Sickle Cell Trait as a Tag for Bone Marrow Transplantation

By Daniel G. Miller

This communication describes the use of the sickle cell as a tag for determining the fate of transplanted homologous bone marrow. Ten transplantations have been carried out in nine patients using bone marrow obtained from donors with sickle cell trait.

Material and Methods

The bone marrow donors were professional blood donors with normal hematological findings and negative physical examinations whose red cells showed more than 80 per cent sickling when appropriately examined. The method of obtaining and processing the bone marrow was essentially that described by Thomas, Lochte and Ferreebe. The recipients were patients with neoplastic disease and, in most cases, pancytopenia. Sickle cell studies of the blood of the intended recipients were negative.

Sickle cell determinations were performed by the use of sodium metabisulfite, 2 per cent solution in distilled water, freshly prepared. One drop of blood and one drop of sodium metabisulfite were placed on a glass slide and mixed with an applicator stick. A cover slip was applied and sealed and the preparation was kept at room temperature for one hour. The number of sickle cells per thousand erythrocytes was then enumerated by oil immersion microscopic examination. Various methods of fixation of sickle cells with formalin were attempted. Although it was possible to fix these cells there was some loss of sickling in all preparations following the addition of fixative. Figures la, lb and lc show baseline sickle cell preparations of the blood of a donor and prospective recipient, and a similar preparation of the recipient's blood two months post-transplantation.

Results

The results of transplantation of bone marrow from donors with sickle cell trait to four recipients are given below.

Figure 2 shows red cell survival studies as determined by sickle cell and nonagglutinable cell counts. The patient was a 44 year old white male with lymphosarcoma, extensive bone marrow infiltration with lymphocytes, and pancytopenia who was receiving 15 mg. of prednisone daily. The patient had received 5 mg./Kg. (400 mg.) of cyclophosphamide six days prior to the bone marrow transplantation. On 6/9/60 the patient received 450 cc. of homologous bone marrow aspirate. The total nucleated cell count was 15.2 x 10^9. After subtracting the admixture of peripheral leukocytes, the adjusted marrow yield was 12.1 x 10^9 nucleated cells. The donor was sickle cell positive and M negative; the recipient was negative for sickle cells and positive for blood group M. The nonagglutinable cell counts were performed by the Ashby technic as modified by Dacie and Mollison.

From the Lymphoma Section, Division of Clinical Chemotherapy, Sloan-Kettering Institute for Cancer Research, and the Departments of Medicine, Memorial and James Ewing Hospitals and the Cornell University Medical College, New York, N. Y.

Submitted Jan. 12, 1961; accepted for publication Feb. 6, 1961.
This patient had the diagnosis of chronic lymphocytic leukemia made in February 1956. He had been treated with triethylene melamine, prednisone and chlorambucil, as well as radiation therapy to the spleen and node-bearing areas. At the time of bone marrow transplantation in March 1959, the patient had generalized lymphadenopathy, marked hepatosplenomegaly and a hypercellular bone marrow with lymphocytes predominating. He was on no anti-leukemic therapy at the time of the marrow transplantation. This patient had previously accepted a graft of homologous skin, and it was of interest to determine if he would accept a graft of homologous bone marrow from another donor. On 3/16/59 he received from a compatible donor with sickle cell trait a bone marrow transplantation containing 850 x 10⁶ nucleated cells (474 x 10⁶ when adjusted for peripheral leukocyte count). There was an increasing number of sickle cells in the recipient’s peripheral blood for three weeks, followed by a gradual decline. The replicability of the sickle cell count was evaluated in this patient as illustrated in figure 3.

J. S., 28 year old white male, Hodgkin’s disease (fig. 4)

The diagnosis of Hodgkin’s disease was made by lymph node biopsy in 1949. The patient was treated with radiation therapy to peripheral nodes and
nitrogen mustard. In 1954, spinal cord compression occurred; this was treated with radiation therapy to the spine and nitrogen mustard with satisfactory improvement. In April 1959, a low hemoglobin level was found and two months later pancytopenia was present. A bone marrow aspiration was acellular; repeated transfusions were required. The patient was treated with prednisone, 30 mg a day, and this drug in varying dosage was continued throughout the ensuing course.

On February 18, 1960, the patient was given 320 cc. of bone marrow intravenously from a compatible donor with sickle cell trait. The total nucleated cell count was $7.1 \times 10^6$. Subtracting the admixture of peripheral leukocytes the adjusting marrow yield was $5.4 \times 10^6$ nucleated cells. The number of erythrocytes that one may have expected to find in the recipient as a result of transfusion alone was calculated, on the basis of volume and hematocrit, to be 37 per thousand. The 24 hour post-transfusion sickle cell count in the recipient was 40 per thousand. This figure increased to 252 per thousand erythrocytes in three weeks and then fell continually over the next two months. The patient did not require any transfusions for one month and there was a transient rise in the leukocyte and platelet counts.

Fig. 1b. — Sickle cell preparation of peripheral blood of patient R. J. prior to bone marrow transplantation.
SICKLE CELL TRAIT AS MARROW TRANSPLANT TAG

Fig. 1c. — Sickle cell preparation of peripheral blood of patient R. J. 2 months after bone marrow transplantation from donor with sickle cell trait.

