Persistent Mitosis of Transfused Homologous Leukocytes in Children Receiving Antileukemic Therapy

Abstract. *Temporary homologous bone marrow grafts were observed in three patients with acute leukemia, receiving intensive antileukemic treatment, following transfusion of peripheral blood cells from donors with chronic myelocytic leukemia. Persistent mitosis of the transfused cells, containing the Philadelphia chromosome marker, were detected by cytogenetic techniques 19, 39, and 52 days after transfusion.*

Replacement of circulating human granulocytes by transfusion of homologous blood cells was recently accomplished (*1*). Because of the inability to obtain large quantities of granulocytes for transfusion from normal persons, patients with chronic myelocytic leukemia (CML), having peripheral white blood cell counts (WBC) greater than 100,000 per cubic millimeter, with a high proportion of mature granulocytes, served as leukocyte donors. Leukocytes from the donors were obtained by plasmapheresis (*2*). When 10^{11} granulocytes were transfused there was an increase in circulating granulocytes in the majority of recipients (median, 1000/mm^3). Following transfusion, the granulocytes disappeared from the circulation in a curvilinear manner in 1 to 4 days. Clinical control of serious infection, achieved in the majority of recipients, was directly related to the number of granulocytes transfused and to the increase in the number of circulating granulocytes after transfusion (*1*).

All of the patients who served as donors had the Philadelphia (Ph^1) chromosome in over 85 percent of the bone marrow cells in metaphase which could be examined. This cytogenetic marker, which has been shown to be present in the peripheral blood as well as the bone marrow of patients with chronic myelocytic leukemia (*3*), served as a cell label for following the fate of the transfused granulocytes. Bone marrow and blood of recipients were examined after transfusion, for the presence of the Ph^1 chromosome marker. The cytogenetic studies of the bone marrow were performed by the direct air-dry technique, without prior culture in vitro (*4*) while the short-term culture technique was used for the peripheral blood cells (*5*).

The three recipient patients were children (aged 2½ to 8 years) with acute lymphoblastic leukemia, each of whom received a total dose of 1.9 to 8.8 × 10^{11} nucleated cells in one to six separate leukocyte transfusions. The data for the first patient are shown in Fig. 1. Following granulocyte transfusion on day 0, the transfused leukocytes did not disappear in the expected fashion. Rather, the granulocyte increment persisted, followed by three spontaneous rises and falls in circulating granulocytes with the highest count of 31,000 per cubic millimeter on the 35th day after transfusion. Cytogenetic examination of cultured peripheral blood leukocytes on the 12th, 18th, 29th, and 39th day after the leukocyte transfusion showed that 51, 55, 90, and 38 percent, respectively, of the metaphases which could be examined, contained the Ph^1 chromosome (Fig. 1). The bone marrow was examined five times after transfusion, and the Ph^1 chromosome was found successively in 92, 70, 99, 97, and 28 percent of scoreable metaphases. Morphologic examination of Giemsa-stained marrow smears (Fig. 1) showed that during this period a predominance of granulocytes existed in hypercellular marrow specimens until day 39 when lymphoblasts were seen to account for 80 percent of the marrow cells. Although the patient received amethopterin and prednisone during the time that disappearance of lymphoblasts with replacement by donor leukocytes was observed, there was no real evidence of clinical or hematological remission.

After transfusion of leukocytes to the second patient (Fig. 2), a steady, slow rise in the number of white blood cells was noted over the next 3 weeks, reaching a maximum of 37,700 per cubic millimeter with 90 percent granulocytes. Three days after transfusion, the Ph^1 chromosome was found in 40 percent of marrow cells and 1.5 percent of peripheral blood cells. Donor cells were responsible, at least in part, for the marked leukocytosis, as confirmed cytogenetically by finding the Ph^1 chromosome in 1 percent of cultured blood leukocytes in metaphase 18 days after transfusion. An increase in the proportion of granulocytes in the bone marrow was seen on examination of a biopsy section taken at the same time. Amethopterin and prednisone were administered for 3 weeks prior to the transfusion of white blood cells, and

