Allogeneic Marrow Engraftment Following Whole Body Irradiation in a Patient with Leukemia

By C. DEAN BUCKNER, ROBERT B. EPSTEIN, ROBERT H. RUDOLPH, REGINALD A. CLIFT, RAINER STORB AND E. DONNALL THOMAS

The Demonstration of a Transient Marrow Graft in a leukemic patient in 1956, and the treatment of victims of an irradiation accident in 1958, initiated a number of attempts to utilize allogeneic marrow grafts in patients with marrow failure or with leukemia. Recent reviews of those attempts show a notable lack of success with death due to failure of engraftment or to graft-vs-host disease. Survivors showed a transient engraftment with reversion to host marrow, with the exception of the one long-term engraftment reported by Mathé et al. These previous attempts to transplant marrow in man may have failed because (1) donors were not selected on the basis of histocompatibility testing, (2) patients were terminal at the time of transplantation in most cases, and (3) immunization against transplantation antigens had been induced by multiple prior transfusions.

Extensive studies in outbred dogs have demonstrated the correlation between histocompatibility, graft survival and severity of the graft-vs-host reaction. In addition, studies in dogs and in monkeys have shown that the graft-vs-host reaction may be ameliorated by immunosuppressive therapy. Recent rapid advances in human histocompatibility typing have provided in-

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First submitted December 14, 1969; accepted for publication December 22, 1969.

This investigation was supported by Grant CA-10895 from the National Cancer Institute and by Contract PH-43-67-1435 from the National Institute of Allergy and Infectious Diseases, U. S. Public Health Service, and by Grant T-280 from the American Cancer Society.

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formation of value in the prediction of the success or failure of renal allografts\textsuperscript{12,13} and preliminary evidence indicates that this may also be true for bone marrow transplantation\textsuperscript{14,15}.

The present report describes an allogeneic bone marrow transplant in a patient with leukemia who was in good general condition and who had not been isoimmunized by earlier transfusions. The patient's sister served as the marrow donor and histocompatibility typing of the family was carried out. Although the patient eventually died of disseminated cytomegalovirus infection, the case is of interest in that it illustrates prompt marrow engraftment and control by methotrexate of acute secondary disease despite a "one chromosome" difference between the patient and the marrow donor.

**Case Report**

A 46-year-old man was discovered to have chronic granulocytic leukemia in September of 1968. Treatment with busulfan resulted in clinical improvement and a decrease in the peripheral white cell count. He was asymptomatic until January of 1969 when he developed fever, splenomegaly and an elevated WBC. Irradiation to the spleen (50 rads) was given on January 23 and 24 with a subsequent decrease in spleen size and a lowering of the peripheral WBC to 800/mm\(^3\). He developed nausea, vomiting and a low-grade fever. Signs of liver disease appeared with bilirubin elevation to 3.2 mg. per cent and SGOT of 85 (normal, 10-40 units) on January 24. Symptoms subsided, and on February 4 the bilirubin was 0.8 mg. per cent and the SGOT 37.

On February 24, 1969 he was admitted to the hospital with a temperature of 39\(^\circ\)C, malaise and splenomegaly. The WBC was 20,000/mm\(^3\), the differential showed 92 per cent myeloblasts, the hematocrit was 27.5 per cent and the platelet count was 14,000. A bone marrow aspiration showed hypercellularity with replacement of normal marrow elements by myeloblasts. The bilirubin was 0.55 mg. per cent and the SGOT was 107 units. Vincristine, 3 mg., was given intravenously on February 25 and March 5. Prednisone, 80 mg/day orally, was begun on February 26 and continued until March 10. Two units of fresh whole blood were given on March 6. On March 8 the peripheral WBC was 214/mm\(^3\), the hematocrit was 30 per cent and the platelet count was 7000. Bone marrow aspirations showed a decrease in cellularity, but almost all cells were myeloblasts. Following the initiation of chemotherapy there was a lysis of fever and a decrease in spleen size from 6 cm. below the left costal margin to 2 cm. There was no clinical evidence of infection nor bleeding. Because of the poor prognosis and the availability of a sibling marrow donor of reasonable histocompatibility, it was elected to treat the patient with whole-body irradiation and allogeneic marrow infusion.

