Allogeneic Marrow Grafting for Hematologic Malignancy Using HL-A Matched Donor-Recipient Sibling Pairs


Seven patients with hematologic malignancy refractory to conventional therapy were treated with 1000 rads midpoint tissue dose of whole-body irradiation followed by infusion of marrow from an HL-A matched sibling. Three patients with advanced leukemia showed histologic evidence suggestive of engraftment but the graft did not function and they died after 18, 26, and 30 days. Four patients, three with acute lymphoblastic leukemia and one with Hodgkin's disease, treated while in good clinical condition, showed evidence of a functioning marrow graft within 3 wk. Engraftment was proved by cytogenetic analysis in three cases with donors of the opposite sex. One patient died with graft-vs.-host disease (GVH) after 37 days. The other three had mild to moderate GVH. Two patients showed recurrent leukemia and died after 85 and 102 days. In one of these patients, a girl, the recurrent leukemia was in male donor cells. One patient, a boy, is alive and well after 200 days with only female donor cells in the marrow. He shows no evidence of GVH and, as yet, no leukemia.

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EARLY EFFORTS TO TRANSPLANT MARROW in man and in outbred animals encountered several major problems that impeded the clinical application of this technique. A primary obstacle was histoincompatibility between donor and recipient, expressed either as graft rejection or serious graft-vs.-host disease (GVH). Prolonged periods of marrow dysfunction preceding and accompanying marrow grafting also posed a serious clinical problem. The advancement of knowledge in several areas suggests that some of these problems may be overcome and has caused a resurgence of interest in clinical marrow grafting.

Studies of human histocompatibility have shown the major antigens to be controlled by genes at one chromosomal region, designated HL-A, which has two closely associated segregant series or subloci. Genetic analysis of HL-A typing data in a family permits recognition of siblings who have inherited identical HL-A chromosomes from their parents. Such apparently matched sibling pairs can be confirmed by nonreactivity in the one-way mixed leukocyte test (MLC). Histocompatibility typing procedures developed in the dog serve as a model in an outbred species for studies similar to those in man. Matched canine siblings demonstrate prompt engraftment and prolonged survival even without immunosuppressive treatment after grafting. Preliminary studies in man also suggest that HL-A matched donor-recipient pairs are advantageous.

A second area of advancing knowledge concerns the treatment of GVH, described as the major stumbling block in marrow grafting. In the dog, methotrexate (MTX) has proved useful in controlling GVH even when there is demonstrable histoincompatibility. Cyclophosphamide (CY) has not been effective. In the monkey, MTX, CY, and antilymphocyte serum (ALS) have been shown to ameliorate GVH, but without long-term control. Preliminary evidence in man suggests that all three agents may be useful.

Marrow grafting implies a period of marrow aplasia due to the primary disease and to the conditioning treatment of the intended recipient. Advances in supportive measures for the patient without marrow function include ultrasolation techniques, antibiotics effective against Gram-negative organisms, and improved platelet and granulocyte transfusion techniques.

This report describes the course of events in seven patients with hematologic malignant disease refractory to conventional treatment. Each patient was treated with 1000 rads midpoint tissue exposure of whole-body irradiation followed by marrow infusion from a sibling. In each case, HL-A typing of the patient and 4 to 12 family members indicated that the donor and the recipient had inherited the same HL-A chromosomes. Six pairs were nonstimulatory in MLC and one showed minimal stimulation. Following the marrow transplant, MTX was administered to prevent graft rejection and to ameliorate GVH.

MATERIALS AND METHODS

Blood Genetic Markers

Typing of lymphocytes with cytotoxic isoantisera was carried out using the method of Amos et al. or the method of Mittal et al. The antisera used in these studies were obtained from the NIH Serum Bank (Bethesda, Md.), from Dr. Bernard Amos (Duke University, Durham, N.C.), or from Dr. Paul Terasaki (U.C.L.A., Los Angeles, Calif.). Cells
were also sent to Dr. Terasaki for confirmation of typing results when a marrow graft was to be carried out. MLC was performed using the technique described in detail elsewhere. Typing for red blood cell antigens and for enzyme electrophoretic phenotypes was performed by previously described methods.

**Irradiation**

Whole-body irradiation was carried out with the patient on an aluminum stretcher placed transversely between two opposing 60Co sources, which were 400 cm apart for the first three patients and 600 cm apart for the last four patients. The patient was positioned so that the umbilicus lay in the midline of the radiation field. The exposure rate in air ranged from 5.0 to 5.7 R/min at the midpoint and was determined by a Victoreen R meter model 570 with its associated model 553 high-energy 2500-R chamber bearing a recent certification by the Bureau of Standards and checked for constancy against a Victoreen model 540B radium standard just before use. The readings were corrected for deviations of atmospheric conditions from those at calibration and for shutter time. The tissue/air ratio was calculated on the basis of a 1000 sq cm field and a midpoint tissue depth based on the patient’s measurements at the umbilicus. 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 3%. The average results for the seven patients were as follows: forehead, 1147 rads; umbilicus, 1018 rads; upper-inner thigh, 903 rads; foot, 912 rads.

Preparations of platelets, buffy coat cells and fresh whole blood administered after marrow grafting were given 1500 rad in vitro to inactivate immunologically competent cells that might contribute to GVH. The bag containing the cells was exposed to a single 60Co source at a distance of 29 cm and a dose rate of 280 R/min.

**Marrow Infusion**

Marrow was obtained from the donor by multiple aspirations under general anesthesia as described in detail elsewhere. Immediately after collection, the marrow was administered i.v. to the recipient. In no instance was there major untoward reaction to the infused marrow.

For purposes of time comparisons, we have designated the day of marrow infusion as day 0, and subsequent days are numbered from that point.

**Immunosuppressive Therapy**

Based on the most effective canine regimen, MTX was administered, 10 mg/sq m on days 1, 3, 6, and 11, and weekly thereafter. This schedule was varied somewhat depending upon clinical and hematologic events as described for each individual case.

**Isolation Procedures**

The first and fourth patients were managed with conventional hospital reverse isolation. All other patients were kept in a laminar air flow isolation room with complete bacteriological monitoring. In addition, the last three patients received skin sterilization by frequent pHisoHex scrubs, antibiotic sprays of ears and nose, mouth rinses with vancomycin, gentamicin, nystatin, gut sterilization with gentamicin and vancomycin, and a sterile diet. The regimen was essentially that described by Bodey et al.