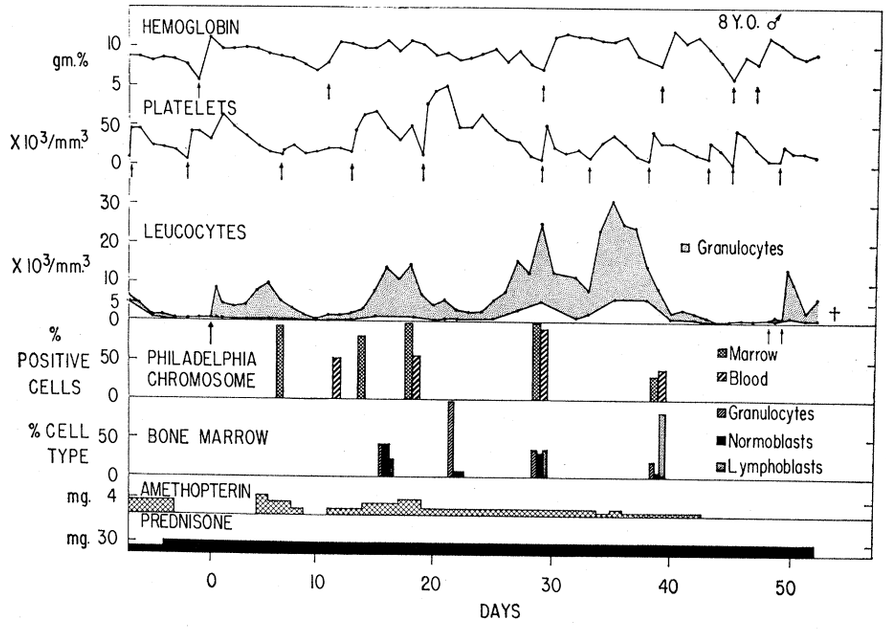

Fig. 1. Changes in the blood and bone marrow of patient No. 1, after leukocyte transfusion. The vertical arrows under hemoglobin, platelet, and leukocyte graphs represent transfusions of the respective blood components. The shaded area under the leukocyte curve represents the granulocyte component of circulating leukocytes. The upper bar graph shows the percentage of metaphases which would be examined in blood or bone marrow, which contained the Ph^1 chromosome. The lower set of bars shows the proportion of different nucleated cell types that were seen in the bone marrow. The abscissa is days after the leukocyte transfusion.