**Histocompatibility Typing**

Red cell typing showed that the patient and his sister were both A, Rh negative. Histocompatibility typing was carried out in this laboratory with the microtest described by Amos et al.\textsuperscript{16} using sera provided by Doctor Bernard Amos and by the NIH tissue typing serum bank. Typing was also carried out in the laboratory of Doctor Paul Terasaki (Table 1). The patient, the donor and the parents were typed initially. The other siblings, who lived in another state, could only be typed later. Donor and recipient were compatible for 11 HL-A groups but incompatible for Group B-10. Analysis of the family data indicated that the donor and recipient inherited the same maternal chromosome but a different paternal chromosome.

The reactivity of the marrow donor's peripheral blood lymphocytes was assessed in a one-way mixed leukocyte culture in which the stimulating cells were inactivated by 2500 R. from a \(^{60}\)Co source.\textsuperscript{17} The uptake of tritiated thymidine by donor lymphocytes in response to stimulating cells, expressed as counts per minute per culture, was as follows: marrow donor cells (control), 882; patient's cells, 4969; unrelated human cells, 3943. This
Table 1.—Results of Cytotoxicity Typing Tests

<table>
<thead>
<tr>
<th>Leukocyte Groups</th>
<th>Sex</th>
<th>Red Cell Type</th>
<th>HLA 1</th>
<th>HLA 2</th>
<th>HLA 3</th>
<th>B 4</th>
<th>HLA 5</th>
<th>HLA 6</th>
<th>HLA 7</th>
<th>B 9</th>
<th>B 11</th>
<th>B 8</th>
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<tr>
<td>Recipient</td>
<td>M A</td>
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<td>-</td>
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<td>-</td>
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<tr>
<td>Donor</td>
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<td>+</td>
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<td>+</td>
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<td>-</td>
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<tr>
<td>Father</td>
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<td>+</td>
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<td>+</td>
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</tr>
<tr>
<td>Mother</td>
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<td>-</td>
<td>+</td>
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reactivity of the donor lymphocytes confirmed a histocompatibility difference. Reactivity of the host lymphocytes could not be tested because of his lymphopenia.

Irradiation

Total body irradiation was administered on March 10, 1969 using opposing 60Co sources. The patient lay on an aluminum stretcher transversely between the two sources which were 400 cm. apart. At the midpoint of the irradiation field, the dose rate in air was 5.8 R./minute and the total dose 1620 R. The tissue/air ratio was calculated to be 0.62 on the basis of an 1100-cm.2 field and a 16-cm. tissue depth. A factor of 0.95 was used to convert from R. to rads. Thus, the calculated midline tissue dose was 954 rads. The exposure rate in air was determined by a Victoreen R. meter model 570 with its associated model 553 high-energy 25-R. chamber bearing a recent certification by the Bureau of Standards and checked for constancy against a Victoreen model 540B radium standard just prior to use. The readings were corrected for deviations of atmospheric conditions from those at calibration and for shutter time. Lithium fluoride radioluminescence dosimeters were taped to the patient's skin at various locations. The dosimeters contained dosimetry grade lithium fluoride powder having grain size between 80 and 200 mesh contained in polyethylene having sufficient wall thickness to produce electron equilibrium and sufficient capacity to contain four aliquots of the powder for readout. The largest standard deviation on these readings was three per cent. The results were as follows: forehead 1156 rads, umbilicus 1380 rads, left midiliac crest 1410 rads, right midiliac crest 1515 rads, inner aspect of left thigh 1114 rads, left foot 885 rads.