R. J., 45 year old white male, lymphosarcoma (fig. 5)

The diagnosis of lymphosarcoma was made by lymph node biopsy in 1954. Peripheral lymphadenopathy was treated with radiation therapy. In 1958, radiation therapy was applied to an abdominal mass and in 1959 to supraventricular and inguinal nodes. In February 1960, pancytopenia was noted. Bone marrow aspiration was hypocellular with a preponderance of lymphocytes. Repeated transfusions were necessary.

On March 10, 1960, the patient received 600 cc. of homologous bone marrow from a compatible donor with sickle cell trait. The total nucleated cell count was 9.9 x 10^6. Subtracting the admixture of peripheral leukocytes, the adjusted marrow yield was 7.1 x 10^6 nucleated cells. Five minutes after the intravenous marrow transfusion, the patient had severe generalized urticaria. This subsided after 50 mg. of Benadryl intramuscularly and did not recur. By July 1960, there were no sickle cells circulating in the recipient. Frequent blood transfusions were necessary and interfered with the accuracy of the sickle cell determinations. There was no significant improvement in the peripheral counts as a result of this bone marrow transplantation.

None of the remaining five patients survived longer than two weeks fol-
Fig. 2.—Comparative red cell survival studies using differential red cell agglutination and sickle cell counts in a patient (J. L.) after bone marrow transplantation.

Fig. 3.—Replicability of the sickle cell phenomenon following bone marrow transplantation in patient H. H. Each point represents the mean and the range of 4 determinations performed by 2 observers.

Following transplantation. Postmortem examinations were performed on two patients; in neither was there any evidence of sickle cell thromboses.

**DISCUSSION**

In other species, the fate of transplanted bone marrow may be followed by a variety of means, e.g., leukocyte alkaline phosphatase (in rat to mouse trans-
Fig. 4.—The hematologic course of patient J. S., a 28 year old male with Hodgkin’s disease, following transplantation of bone marrow.

Sickle cell trait as marrow transplant tag

plantation), differential red cell agglutination, sex chromatin, distinctive chromosomes, and characteristic hemoglobins. In man, differential red cell agglutination and sex chromatin are both used to observe donor cells circulating in the recipient. A high number of nonagglutinable cells may preclude the use of this technic in some situations. A low number of cells positive for sex chromatin may limit the usefulness of this technic. However, within these limitations the above are reliable and useful tags. Sickle cell trait also appears to be a reliable and useful tag for bone marrow transplantation. We have found the sickling phenomenon to be replicable. The comparison of red cell survival using sickle cell counts and nonagglutinable cell counts showed good agreement.
With regard to the possibility that sickle cell marrow in a normal environment would no longer produce sickle cells, Popp et al.7 have shown that erythrocytes of mouse bone marrow chimeras maintain hemoglobin of the donor strain.

The rise in the number of sickle cells post-transplantation could be due to production of new cells by the transplanted marrow or due to sequestration and release of donor red cells. In J. S., the calculated number of erythrocytes transfused and the number found in 24 hours agreed closely and this value was subsequently exceeded approximately sixfold.

It would be advisable to perform hemoglobin electrophoresis on each sickle cell bone marrow donor lest the anemic and thereby hypoxic recipients inadvertently receive marrow from a donor with a combination of sickle cell trait and some other hemoglobinopathy or a high percentage of hemoglobin S. Hemoglobin electrophoresis may also be useful to quantitate donor hemoglobin in the recipient rather than, or in addition to, enumerating sickle cells. This was attempted in one patient, H. H., but in this case the quantity of circulating hemoglobin S was too small to detect. If the recipient requires blood transfusions postmarrow transplantation, this blood should be checked for sickling.

*Performed by Dr. James L. German, III, of The Rockefeller Institute, New York, N. Y.
SICKLE CELL TRAIT AS MARROW TRANSPLANT TAG

SUMMARY

The sickle cell trait appears to offer a reliable and replicable tag for bone marrow transplantation. Red cell survival curves using sickle cell and nonagglutinable cell counts show good agreement. The recipients exhibited no untoward effects as a result of receiving marrow from a donor with sickle cell trait.

SUMMARIO IN INTERLINGUA

Cellulas a character falciforme pare poter servir, con alte grados de fidelitate e de reproductibilitate, como "cellulas marcate" in transplantationes de medulla de osso. Curvas de superviventia de erythrocytos basate super le numeratio de cellulas falciforme e de cellulas nonagglutinabile monstra un bon grado de congruentia. Nulle reactiones adverse eseva exhibite per le recipientes de medulla de un donator con le character de cellula falciforme.

ACKNOWLEDGMENT

The author expresses his appreciation to Miss Deli Strummer and Mrs. Mary Tokes for technical assistance. We are indebted to Dr. Amos Cahan and the Knickerbocker Foundation, Inc., New York City, for cooperation in obtaining suitable bone marrow donors. The clinical studies were facilitated by the kindness of Dr. L. F. Craver and Dr. H. D. Diamond.

REFERENCES


Daniel G. Miller, M.D., Assistant Professor of Medicine, Cornell University Medical College; Assistant Attending Physician, Memorial Hospital for Cancer and Allied Diseases; Associate, Sloan-Kettering Institute for Cancer Research, New York, N. Y.
Sickle Cell Trait as a Tag for Bone Marrow Transplantation

DANIEL G. MILLER