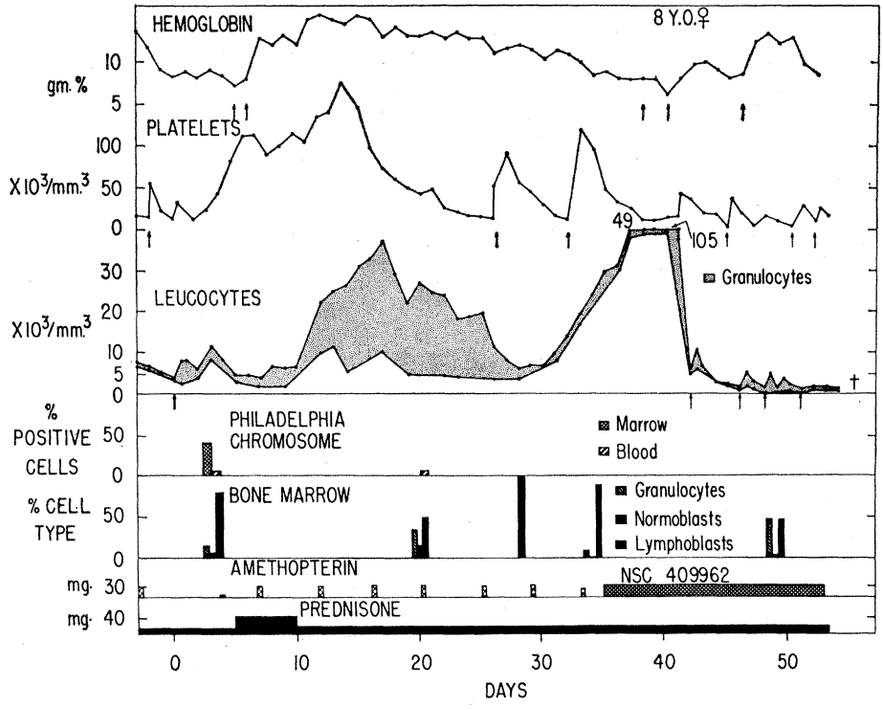

Fig. 2. Changes in the blood and bone marrow of patient No. 2 after leucocyte transfusion. Initially, a leucocytosis of granulocytes occurred whereas later, when evidence of clinical and hematologic improvement had passed, a leucocytosis consisting primarily of lymphoblasts was seen. Hemoglobin and platelet levels spontaneously reached normal after transfusion of white blood cells.

continued afterward. After transfusion of white blood cells, the hemoglobin and platelet levels spontaneously returned to normal and transfusion of these blood elements was not required.

A third patient received six leukocyte transfusions for recurrent severe infection and septicemia associated with granulocytopenia. His marrow was examined cytogenetically 54 days after the last of his six transfusions. At that time, the bone marrow was morphologically normal, but 5 percent of the metaphases contained the Ph¹ chromosome. Further cytogenetic examinations were performed at monthly intervals but the Ph¹ chromosome was not found after the initial observation. The hematological remission lasted 4 months and was indistinguishable from that usually induced by 6-mercaptopurine and prednisone. In spite of the prolonged persistence of donor cells and the length of remission afterward, the patient did not suffer any detectable adverse clinical effects.

The persistence of Ph¹-labeled cells in the marrow following leukocyte transfusion provides conclusive evidence of a replicating homograft of transfused leukocytes. In addition, an erythroid homograft resulting from transfusion of nucleated red blood cells or by the erythropoietic stem cells in the donor blood is suggested by the cytogenetic findings in the first patient on day 29 (Fig. 1). At this time 99 percent of the scoreable metaphases in the marrow were Ph¹ positive. However, 40 percent of the nucleated marrow cells and 60 percent of the mitoses counted were erythroid cells. This suggests that the erythroid cells must contain the Ph¹ chromosome, as has been demonstrated in patients with chronic myelocytic leukemia (*3*). However, the erythropoiesis was ineffective, as there was no increase in reticulocytes and the transfusion requirement for red blood cells was affected only slightly. Donor red cells were searched for in the recipient at this time by immunologic grouping and typing, but none were found.

Skin grafts were applied to the forearm of this patient, taken from the leukocyte donor and from a normal volunteer. Both were of the same sex and were applied 15 days after transfusion. The grafts from the leukocyte donor survived for 24 days, until day 39. The grafts from the normal person were viable at the time the patient died on day 52; these grafts thus survived 37 days and might have survived longer had the patient lived. The rejection of the leukocyte donor's skin occurred at the time when the proportion of Ph¹ positive cells in the marrow was decreasing in association with increasing numbers of leukemic cells.

The intracellular alkaline phosphatase (LAP) activity is known to be decreased or absent in the circulating leukocytes of patients with chronic myelocytic leukemia. The determination was performed on cells of the CML patients who were used as leukocyte donors (*7*). The cells of the donor for patient No. 1 had LAP activity in the low normal range [37 *p*-nitrophenol (PNP) per hour per 10^9 cells]; however, in the recipient, 12 days after transfusion, when his WBC was 1900/mm³ with 75 percent granulocytes, the LAP was 773 PNP/hr per 10^9 cells. Three days earlier (Fig. 1) 90 percent of the peripheral blood cells were Ph¹ positive. The same phenomenon was observed in the second patient 19 days after transfusion at the height of the leukocytosis (Fig. 2). The LAP was 159 PNP/hr per 10^9 cells whereas the LAP of the donor's cells was 2 PNP/hr per 10^9 cells. Block *et al.* have shown that in CML patients who are treated with chemotherapeutic agents and achieve remission, LAP activity reverts to normal (*8*). Similarly the LAP of the donor cells rose to normal values after replication of the injected cells in these two recipients.