Two hours before irradiation, the patient was given 100 mg. of pentobarbital and 100 mg. of chlorpromazine. The irradiation lasted approximately five hours and was interrupted for brief intervals on six occasions because of nausea and vomiting. These symptoms ceased at the end of the irradiation. Following completion of the irradiation, the patient was transferred to a regular hospital room and placed on reverse isolation (personnel wore mask, gown and gloves). No attempt was made to sterilize the gastrointestinal tract, and he received the regular hospital diet.

Marrow Infusion

Approximately one-half hour before the end of irradiation, the marrow donor was taken to the operating room and marrow aspirations were carried out under general anesthesia. Marrow was aspirated from approximately 100 sites on the sternum and the anterior and posterior iliac crests. As each aspiration was performed, the marrow was transferred immediately to a beaker containing aliquots of 100 ml. of TC-199 with 3500 units of heparin (Connaught Laboratories, Toronto, Canada). The marrow suspension was passed through stainless steel screens of 300 and 200 microns square. The volume aspirated was 236 ml. The number of nucleated cells obtained was \(1.90 \times 10^8\), of which \(1.4 \times 10^6\) were calculated to be peripheral blood cells, leaving \(1.76 \times 10^8\) as the total number of bone marrow cells. Of this total, \(3.2 \times 10^6\) came from the sternum, \(3.9 \times 10^6\) came from the anterior iliac crests and \(10.5 \times 10^6\) from the posterior iliac crests.

The total volume of 360 ml. was given to the recipient intravenously over a 75-minute period.
period beginning one hour after irradiation. There was no immediate reaction to the infused marrow and respiratory problems did not occur. However, one hour following the infusion the patient developed shaking chills and a temperature of 39°C. Blood cultures taken at this time grew out a few colonies of alpha-hemolytic streptococcus.

**Allogeneic Marrow Engraftment**

From the fifth to the 12th postirradiation day, the patient's WBC was less than 20 cells/mm³. On the 13th day the WBC began to rise, going above 200/mm³ on day 18 and above 900/mm³ on day 20. On that day the differential showed 37 per cent polymorphonuclears, 49 per cent band forms, six per cent metamyelocytes, two per cent myelocytes, three per cent lymphocytes and three per cent monocytes. The recovery of peripheral white blood cells, platelets and reticulocytes is shown in Fig. 1. Table 2 shows the results of bone marrow cytogenetic studies documenting the repopulation by female donor cells.

**Table 2.—Results of Cytogenetic Analyses of Direct Marrow Preparations**

<table>
<thead>
<tr>
<th>Day After Marrow Graft</th>
<th>&lt; 45</th>
<th>46</th>
<th>92</th>
<th>&gt; 92</th>
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<td>10</td>
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</table>
Fig. 2.—Clinical and biochemical events in patient given 950 rads whole-body irradiation and allogeneic bone marrow.

Immunosuppressive Therapy

Following total body irradiation and marrow infusion, the patient was given hydrocortisone 60 mg./day until day 21, and then prednisone 10 mg./day. Methotrexate was administered in a fashion similar to that used in laboratory animals for prevention of graft rejection and amelioration of graft-vs-host disease.10,18 Twenty-five mg. was given on days one, three, six, 12, 18 and 23, 10 mg. was given on day 32 and 25 mg. on days 36, 44 and 54.

Clinical Events

The clinical course of this patient may be divided into three rather definite periods of time. During the first period (irradiation to day 14), the patient was profoundly leukopenic. Anemia and thrombocytopenia were corrected by fresh whole blood and platelet trans-
fusions that were given 1400 R. prior to infusion. During this period the patient was moderately febrile (Fig. 2), but no additional chills were observed. Blood cultures taken on days two and three were negative, but those taken on days four through seven were positive for alpha-hemolytic streptococcus. Penicillin was begun on day seven and continued for six days. During the first four days he was given nothing by mouth except occasional chips of ice. Fluids and electrolytes were given intravenously. Serum uric acid levels were 3.9–5.0 mg. per cent. During that time he did not have nausea or vomiting. On the fifth postirradiation day the patient ate breakfast without difficulty. He then had the gradual development of pharyngitis, gingivitis and mucositis so that he was unable to swallow by the end of the second week. No specific infective organisms were cultured from the oropharynx. During this period the patient was maintained on parenteral fluids.