**Informed Consent**

The protocol used here has been the subject of initial approval and periodic review by the Clinical Investigation Committees of the University of Washington School of Medicine and the USPHS Hospital. The hazards and uncertainties of these procedures along with alternative approaches are discussed in detail with the patient, responsible family members,
Table 1.—Summary of Recipient Data, Marrow Graft Function, GVH, and Outcome

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Diagnosis</th>
<th>State of Disease at Time of Grafting</th>
<th>Evidence of “Take”</th>
<th>Function of Graft (White Blood Cell Count &gt;1000/cu mm)</th>
<th>Evidence of GVH</th>
<th>Recurrence of Malignant Disease</th>
<th>Survival (days)</th>
<th>Cause of Death</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AML</td>
<td>Advanced</td>
<td>?</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>26</td>
<td>Uremia</td>
<td>Renal failure due to urate uropathy</td>
</tr>
<tr>
<td>2</td>
<td>AML</td>
<td>Advanced</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>30</td>
<td>Septicemia</td>
<td>Septicemia</td>
</tr>
<tr>
<td>3</td>
<td>CML Blast crisis</td>
<td>Advanced</td>
<td>?</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>18</td>
<td>Septicemia</td>
<td>Probable splenic abscess before irradiation</td>
</tr>
<tr>
<td>4</td>
<td>ALL</td>
<td>CNS relapse</td>
<td>Yes</td>
<td>Yes</td>
<td>++</td>
<td>Yes</td>
<td>102</td>
<td>Leukemia</td>
<td>Patient with recurrence of leukemia in donor cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Marrow remission</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Septicemia</td>
<td>Recurrent leukemia, no marker to distinguish donor from host cells</td>
</tr>
<tr>
<td>5</td>
<td>ALL</td>
<td>Early relapse</td>
<td>Yes</td>
<td>Yes</td>
<td>+</td>
<td>Yes</td>
<td>85</td>
<td>Leukemia</td>
<td>Patient in reasonably good health and attending school 200 days after grafting.</td>
</tr>
<tr>
<td>6</td>
<td>ALL</td>
<td>Early relapse</td>
<td>Yes</td>
<td>Yes</td>
<td>±</td>
<td>No</td>
<td>&gt;200</td>
<td>Septicemia</td>
<td>Only donor cells in marrow</td>
</tr>
<tr>
<td>7</td>
<td>Hodgkin's</td>
<td>Stage IV</td>
<td>Yes</td>
<td>Yes</td>
<td>+++</td>
<td>No</td>
<td>37</td>
<td>GVH</td>
<td>Fatal GVH despite HL-A matching confirmed by family study and by MLC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Early relapse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AML, acute myelocytic leukemia; CML, chronic myelocytic leukemia; ALL, acute lymphocytic leukemia.
and the marrow donor. Although marrow donation by minors is of particular concern, no detrimental effect on the donor has yet been observed. Removal of a small fraction of a replicating cell population is unlikely to have any lasting effect. The practice of autotransfusion eliminates the risk of hepatitis. Anesthetic accident remains a legitimate concern.

**RESULTS**

**General Summary**

Table 1 summarizes the important features of these seven cases. Table 2 summarizes the marrow transplantation data. Because of the importance of the transfusion history in marrow grafting, Table 3 gives these data in detail. Table 4 summarizes the results of histocompatibility typing and of the one-way MLC.

**Irradiation Effects**

Table 5 summarizes the immediate effects of irradiation in these seven patients. One hour before irradiation all patients were given pentobarbital, 100 mg, i.v., and chlorpromazine, 25 mg i.v., with appropriate reduction in dose for children. All patients developed nausea toward the end of the irradiation period. In the first 48 hr, vomiting and diarrhea ranged from none to moderately severe. After the second day vomiting was rare and oral intake of liquids and soft food began. One to four daily liquid stools continued through the fifth day when gastrointestinal symptoms ceased. Four previously afebrile patients developed chills and fever at the end of irradiation with return to normal in 8 to 24 hr. The severity of the febrile response seemed to be related to the body mass of tumor cells at the time of irradiation.

**Case Reports**

**Case 1**

An 18-yr-old male with acute myeloblastic leukemia was treated with vincristine, MTX, 6-mercaptopurine, and prednisone (VAMP) from June to September 1969. He had a partial response followed by recurrence while on treatment. He was admitted to the USPHS Hospital on September 23, 1969, for marrow transplantation. Physical examination showed no enlargement of lymph nodes, spleen, or liver. The white blood cell count was 20,300/cu mm with 68% blasts, hematocrit 38%, and platelet count 96,000/cu mm. His marrow showed more than 90% blast cells. He was given whole body irradiation on September 28, immedi-

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**Table 2.—Summary of Marrow Transplantation Data**

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Recipient</th>
<th>Sibling Donor</th>
<th>Volume of Marrow Cell Suspension (ml)</th>
<th>Total Nucleated Cells Infused x 10^8</th>
<th>Corrected Marrow Cells Infused x 10^6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>78 18 M</td>
<td>28 M</td>
<td>995</td>
<td>28.1</td>
<td>23.5</td>
</tr>
<tr>
<td>2</td>
<td>71 31 M</td>
<td>27 M</td>
<td>896</td>
<td>23.1</td>
<td>18.2</td>
</tr>
<tr>
<td>3</td>
<td>83 48 M</td>
<td>33 F</td>
<td>1,058</td>
<td>34.9</td>
<td>25.9</td>
</tr>
<tr>
<td>4</td>
<td>60 16 F</td>
<td>10 M</td>
<td>471</td>
<td>17.4</td>
<td>14.6</td>
</tr>
<tr>
<td>5</td>
<td>39 9 M</td>
<td>14 M</td>
<td>770</td>
<td>28.1</td>
<td>24.4</td>
</tr>
<tr>
<td>6</td>
<td>36 10 M</td>
<td>8 F</td>
<td>483</td>
<td>15.0</td>
<td>15.2</td>
</tr>
<tr>
<td>7</td>
<td>63 24 M</td>
<td>33 F</td>
<td>738</td>
<td>17.4</td>
<td>13.0</td>
</tr>
</tbody>
</table>
Table 3.—Summary of Transfusions of Platelets, Buffy Coat Cells and Blood