The administration of antimetabolite drugs prior to and during the period of persistence of transfused cells certainly contributed to the prolonged survival of homologous cells, as immunosuppressive effects of these agents have been documented in man (*9*). Hypogammaglobulinemia was noted in patients Nos. 1 and 3, with levels of less than 500 mg/100 ml and in patient No. 2 with 800 mg/100 ml, during the post-transfusion period. This finding supports our belief that immune capability in these recipients was impaired.

"Secondary" or "homologous" disease has been studied in primates (*10*) and has been described in two patients by Mathé *et al.* (*11*). The pathologic and clinical stigma of secondary disease are not specific and most of them are frequent autopsy findings in patients with acute leukemia. The findings at necropsy of both our patients were quite consistent with changes known to occur in patients with acute leukemia, and no evidence of "secondary" disease could be established.

Successful transplantation of homologous bone marrow has been reported in man, after whole-body irradiation or chemotherapy in the treatment of malignant disease (*11, 12*). Prolifera-

tion of transfused stem cells in peripheral blood from normal and leukemoid rodents has been successful in radiation protection experiments (*13*). The number of circulating leukocytes required for successful radiation protection in rodents has been reported to be 100 times that necessary for radiation protection by bone marrow infusions. It is of interest that the dose of leukocytes transfused to our patients was in the range of 10^{11} cells, while the number of bone marrow cells transfused in the few cases in which "takes" have been demonstrated in man was 10^{9} cells.

ROBERT H. LEVIN, JACQUELINE WHANG
J. H. TJIO, PAUL P. CARBONE
EMIL FREI III, EMIL J. FREIREICH
Medicine Branch, National Cancer Institute, and *National Institute of Arthritis and Metabolic Diseases, Bethesda 14, Maryland*

References

1. E. E. Morse, W. Bronson, P. P. Carbone, E. J. Freireich, *Clin. Res.* **9**, 332 (1961).
2. A. Kliman, L. A. Gaydos, L. R. Schroeder, E. J. Freireich, *Blood* **18**, 303 (1961).
3. J. Whang, E. Frei III, J. H. Tjio, P. P. Carbone, G. Brecher, *ibid.*, in press.
4. J. H. Tjio and J. Whang, *Stain Technol.* **37**, 17 (1962).
5. P. S. Moorhead, P. C. Nowell, W. J. Mellman, D. M. Battips, D. A. Hungerford, *Exptl. Cell. Res.* **20**, 613 (1960).
6. W. N. Valentine and W. S. Beck, *J. Lab. Clin. Med.* **38**, 38 (1951).
7. A. C. Peacock, G. Brecher, E. Highsmith, *Am. J. Clin. Pathol.* **39**, 80 (1958).
8. J. B. Block, P. P. Carbone, J. J. Oppenheim, E. Frei III, *Ann. Internal Med.*, in press.
9. R. H. Levin, E. Frei III, M. Landy, *Clin. Res.* **11**, 204 (1963).
10. M. J. De Vries, B. G. Grouch, L. M. Van Pritten, D. W. Van Bekkan, *J. Natl. Cancer Inst.* **27**, 67 (1961).
11. G. Mathé, J. Bernard, M. J. De Vries, L. Schwarzenberg, M. J. Larrieu, C. M Lalanne, J. L. Dutreix, T. Amil, J. Surmont, *Revue d'Hematol.* **15**, 115 (1960).
12. J. O. W. Bielby, I. S. Code, A. M. Jelliffe, D. M. Parben, J. W. Stewart, *Brit. Med. J.* **I**, 96 (1960).
13. J. W. Goodman, G. S. Hodgson, *Blood* **19**, 702 (1962).