During the second time period (days 15–41) granulocytes, platelets and reticulocytes appeared in the peripheral blood in increasing numbers (Fig. 1). Concurrently, the pharyngitis and the gingivitis improved. A maculopapular, moderately pruritic skin rash appeared on day 13 and reached its maximum intensity on days 15–18 (Fig. 2). This rash originated on the trunk and spread to involve the extremities, the head, and especially the ears. As the rash faded there was desquamation. The rash was quiescent from day 22 to day 30 when it recurred, but again gradually subsided (Fig. 2) leaving a residual pigmentation. The volume and number of stools is also shown in Fig. 2. A moderate diarrhea at the end of the second week slowly subsided and bowel movements were nearly normal during days 22–34. During this period there were minimal abnormalities in liver function tests (Fig. 2).

In the third and final period (days 42–56) the patient developed a low-grade fever that increased progressively (Fig. 2). Repeated blood cultures were negative. Platelet counts declined, although megakaryocytes were observed in the marrow. Diarrhea became severe and presented a major problem in clinical management (Fig. 2). Stool volumes increased to two liters/day. The diarrhea was bloody during the last few days. Hypokalemia required vigorous replacement therapy. The patient lost weight progressively (Fig. 2). A superior vena cava catheter was implanted four days prior to death that permitted an increase in caloric intake and potassium supplementation. Culture of the sputum showed the predominant organism to be Neisseria catarrhalis. Penicillin therapy was initiated, but the patient became increasingly dyspneic and died on the 56th day after marrow transplantation.

**Autopsy**

The most prominent features on gross autopsy were pulmonary edema and ulcerative lesions in the colon. Histological examination showed cells with nuclear and cytoplasmic inclusions bodies in all organs except the kidney. Inclusion bodies were particularly prominent in the intestine, the lymphatic tissues, the adrenals and the lungs. The bone marrow was moderately cellular without evidence of leukemia. The spleen and the lymph nodes showed marked lymphoid atrophy. The skin showed follicular hyperkeratosis but no dyskeratosis. The liver was not particularly abnormal showing only some increase in fat and occasional small focal zones of neutrophilic infiltration. Attempts to isolate virus from frozen lung tissue were unsuccessful. It was concluded that the cause of death was disseminated viral disease, probably cytomegalovirus.

**Discussion**

This report describes an allogeneic bone marrow transplantation in a patient with blastic crisis of chronic myelogenous leukemia. The patient was considered to be suitable for attempted marrow transplantation for several reasons. He was very unlikely to live for more than a few weeks with blastic crisis of chronic myelogenous leukemia. The possibility of immunization to transplantation antigens by prior transfusions was minimal, since his transfusion history
was limited to two units of blood given four days before irradiation and during a time of intensive chemotherapy. The patient was not infected nor had he been treated for infection in the recent past. The patient's sister, who volunteered to be a marrow donor was ABO compatible and identical to the patient in 11 of 12 HL-A groups.

The patient was given whole-body irradiation of 950 rads calculated as a midline tissue dose. Major problems with radiation sickness and the acute gastrointestinal syndrome were not observed. This dose of radiation was selected because work in dogs has shown that lower doses may not permit successful engraftment. It is somewhat larger than the irradiation dose customarily administered for allogeneic engraftment in monkeys and in man. Higher doses have been administered to human patients when an identical twin was available to serve as marrow donor. No major problems with bacterial infection were encountered despite the less-than-optimal facilities for isolation and the decision not to use sterile food nor prophylactic antibiotics of any kind.