<table>
<thead>
<tr>
<th>Patient</th>
<th>Number of Units</th>
<th>Type</th>
<th>Donor</th>
<th>Number of Units</th>
<th>Type</th>
<th>Donor</th>
<th>Number of Units</th>
<th>Type</th>
<th>Donor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>Packed RBC</td>
<td>Random</td>
<td>0</td>
<td></td>
<td></td>
<td>18</td>
<td>Whole blood</td>
<td>Random</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Platelets</td>
<td>Random</td>
<td>46</td>
<td></td>
<td>Random</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>B.C.</td>
<td>Marrow donor</td>
<td>44</td>
<td></td>
<td>Parent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>B.C.</td>
<td>Other siblings</td>
<td>96</td>
<td></td>
<td>Other siblings</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>Whole blood</td>
<td>Random</td>
<td>6</td>
<td>Whole blood</td>
<td>Random</td>
<td>2</td>
<td>Whole blood</td>
<td>Random</td>
</tr>
<tr>
<td></td>
<td>92</td>
<td>Platelets</td>
<td>Random</td>
<td>26</td>
<td>Platelets</td>
<td>Random</td>
<td>59</td>
<td>B.C.</td>
<td>Marrow donors</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>B.C.</td>
<td>Marrow donors</td>
<td>1</td>
<td>Platelets</td>
<td>Marrow donor</td>
<td>13</td>
<td>Platelets</td>
<td>Marrow donors</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td></td>
<td></td>
<td>13</td>
<td>Platelets</td>
<td>Random</td>
<td>2</td>
<td>Whole blood</td>
<td>Random</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Platelets</td>
<td>Other siblings</td>
<td>26</td>
<td></td>
<td>Other siblings</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>B.C.</td>
<td>Other siblings</td>
<td>86</td>
<td></td>
<td>Other siblings</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td>Platelets</td>
<td>Marrow donor</td>
<td>20</td>
<td>Marrow donor</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Whole blood</td>
<td>Random</td>
<td>0</td>
<td></td>
<td></td>
<td>6</td>
<td>B.C.</td>
<td>Marrow donor</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Platelets</td>
<td>Random</td>
<td></td>
<td></td>
<td></td>
<td>32</td>
<td>Platelets</td>
<td>Father</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>Packed RBC</td>
<td>Father</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>Packed RBC</td>
<td>Random</td>
<td>0</td>
<td></td>
<td></td>
<td>12</td>
<td>Platelets</td>
<td>Mother</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
<td></td>
<td></td>
<td>4</td>
<td>B.C.</td>
<td>Marrow donor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td>Platelets</td>
<td>Parent</td>
<td>1</td>
<td>Parent</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>Whole blood</td>
<td>Random</td>
<td>0</td>
<td></td>
<td></td>
<td>4</td>
<td>Platelets</td>
<td>Wife</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12</td>
<td>Platelets</td>
<td>Wife's mother</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B.C., buffy coat cells plus platelets. Each unit prepared from 500 ml fresh whole blood.

* All fresh blood products infused after grafting were given 1500 R in vitro.
**Table 4.—Summary of Histocompatibility Typing of Patients and Their Marrow Donors**

<table>
<thead>
<tr>
<th>Blood Type</th>
<th>Number of Family Members Studied To Determine HL-A Genotype*</th>
<th>HL-A Genotype†</th>
<th>Mixed Leukocyte Culture‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parents</td>
<td>Siblings</td>
<td>Children</td>
</tr>
<tr>
<td>Patient 1</td>
<td>O</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Donor</td>
<td>O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 2</td>
<td>A</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Donor A</td>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor B</td>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 3</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Donor ‡</td>
<td>O</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 4</td>
<td>O</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Donor</td>
<td>O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 5</td>
<td>A</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Donor</td>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 6</td>
<td>O</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Donor</td>
<td>O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 7</td>
<td>O</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Donor</td>
<td>O</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Numerals indicate HL-A designations or Terasaki designations. x = any unknown antigenic group.
† Means of duplicate cultures expressed as counts per minute of tritiated thymidine per culture.
‡ Two tests performed on different days.

Patient was followed by marrow from his brother. On the day before, the day of and the day after marrow infusion, the patient received i.v. fluids in the amount of 3500–4000 ml daily. On day 1, 10 mg/sq m of MTX was given. Later that day he developed flank pain, and the urine contained red blood cells and urate crystals. Despite 600 mEq of sodium bicarbonate, the urine pH remained at 5.0. On day 2, the renal pelvis was flushed at cystoscopy and hemodialysis was carried out. Despite these measures he became anuric. He was then supported by hemodialysis and by multiple infusions of platelets and Buffy coat cells. On day 4 the temperature rose to 38.9°C and thereafter ranged from 38° to 40°C. Blood cultures were sterile until day 17 when *Escherichia coli* was cultured. This organism remained in the blood until day 22. Blood cultures were sterile until day 26 when he developed a *Pseudomonas aeruginosa* septicemia and died. At no time was there a rise in reticulocyte, platelet, or white blood cell counts.

Autopsy showed a hypoplastic marrow with scattered myelopoietic foci. There was marked lymphoid atrophy of spleen and lymph nodes with focal extramedullary hemopoiesis, visceral...

**Table 5.—Summary of Immediate Irradiation Effects**

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Before Irradiation</th>
<th>Temperature (°C)</th>
<th>Enuresis (times/volume)</th>
<th>Stools (times/volume)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37.2</td>
<td>41.1</td>
<td>7/75</td>
<td>17/450</td>
</tr>
<tr>
<td>2</td>
<td>36.8</td>
<td>39.2</td>
<td>4/250</td>
<td>5/505</td>
</tr>
<tr>
<td>3</td>
<td>39.4</td>
<td>37.8</td>
<td>7/75</td>
<td>17/450</td>
</tr>
<tr>
<td>4</td>
<td>36.8</td>
<td>37.0</td>
<td>4/250</td>
<td>5/505</td>
</tr>
<tr>
<td>5</td>
<td>36.8</td>
<td>38.9</td>
<td>3/70</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>36.5</td>
<td>37.4</td>
<td>1/10</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>37.2</td>
<td>37.4</td>
<td>1/10</td>
<td>0</td>
</tr>
</tbody>
</table>
aspergillosis, and bilateral bronchopneumonia. Gross inspection of the kidneys showed yellow streaking typical of uric acid uropathy. Microscopically, they showed congestion and edema and granular protein casts in the tubules.

