjio, J. H., and Whang, J.: Chromosome preparations of bone marrow cells
without prior in vitro culture or in vitro colchicine administration. Stain


10. Schmidt, P. J., Yokoyama, M., McGinniss, M. H., and Levin, R. H.: Eryth-
roid homograft following leukocyte transfusion in a patient with acute
leukemia. II Serologic and immuno-
chemical studies. Blood

(VAMP) for acute lymphocytic leu-
Cancer Res. 5:20, 1964.

12. Kadull, P. J., Reames, H. R., Coriell, L. L., and Fahey, L.: Studies on Tulare-
emia V. Immunization of man J. Im-

13. Levin, R. H., Landy, M., and Frei, E., III.: Effect of 6-mercaptopurine on


Erythroid Homograft Following Leukocyte Transfusion in a Patient with Acute Leukemia: I. Clinical Studies and Implications

ROBERT H. LEVIN, JACQUELINE WHANG, PAUL P. CARBONE and EMIL J. FREIREICH