18 September 1963

Galactose Metabolism by Rat Liver Tissue: Influence of Age

Abstract. *The metabolism of galactose by liver tissue from fetal, new-born, pre-adult, and adult rats was studied in vitro by determining the extent of conversion of galactose to CO_2 and the disappearance of the sugar from the incubation medium. Results indicate that the utilization of galactose by liver tissue decreases as the organism grows older. The explanation of the tendency of patients with congenital galactosemia to improve symptomatically with age on the basis of "maturation" of galactose metabolizing pathways in their livers does not appear to be consistent with these observations.*

Patients with congenital galactosemia have been shown to lack the enzyme, galactose-1-phosphate uridyl transferase (Gal-transferase) (*1*). The absence of this enzyme, which catalyzes the reaction,

galactose-1-phosphate (Gal-1-P) + uridinediphosphate-glucose (UDPG) → glucose-1-phosphate (G-1-P) + uridinediphosphate-galactose (UDPGal),

results in an inability to metabolize galactose, and this deficiency is associated with a syndrome in infants, characterized by malnutrition, vomiting, diarrhea, enlargement of the liver and spleen, jaundice, cataracts, and mental deficiency. It has been learned from clinical experience that as these patients grow older, especially after puberty, the ingestion of galactose may fail to elicit the symptoms typical of galactose intoxication. An explanation for this apparent amelioration of the clinical disease appeared when Isselbacher (*2*) reported the existence in mammalian tissue of an enzyme, uridine diphosphate galactose pyrophosphorylase (UDPGal-pyrophosphorylase), which could circumvent the enzymatic deficiency believed to be responsible for the galactosemic syndrome by catalyzing the reaction,

Gal-1-P + uridinetriphosphate → pyrophosphate + UDPGal

The activities of both enzymes, Gal-transferase and UDPGal-pyrophosphorylase, were shown to be five times greater (per milligram of tissue) in the livers of adult rats than in the livers of newborn rats (*2*). It seemed highly plausible that the galactosemic patient, though lacking the transferase, was capable of developing the pyrophosphorylase as he matured, thereby acquiring at least a partial ability to metabolize galactose.

Recent quantitative measurements of galactose oxidation in galactosemic subjects, in which galactose-1-C^{14} was used, revealed (*3*) that there exists among galactosemic patients a subgroup whose members develop, at some time during childhood, a substantial ability to metabolize galactose. Such acquisition of pathways for the metabolism of galactose was, however, observed in only a small proportion of the patients tested, and the majority of postpubertal galactosemic patients tested were unable to metabolize galactose, although they too exhibited the clinical amelioration of the syndrome described above. Therefore, it seemed worthwhile to evaluate the functional significance of the increased Gal-transferase and UDPGal-pyrophosphorylase activities observed in adult liver tissue, and to establish whether these heightened enzymatic activities were, in fact, reflected in augmentation of galactose metabolism by the tissue, as postulated (*2*). Experiments were performed to determine the ability of liver tissue from rats of various ages to consume galactose and to convert galactose to CO_2; consumption being taken as an index of the overall utilization of the sugar, and CO_2 production as an index of the extent of operation of the sequence of reactions participating in the catabolism of galactose to CO_2.

Figure 1 shows the striking extent to which galactose consumption by liver from newborn rats exceeded galactose consumption by adult liver tissue. Results from the first three experiments also showed that galactose consumption rises immediately after birth, and thereafter declines, so that liver tissue from 20-day-old rats yields intermediate values, and galactose consumption by adult rat liver is less than 50 percent of that by liver from newborn rats. In the experiments shown in Fig. 1, liver tissue was obtained from individual fetuses and newborns; other experiments, in which pooled liver tissue from entire litters was used, yielded results similar to those shown graphically.

Figure 2 shows that the rate of uptake by the younger tissue greatly exceeds that of the older tissue over an extended interval. (The linearity of uptake by the newborn tissue of one strain appears to fall off after 90 minutes, but this apparent effect is believed to be artifactual, and is attributed to the fact that the rapid rate of utilization by this tissue caused the concentration of substrate to fall below the level necessary to maintain saturation of the system and linear kinetics. The saturating con-