**Comment**

The development of urate uropathy in this patient set the stage for an extremely complicated course. Presumably, failure of the marrow graft to function may be ascribed to uremia and to infection. A contributing factor may have been augmented toxicity by the one dose of MTX given just before the onset of renal failure. To avoid the complication of urate uropathy, subsequent patients were given allopurinol until the day before irradiation when i.v. fluids and sodium bicarbonate were started to assure an alkaline urine at the time of irradiation. When necessary, diuretics were administered to maintain a good urine flow.

**Case 2**

A 30-yr-old male was found to have acute myelogenous leukemia in August 1969. After 1 mo on a VAMP program he showed no response and he had developed a severe *Pseudomonas aeruginosa* cellulitis of his left hand. He was admitted to the USPHS Hospital and treated with cytosine arabinoside. For 1 mo he was profoundly leukopenic and thrombocytopenic. He was supported by multiple platelet transfusions from random donors and from his two HL-A matched brothers. By mid-November there was a partial remission of his leukemia, infections had cleared and he was discharged from the hospital.

He entered the hospital on December 15 in relapse. The white blood cell count was 930/cu mm with 20% blasts, the platelet count was 7,500/cu mm, and hematocrit 24%. The marrow was hypoplastic but consisted almost entirely of blast cells.

Whole-body irradiation was administered on December 19, 1969. Shortly thereafter, irradiation marrow from the first brother was administered and, the following day, marrow from the second brother was administered. MTX, 10 mg/sq m, was administered on days 1, 3, 6, and 11 following the second marrow infusion. The patient was afebrile and in good clinical condition for the first 10 days. He then became febrile and was treated with methicillin and gentamicin, which were discontinued when all blood cultures were negative. Throughout the postirradiation period he was supported by irradiated buffy coat cell and platelet transfusions from the two marrow donors. On day 25 marrow aspirated from the iliac crest showed moderate cellularity with normal myeloid tissue including mature granulocytes and an occasional megakaryocyte. On that day the white blood cell count was 88/cu mm, the platelet count 33,000/cu mm, and the reticulocyte count 0.3%. An alpha-streptococcus and a Klebsiella were grown from the blood on days 26 and 27. In spite of treatment with gentamicin, penicillin, prednisone, and general supportive care, he died on day 30. There was no clinical evidence of GVH.

At autopsy the marrow showed about 50% of normal cellularity with many plasma cells. There were some mature granulocytes and many erythroid precursors. Megakaryocytes were present but reduced in number. There was moderate lymphoid atrophy of spleen and lymph nodes. There was septic necrosis of the esophagus, pulmonary edema, and bleeding in the lung and gastrointestinal tract. The skin showed mild vacuolation in the basal cells of the epidermis.

**Comment**

This patient received a large number of marrow cells from two HL-A matched siblings. He was then in good clinical condition for a period of time long enough to permit beginning function of the grafted marrow. Although the
marrow became moderately cellular, function of the graft was not evident in the peripheral blood, and he died of hemorrhage and infection.

**Case 3**

A 48-yr-old man with chronic myelogenous leukemia had been treated with busulfan since March 1962. In March 1970 he developed fever, splenomegaly, and a white blood cell count of 44,000/cu mm with 14% blast cells. His white blood cell count then rose progressively and did not respond to treatment with hydroxyurea, prednisone, vincristine, and allopurinol. He continued to have fever and left upper quadrant pain, and the platelet count remained below 20,000/cu mm despite transfusions of platelets from random donors.

Histocompatibility typing of the patient and his family suggested, but did not prove, that a sister was compatible at the HL-A locus. The patient’s parents were dead. Typing of the nine siblings indicated one parent to have had HL-A haplotypes 3, 7 and 9, Te54 and the other parent to have had 9, Te50 and 10, 12. The marrow donor inherited haplotypes 3, 7 and 10, 12. At the time of study, the patient’s cells were all leukemic blasts. HL-A 3 and 10 were present but antigens determined by the second sublocus (7 and 12) could not be demonstrated. However, the patient’s wife did not have HL-A7, but one of their children did. Apparently the inability to detect antigens at the second sublocus was a peculiarity of the leukemic cells. In one-way MLC, response of the patient’s cells could not be evaluated because of absence of lymphocytes. The patient’s irradiated leukemic cells stimulated lymphocytes of the donor minimally on two occasions (Table 4).

On April 10, 1970, the patient was admitted to USPHS Hospital. The spleen was enlarged 13 cm below the left costal margin. The hematocrit was 34%, the white blood cell count 90,000/cu mm. with 70% blasts and the platelet count 15,000/cu mm. On April 12 CY, 15 mg/kg and hydroxyurea, 4 g, were administered. On April 14 the patient was given total body irradiation followed by marrow infusion.

MTX was administered, 10 mg/sq m on days 1 and 3, and 5 mg/sq m on days 6 and 11. Hydrocortisone, 50 mg, was administered daily. On day 8 a transfusion of 51Cr-labeled platelets from the marrow donor showed a recovery of 12% and a survival of 1.2 days. At this time the spleen size had decreased to 5 cm below the left costal margin. At no time was the uric acid above 12 mg/100 ml and there was no evidence of uric acid nephropathy. Throughout the posttransplantation course the patient was febrile. Repeated blood cultures were negative. He received two courses of carbenicillin and cephalothin from day 6 to 9 and from day 10 to 14 for presumed infection. The white blood cell count fell to below 100/cu mm on day 7 and thereafter remained below this level. Marrow examination on day 11 and day 16 showed aplasia. On day 17 he developed a bacteroides septicemia, treated with penicillin and tetracycline. He died on day 18 in shock.

The autopsy showed a 1700-g spleen with multiple septic infarcts, marrow hypoplasia with scattered foci of hemopoiesis with lymphocytes predominating, septic necrosis of the colon, congestive hepatomegaly, severe lymphoid hypoplasia, and acute tracheobronchitis with terminal aspiration.