Marrow engraftment occurred promptly and a subsequent rejection of the graft did not occur. Cytogenetic analysis of the bone marrow from the 14th postirradiation day onward showed only female donor cells. It should be noted that the marrow transplant was carried out immediately after irradiation. In this laboratory, marrow engraftment immediately after irradiation has been consistently successful in the dog, although others have suggested that there may be an advantage in waiting one or more days after irradiation before marrow engraftment. Methotrexate was given to the patient on days one, three, 6, 11, and subsequently at approximately weekly intervals in an effort to approximate the immunosuppressive regimen that has been shown to be optimal for amelioration of secondary disease in the dog. It is evident that the early administration of methotrexate did not interfere with successful engraftment although it probably did contribute to the slow rise in the white blood cell count and the periodic drops in platelet count observed shortly after methotrexate administration.

Secondary disease appeared in this patient as was to be expected from studies in dog, monkey, and man. The typical clinical features of skin rash and diarrhea were evident by the end of the second week. The low-grade disturbance of liver function was also presumed to be a part of the graft-vs-host syndrome, although the patient had had liver disease of unknown etiology in the month before transplantation. Methotrexate appeared to control the secondary disease in this patient with subsidence of skin rash and diarrhea by the end of the third week. Methotrexate was given on day 23 and then withheld for nine days. During that time the skin rash flared up again but subsided after methotrexate was resumed. The complex events in the last two weeks of the patient’s life were thought at the time to be associated with active graft-vs-host disease. However, autopsy disclosed a disseminated viral infection without evidence of acute secondary disease. Susceptibility to viral infections during the period of immunological incompetence following allogeneic marrow engraftment is well recognized. However, dogs that are isolated during the
first three months after engraftment slowly regain their ability to make anti-distemper antibodies, and they regain good health. Since the source of this patient's viral infection is unknown, it is not possible to predict whether ultraisolation techniques, such as the life island or laminar flow room, would have prevented the infection. Cytomegalovirus may have been introduced during the blood and platelet transfusions.

HL-A identical siblings, where the siblings have inherited the same HL-A chromosome from each parent, represent optimal pairings for marrow engraftment in the absence of malignant disease as has been observed in the treatment of immunologic deficiency. Since it is known that leukemia will recur after supralethal whole body irradiation and isogeneic human marrow engraftment, it may well be that a similar recurrence of leukemia will be observed with HL-A identical sibling marrow engraftment. In this event, a one-chromosome difference, as was the case in the patient reported here, may be necessary for eradication of the disease. A one chromosome difference, however, will result in secondary disease that may or may not be controllable. Recent studies in dogs show that in some animals immunosuppressive therapy in the first 100 days can abolish secondary disease despite known histoincompatibility. Additional studies of marrow grafts from HL-A identical siblings, as well as marrow grafts from siblings with a one-chromosome difference are necessary before these questions can be answered.

SUMMARY

A 46-year-old man with blastic crisis of chronic myelogenous leukemia was given 950 rads whole-body irradiation followed immediately by $17.6 \times 10^6$ marrow cells. The marrow donor was the patient's sister who matched the patient in 11 of 12 HL-A groups and whose leukocytes reacted to the patient's leukemic cells in mixed leukocyte culture. The patient's white blood cell count began to go up on the 13th day and marrow engraftment was confirmed by repeated cytogenetic analyses. Secondary disease with skin rash and diarrhea was evident by the end of the second week but appeared to be controlled by methotrexate. The patient then developed fever, recurrent diarrhea and pneumonitis and died 56 days after irradiation. Autopsy showed inclusion bodies typical of cytomegalovirus. There was no histological evidence of acute secondary disease nor of leukemia.

ACKNOWLEDGMENTS

We are grateful to Dr. Burton S. Eggertsen, Dr. Bruce Beckwith and Dr. M. J. de Vries for review of the autopsy slides, and to Mr. Peter Wootton who performed the radiation dosimetry. We are indebted to Drs. Russell E. Larson and Alexander R. Stevens, Jr., for referral of this patient.

REFERENCES

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