**COMMENT**

This patient had a large body burden of leukemic cells indicated by the high white blood cell count, the packed marrow, and the large spleen. Following irradiation, vigorous treatment prevented problems with uric acid. The patient destroyed platelets rapidly after the marrow graft, including platelets from the marrow donor. Although there was suggestive histological evidence of marrow engraftment, the graft did not function as judged by failure of the white blood cell count to go up. One can only speculate that the destructive process observed with donor platelets extended as well to other donor cells. The very large spleen may have played a role in this process.
Case 4

A 15-yr-old girl developed a mass in the left neck in July 1969. A biopsy diagnosis of lymphosarcoma was followed by treatment with 1000 rads. Several weeks later she was found to have a white blood cell count of 53,000/cu mm with 44% lymphoblasts, and the diagnosis was changed to acute lymphoblastic leukemia. From August 1969 until April 1970 she was treated with various combinations of drugs, including prednisone, oral and parenteral MTX, 6-mercaptopurine (6-MP), vincristine, and cytosine arabinoside with several brief remissions. During relapse her illness was complicated by urinary tract infections, one episode of septicemia with Candida albicans and one episode of leukemic meningitis treated with intrathecal MTX. Continuous high dose prednisone therapy resulted in severe iatrogenic Cushing's disease. Attempts at steroid withdrawal produced symptoms of adrenal crisis. The patient was in apparent complete remission and doing well until 2 days before admission when she noted decreased vision in the left eye that progressed to total blindness.

She was admitted to the USPHS Hospital on May 30, 1970. The hematocrit was 34.5%, the platelet count 220,000/cu mm, and the white blood cell count 3900/cu mm with a normal differential. Lumbar puncture showed an opening pressure of 150 mm. The clear spinal fluid contained one lymphoblast. Marrow examination showed about 15% lymphoblasts. The patient was considered to have active CNS leukemia and early marrow relapse. She was treated with intrathecal MTX and with 500 R of irradiation to the skull. After this treatment the papilledema cleared, although she remained blind in the left eye.

On June 15, 1970, the patient was given total-body irradiation. Twenty-four hours after irradiation, marrow from her brother was infused i.v. Hematologic events and MTX ad-

![Hematologic events and MTX administration in Case 4.](image-url)
ministration are shown in Fig. 1. MTX was omitted on day 11 because of mucositis, and other doses were delayed or reduced during the course of her therapy because of mucositis or low peripheral blood counts. Sensitivity to MTX was found to be related to documented folate deficiency, and regularly scheduled doses were well tolerated when dietary folate was restored.

She developed oral candidiasis and an inguinal rash from which *Pseudomonas* was cultured. Her platelet count was maintained above 20,000/cu mm by means of transfusion of platelets from her father beginning in the second week. With the rise in the patient's circulating granulocyte count there was rapid healing of the infection of the groin.

On day 27 the patient developed a papular, erythematous, pruritic skin rash on the face and trunk. Biopsy examination showed mild lymphocytic infiltration and lysis of the basal cell layer of the epidermis consistent with GVH. There was no diarrhea nor were there changes in liver function. The rash appeared to improve 24–48 hr after a dose of MTX and to worsen just before the next dose. In addition to the skin changes the patient demonstrated susceptibility to infection with several episodes of oral and vaginal candidiasis, one episode of urinary tract infection, and staphylococcal and pneumococcal pharyngitis. On day 77, determination of circulating immunoglobulins by immunoelectrophoresis and immunodiffusion showed no detectable IgM, 0.02 g/100 ml of IgA and 0.55 g/100 ml of IgG. Additional problems were encountered with episodes of nausea, vomiting, mild hypotension and fever associated with plasma cortisol levels under 5 µg/100 ml on several occasions. These were controlled by adjustment of the patient's dose of steroids.

The patient was discharged on day 52. On day 62 marrow examination showed normal cellularity with islands of lymphoblasts occupying 20–30% of the marrow. By day 69 marrow examination showed over 50% lymphoblasts. By day 76 the marrow showed 80% leukemic cells. By day 80 the lymphoblasts in the peripheral blood had risen to 45,600/cu mm and the patient was started on chemotherapy with vincristine, prednisone, and 6-MP. This therapy produced severe pancytopenia and marrow hypoplasia, but lymphoblasts continued to persist in both marrow and peripheral blood. Despite support with platelet and granulocyte transfusions the patient developed first staphylococcal and finally *E. coli* septicemia and died on day 102.

Cytogenetic studies were performed eight times beginning on day 15 and ending on day 84. All 240 mitoses examined in preparations of uncultured marrow and blood had sex chromosomes compatible with 46,XY. Of particular note is the fact that at the time of florid leukemia, day 76 to 79, each of 24 dividing uncultured cells from the marrow and each of 24 such cells from the blood had a Y chromosome as judged by morphologic and fluorescent criteria. Details of the cytogenetic studies are given elsewhere.25

At autopsy there was hypoplasia of the marrow, lymph nodes, and spleen. In these organs, and in the thymus, kidney, liver, meninges, and optic chiasm, there were many leukemic cells. There were wide spread visceral foci of septic necrosis containing bacteria and/or *Candida*. The skin showed mild dermal, interstitial, and perivascular chronic inflammatory cell infiltrate.

**COMMENT**

This patient, with acute lymphoblastic leukemia, had CNS leukemia but was otherwise in good clinical condition at the time of irradiation. Irradiation and marrow infusion were well tolerated and prompt engraftment occurred. Leukemia recurred by day 62 and cytogenetic studies showed only male (donor) cells. A modest GVH occurred and appeared to be controlled by MTX.

**Case 5**

A 5-yr-old boy had the diagnosis of acute lymphoblastic leukemia made on June 24, 1966. He was treated with prednisone and 6-MP with complete marrow remission maintained with 6-MP from August 1966 until August 1969. His first relapse was treated successfully with prednisone and vincristine, and his second remission was maintained on
oral MTX from September to November 1969, at which time he suffered marrow relapse. Cytosine arabinoside and prednisone produced a third marrow remission by January 1970 that lasted for 6 wk. A short fourth marrow remission was achieved with L-asparaginase. An attempt at remission induction with prednisone and daunomycin produced marrow hypoplasia, but with 40 to 50% lymphoblasts.

He was admitted to the USPHS Hospital on July 24, 1970. The physical examination showed no abnormality except for a mildly Cushingoid habitus. Laboratory data: hematocrit 28%, white blood cell count 2600/cu mm with 14% granulocytes, 76% lymphocytes, and 8% lymphoblasts. The platelet count was 86,000/cu mm.

On July 27, 1970, he received total-body irradiation. On the day following irradiation marrow was infused i.v. Hematologic events and MTX administration are shown in Fig. 2. By day 29 he was removed from isolation and was managed as an outpatient. Evidence of GVH began by day 24 with a rise in his SGOT to 174 IU, associated with the onset of a pruritic generalized erythematous papular skin rash. The rash was worse just before doses of MTX and resolved nearly completely within 48 hr after MTX. The liver was enlarged 4 to 6 cm below the right costal margin. The alkaline phosphatase was 154 IU, the LDH fluctuated between 1000 and 5800 IU, the SGOT fluctuated between 150 and 300 IU, and the bilirubin rose to 1.7 mg/100 ml. Diarrhea was not observed. On day 24 marrow examination showed hypoplasia with foci of regeneration and 10% lymphoblasts. Even though megakaryocytes were present in this and in all subsequent marrow specimens the patient required continuing platelet support to maintain an adequate peripheral platelet count. The survival of transfused platelets was 24-26 hr even when the marrow donor was utilized as a donor for platelets. By day 39 the white blood cell count had risen to 4800/cu mm, with 3000 lymphoblasts. The patient developed malaise and a temperature to 39.4°C. Marrow examination showed almost total replacement by lymphoblasts. He was admitted
ALLOGENEIC MARROW GRAFTING

To the hospital and treated with prednisone and vincristine from day 42 until day 66. On this regimen he developed pancytopenia with the persistence of large numbers of lymphoblasts in the marrow. He developed E. coli septicemia and was treated with ampicillin. Subsequently, septicemia due to Pseudomonas and Klebsiella was treated with carbenicillin and gentamicin. In the face of this deteriorating clinical course a second marrow transplantation was attempted. CY was administered at a dose of 50 mg/kg daily for 4 days followed by infusion of $1.45 \times 10^9$ marrow cells from the same marrow donor. Despite these measures combined with systemic antibiotics and the infusion of irradiated buffy coat cells, the patient succumbed to Klebsiella sepsis on day 85, 14 days following the second marrow infusion. At the time of demise there was no evidence of recovery of peripheral blood counts.

Autopsy showed marrow hypoplasia with small clumps of immature red cells. There was lymphoid atrophy of spleen, thymus, lymph nodes, and small intestine. There was bacterial necrosis of the rectum, multiple pulmonary aspergillomas, and small foci of Pneumocystis carinii. The liver showed marked fibrosis and bile stasis.

COMMENT

This patient and his donor had a red blood cell antigenic difference involving C and c of the Rh system but frequent transfusions prevented study of this difference. There was no other blood genetic marker to distinguish donor from host. However, prompt recovery of marrow cellularity following 1000 rads whole-body irradiation is presumptive evidence of successful engraftment and was confirmed by the appearance of mild GVH. Leukemia recurred very rapidly but no marker existed to identify the leukemia as involving donor or host cells.

A second marrow graft was attempted under adverse circumstances and was unsuccessful. Autopsy showed no evidence of GVH.

Case 6

A 10-yr-old boy had the diagnosis of acute lymphoblastic leukemia made in July 1964. Therapy with 6-MP resulted in complete remission, which was maintained with oral 6-MP until March 1965. Over the next 5 yr he received various combinations of MTX, prednisone, 6-MP, vincristine, CY, cytosine arabinoside and daunomycin. Five complete remissions were achieved with the last relapse in October 1969. L-asparaginase produced a complete remission lasting until December 1969. L-asparaginase combined with 6-MP produced a remission lasting from January until August 1970.

At time of admission to the USPHS Hospital on August 17, 1970, the patient had a history of seven complete remissions spanning a period of 6 yr. He had not required a previous transfusion. Physical examination showed no hepatosplenomegaly or lymphadenopathy. The hematocrit was 34%, and the total white blood cell count was 19,900/ cu mm, with 3100 granulocytes, 4200 lymphocytes, and 12,000 lymphoblasts. The platelet count was 156,000/cu mm. A marrow aspirate showed 90% lymphoblasts. To reduce the load of leukemic tissue he was given a single dose of 15 mg/kg of CY and 5 daily i.v. doses of prednisolone, 500 mg/sq m. This decreased the lymphoblasts in the peripheral blood but had no effect on the marrow. On August 24, 1970, the patient received whole-body irradiation and, 24 hr later, marrow from his sister.

Hematological values and MTX administration are shown in Fig. 3. The first 30 days were complicated only by esophagitis in the first week, which responded to oral antacids. The patient's platelet count was maintained above 50,000/cu mm by transfusion of platelets obtained from his parents. He was removed from isolation on day 25. On day 29 the marrow was hypocellular but showed islands of normal marrow elements.

The patient was discharged on day 37 and followed as an outpatient. Cutaneous and gastrointestinal manifestations of GVH were not observed. However, an elevation of serum bilirubin and alkaline phosphatase were first noted on day 37. Over a period of 2 wk the bilirubin rose to 9.5 mg/100 ml, the alkaline phosphatase to 610 IU, and the SGOT to
471 IU. The patient noted malaise associated with poor appetite. The liver was palpable 5 cm below the right costal margin. On day 65 his bilirubin had fallen to 2.5 mg/100 ml, his liver was smaller, and the SGOT had dropped to 220 IU. The alkaline phosphatase remained elevated. He was symptomatically improved. Diagnostic studies included an assay for Australia antigen, E-B virus antibody titers, cytomegalovirus complement fixation titers, Sabin and Feldman dye test for toxoplasmosis, serum heterophile titer, and complement fixation tests for mycoplasma antibodies. All of these were negative. The hepatic dysfunction was accompanied by an hemolytic anemia with a rise in the corrected reticulocyte count to 7%, and a titer of 1:1000 of a cold reacting anticomplementary RBC agglutinin with I specificity. The patient's marrow was examined on day 86 and no evidence of leukemia was present. The bilirubin was 1.2 mg/100 ml, the alkaline phosphatase 300 IU, and the SGOT 115 IU.

For 4 wk the patient was in good clinical condition except for some fatigability. On a hunting trip he developed a cold followed by chest pain. On examination on day 113 he had a temperature of 39.5°C and bilateral lower lobe pulmonary infiltrates with pneumococci in the sputum. The white blood cell count was 17,800/cu mm with 8900 polymorphonuclears, 3380 band forms and 2670 lymphocytes. The pneumonia resolved on penicillin therapy which was discontinued on day 126 following 4 days without fever. The white blood cell count then was 4300/cu mm with 2400 polymorphonuclears and 1290 lymphocytes.

The patient then continued to be clinically well except for some fatigability. The bilirubin...

Fig. 3.—Hematologic events and MTX administration in Case 6.
was normal after day 120, but the SGOT had ranged from 81 to 85 IU and the alkaline phosphatase from 103 to 265 IU. Peripheral blood counts have been within normal limits except for a hemoglobin of 10 g. Marrow examination on day 167 showed normal cellularity but there were scattered islands of lymphoblasts. A repeat marrow examination on day 195 was interpreted as being within normal limits.

Cytogenetic studies showed that all 20 mitoses examined on day 86 and all 18 examined on day 167 in preparations of uncultured marrow had the female donor XX sex chromosomes. Also on day 167, marrow was cultured with phytohemagglutinin and all 40 mitoses examined were of donor type. Starch gel electrophoresis of leukocyte lysates before grafting showed the patient to have adenosine deaminase 1 and the donor to have adenosine deaminase 2-1. Examination of the patient’s leukocytes on days 29, 35, 43, 49, and 86 showed donor-type adenosine deaminase 2-1. In the Duffy system, the donor was Fy*-positive and the recipient was Fy*-negative. Examination of the patient’s red cells on day 86 showed them to be Fy*-positive.

The patient continues to do well 310 days after grafting. His performance has been normal including school and sports. He has no skin rash and no diarrhea. The liver is palpable 2 cm below the right costal margin, but liver function tests are normal. The white blood cell count ranges between 9700 and 12,000/cu mm with 29%-45% lymphocytes of normal morphology. The hemoglobin is 12.6 g/100 ml and the platelet count 200,000/cu mm. The marrow is of normal cellularity without evidence of leukemia.

**COMMENT**

At the time of writing, day 310, the patient is clinically well. There is evidence of complete marrow chimerism without persistence of host cells. There is no evidence of GVH, although the minimal disturbance of liver function might be due to GVH. Evaluation of recurrent disease must await the passage of time.

**Case 7**

In August 1969 a 24-yr-old man was found to have Stage IV-B Hodgkin’s disease (lymphocyte depletion type) involving mediastinum, lung, and bone. He was treated with two courses of BCNU, and then with nitrogen mustard, vincristine, methyhydrazine, and prednisone, without response. A subsequent course of vinblastine produced only pancytopenia. He also received irradiation to the left thoracic cage, left axilla, right chest, right hip and pelvis, and spleen. These treatments provided pain relief of short duration.

At the time of admission to the USPHS Hospital on September 17, 1970, his detectable disease included a 3-cm mass in the right lower lung field, several palpable masses on the right anterior chest wall, and lytic lesions of the left first and left 10th ribs and right pelvis. Laboratory examination showed an hematocrit of 32%, white blood cell count 8500/cu mm, and platelet count of 171,000/cu mm. Blood chemistry studies were within normal limits. On September 21, total-body irradiation was given. On the following day marrow from his sister was infused i.v.

The hematologic events and MTX administration are shown in Fig. 4. The patient remained afebrile and free of detectable infection. By day 16 platelet support was no longer required. On day 18 he was removed from isolation. On day 20 marrow examination showed actively proliferating marrow. On day 37 cytogenetic studies showed that all 20 mitoses examined in preparations of uncultured marrow had the female donor XX chromosomes.

Postirradiation immunosuppression was not started until day 5 (Fig. 4). On day 9 a fine erythematous macular rash was noted on the patient’s right forearm. The following day this rash involved the posterior thighs, knees, and buttocks, and was increasingly pruritic. By day 17 it had spread to involve his entire body. On day 20, an elevated alkaline phosphatase and SGOT were noted for the first time, and on day 27 the serum bilirubin became elevated. On day 24, diarrhea developed and became a major problem in the patient’s clinical management with stool volumes ranging between 1 and 2 liters per day. There was diffuse desquamation of skin, and a skin biopsy showed necrosis of the dermal-
epidermal junction with epidermolysis. During this time the detectable tumor decreased in size, and by day 23 was not palpable nor detectable on chest X ray. On day 34 a mixed-organism septicemia developed and the patient died on day 37.

At autopsy the marrow was of normal cellularity. There was marked lymphoid atrophy of nodes, spleen, and small intestine. The gastrointestinal tract showed diffuse infiltration by mononuclear cells with partial loss of epithelium and multiple foci of fungal and bacterial necrosis. The liver showed congestion and bile stasis. The skin showed multiple ulcerations with almost complete loss of the epidermis and marked necrosis of the basal layer and subepidermal collagen. There was no evidence of viable tumor anywhere. In areas previously containing tumor there was only fibrosis and necrosis.

COMMENT

This patient had a prompt marrow graft proved by cytogenetic analysis. He then developed fatal GVH. Clinical evidence of Hodgkin’s disease disappeared completely during the first 3 wk after irradiation and there was no apparent tumor at autopsy.

DISCUSSION

Of the first three patients in this series, one showed moderate marrow cellularity at necropsy, a presumptive indicator of engraftment after 1000 rads
whole-body irradiation. The other two showed scattered foci of hemopoiesis, but the origin of these cells could not be determined. They survived for 18, 26, and 30 days, a period long enough for function of the graft to have occurred, but beginning recovery of peripheral blood counts was not observed. The first patient became anuric because of urate uropathy. The second patient had had previous platelet and buffy coat cell transfusions from both his sibling marrow donors, a procedure known to diminish the chances of engraftment or to cause graft rejection in canine matched siblings\textsuperscript{24} and in mice of different strain but compatible at the H-2 locus.\textsuperscript{26} Graw et al.\textsuperscript{8} observed a successful marrow graft in a patient who had received platelet transfusions from the marrow donor, but the patient was being treated with immunosuppressive chemotherapeutic agents at the time of transfusion. In our second patient, the graft may have failed to function because of prior sensitization to donor antigen, although evaluation is difficult in view of the concurrent septicemia. The third patient had a large spleen. Following the marrow infusion he was found to destroy rapidly platelets from the marrow donor. Presumably the infused marrow was destroyed, or its function was inhibited, in a similar fashion. All three of these patients had far-advanced disease at the time of irradiation and marrow grafting, and their severe illness appeared to make an unfavorable outcome likely.

The next four patients were in good clinical condition and all had a relatively smooth clinical course in the first 2 wk after marrow infusion and all showed prompt marrow graft function. Successful marrow engraftment was proved by cytogenetic analysis of marrow and peripheral blood in three patients of opposite sex to their donors. The fourth patient was of the same sex as his donor and no other blood genetic marker difference was present. However, prompt recovery of marrow function following 1000 rads whole-body radiation constitutes evidence for engraftment, since recovery would not be expected otherwise.

One of our marrow graft patients reported earlier\textsuperscript{14} and three of the patients of Graw et al.\textsuperscript{4} developed cytomegalovirus infections. All of these patients had had multiple transfusions of platelets from random donors, a possible source of cytomegalovirus infection.\textsuperscript{27} In order to reduce this risk our Cases 4–7 reported here were given transfusions only from the marrow donor or immediate family members (Table 2). It may be significant that none of these patients developed cytomegalovirus infection.

Of the four patients with functional marrow engraftment, one showed mild disturbance of liver function (Case 6), one had a mild skin rash and mild disturbance of liver function (Case 5), and one showed a moderate skin reaction without gut or liver difficulty (Case 4). These three patients all received MTX on days 1, 3, and 6. Only one patient (Case 7) had severe GVH and, because of an oversight, he did not receive MTX until day 5. The early administration of MTX has been shown to be of great importance in ameliorating GVH in the mouse\textsuperscript{28} and in the dog.\textsuperscript{29} Whether or not the few days' delay in starting MTX contributed to the severity of the GVH reaction cannot be determined. Reports by Santos et al.\textsuperscript{30} and Graw et al.\textsuperscript{8} indicate a considerable variation in the severity of GVH in patients with marrow grafts following CY, but interpretation of these variations is not always clear because of differences in histocompatibility matching and in immunosuppression following engraftment. With grafts be-
between HL-A matched siblings, confirmed by genotyping and MLC, it seems reasonable to expect a spectrum of severity of GVH depending on other histocompatibility loci not yet identified. Canine siblings matched by serotyping and MLC but not given immunosuppressive therapy after marrow grafting showed a high mortality from GVH. The fact that fatal GVH has occurred in human patients despite HL-A matching emphasizes the importance of immunosuppressive therapy following grafting.

Santos et al. reported six patients with acute leukemia given 50 to 60 mg/kg of CY each day for 4 days followed by marrow infusion. Two patients lived long enough to be evaluated for recurrent leukemia: 75 and 215 days. By cytogenetic analysis their marrow cells were entirely of the donor type and they did not show recurrent leukemia. However, Graw et al. reported seven patients with acute leukemia similarly treated except that they were given 45 mg/kg of CY on each of 4 days. Three of their patients with donors of opposite sex showed a mixture of donor and host cells on cytogenetic analysis of the marrow (E. S. Henderson, personal communication). They showed recurrence of leukemia in 21 to 84 days, and the recurrence was in host cells with disappearance of donor cells. The mixed chimerism observed by Graw et al. is not unexpected since allogeneic marrow grafts following CY in dogs resulted in a persisting mixture of donor and host cells. Some monkeys also showed mixed chimerism, but early death from GVH prevented definitive evaluation. In contrast, dogs given supralethal whole body irradiation and allogeneic marrow grafts showed only donor cells with no surviving host cells in marrow or peripheral blood. The complete disappearance of host cells in these animals is in agreement with Mathé’s observation in one patient with a successful marrow graft following 800 rads whole-body irradiation who survived 20 mo, with his graft and without leukemia. It was reasonable to hope, therefore, that host leukemic cells would be eliminated in our patients given 1000 rads whole-body irradiation in preparation for marrow grafting. In fact, leukemia did recur in two patients. In one instance no marker was available to identify the leukemic cells as of donor or host origin. In the other case the host was female, whereas all cells in marrow and peripheral blood were male. Thus, although the first objective of eliminating host cells was achieved, recurrence of leukemia was not prevented in these two cases.

Barnes et al. first reported apparent cure of some mice by lethal irradiation and allogeneic marrow grafting. They pointed out that the irradiation alone would not kill all leukemic cells but that the residual leukemic cells might be eliminated by the colonizing cells through a reaction of immunity. Mathé has used the term “adoptive immunotherapy” to describe the antileukemic effect of the immunologically foreign marrow. A number of studies have been reported involving treatment of murine lymphomas with lethal irradiation and transplantation of allogeneic marrow and spleen cells. Although a few mice have survived, the reported successes with this approach have been few indeed, and most animals have died of recurrent tumor or of GVH. The most notable and recent report was that of Boranic who treated mice bearing a transplanted myeloid leukemia with sublethal irradiation and histoincompatible marrow and lymphoid cells, treated the resultant GVH in
a variety of ways and achieved a significant number of cures. The recurrent leukemia despite the presence of allogeneic marrow cells in our cases and in those of Graw et al. indicates that adoptive immunotherapy by marrow cells from normal HL-A matched donors is not therapeutically operative in all cases of human lymphoblastic leukemia. The number of cases is yet too few for definitive conclusions, and more experience is needed with lymphoblastic leukemia and with other hematological malignancies. The two cases of Santos et al. with acute myelogenous leukemia, the one case of Mathé et al. and our Case 6 with acute lymphoblastic leukemia did not show recurrent leukemia following allogeneic marrow grafting.

The unexpected recurrence of leukemia in donor-type cells is of far-reaching significance. It indicates the presence of an agent capable of inducing malignant transformation in normal cells and constitutes strong evidence that leukemia in man is due to a virus. As a result, additional therapeutic possibilities arise including the delay of marrow grafting until virus released from irradiated cells has been cleared, the use of antiviral agents or interferon inducers, and the use of lymphoid cells specifically immune to viral antigen. The exponential “cell kill” theory has played an important role in the rationale of the chemotherapy of malignant disease over the past decade. This theory appears to be of value when applied to a cell population that has already undergone malignant transformation. However, it obviously does not apply when an agent capable of inducing malignancy in normal cells is still at large. If this is a general phenomenon, interpretation of the duration of remission in acute lymphoblastic leukemia in terms of log cell kill by chemotherapeutic agents is not valid.

One of the patients described here has not, as yet, demonstrated recurrence of leukemia. Human siblings matched at the HL-A locus still represent a wide range of genetic diversity at other loci. Human leukemias may prove to be diverse in etiologic, antigenic, and metabolic characteristics. In view of these unknown variables in host and in disease no prediction can yet be made about the spectrum of end results that may be seen. The cases reported here indicate the feasibility of the irradiation and marrow grafting procedure for patients with hematological malignancy who have had all benefit of conventional therapy provided that they have a HL-A matched sibling willing to serve as marrow donor, that they have not had prior platelet or buffy coat cell transfusions from the intended donor, and that they are in good clinical condition at the time of grafting. Such therapeutic efforts may also yield new insights into the nature of these diseases.

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Allogeneic Marrow Grafting for Hematologic Malignancy Using HL-A Matched Donor-Recipient Sibling Pairs