Successful Donor Cell Engraftment in a Recipient of Bone Marrow from a Cadaveric Donor

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A 12-year-old male with acute lymphocytic leukemia received donor bone marrow from his histocompatible father whose marrow was harvested 40 minutes postmortem after he suffered a myocardial infarction. The marrow was stored in liquid nitrogen for 17 days prior to infusion into the recipient. Trypan blue viability was >99% for the fresh marrow. Progenitor cell assays revealed that 20% of the CFU-MIX, 16% of the BFU-E, 10% of the CFU-E, and 17% of the CFU-GM were spared during the cryopreservation period. Posttransplantation, the recipient had a leukocyte count >10^5/L by day 26. Southern blotting analysis documented the donor origin of the peripheral blood mononuclear cells and granulocytes isolated 46 days posttransplantation. Unfortunately, the patient died of complications relating to graft-host disease 67 days following transplantation. This case demonstrates the feasibility of cadaveric marrow as a source of donor cells and is the first reported case of documented leukocyte engraftment in a recipient of cadaveric marrow.

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MATERIALS AND METHODS

Case report. A 5-year-old boy with acute lymphocytic leukemia attained a remission with conventional therapy that continued for 3 years. Subsequently, he experienced three bone marrow relapses, all successfully treated with standard reinduction and maintenance therapies. His course was notable for methotrexate-associated hepatic cirrhosis, portal hypertension, colitis, hemorrhagic cystitis, and Streptococcus viridans septicemia with cardiac arrest. At age 12, upon recovery, he was transferred to the University of Minnesota Hospital for BMT.

No histocompatible siblings were available to serve as a bone marrow donor, but his father was phenotypically histocompatible for all class 1 (HLA-A, -B, -C) and class 2 (HLA-DR, -DQ, -DP) antigens tested and was, therefore, selected as the donor. The donor and recipient were mutually nonreactive in MLC, with relative response rates of less than 5%. The donor was 40 years old, previously healthy, and had no risk factors for cardiac disease; however, while awaiting marrow harvesting, he suffered an acute myocardial infarction. Vigorous resuscitative efforts were unsuccessful in restoring cardiovascular pressure or respiration. No red cell transfusions were administered during resuscitation or harvesting. In accord with the father’s last expressed desires, 900 mL of bone marrow was harvested 40 minutes after cessation of cardiopulmonary resuscitation. After transportation for two hours at room temperature, the marrow was processed, and bone marrow mononuclear cells were stored at ~180°C. Aliquots were thawed and tested in cell culture on the day of marrow infusion. With the consent of the University of Minnesota Human Subjects Committee, the family was informed of the potential risks of graft rejection and partial engraftment because of the circumstances of the marrow procurement and requested that the BMT procedure proceed as

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originally planned. Pretransplant conditioning consisted of cytosine arabinoside (3 g/m²/dose × 12) beginning on day −8, and 8.5 Gy single-dose total-body irradiation (TBI) delivered at 26 cGy/min on day −1. On the day of BMT, 2.96 × 10⁶ cells/kg bone marrow were infused. Methotrexate, antithymocyte globulin, and prednisone were administered as GVHD prophylaxis.¹⁶

The initial month after BMT was complicated by gastrointestinal mucositis, ileus, bacterial sepsis, and persistent fevers. Normalization of peripheral blood counts occurred 26 days post-BMT (Fig 1). An absolute reticulocyte count of greater than 70,000 occurred on day 27 post-BMT. However, the patient was platelet transfusion dependent at this time and on all subsequent days post-BMT. A bone marrow biopsy specimen 28 days post-BMT was hypocellular with a decrease in erythroid cells, myeloid cells, and megakaryocytes. Concurrently with a sudden rise in the WBC count on day 37 post-BMT, profuse diarrhea and abdominal pain developed along with a reticular, morbilliform erythematous eruption over the trunk, extremities, and soles. Biopsy specimens of the skin, esophagus, stomach, and rectum were all consistent with acute GVHD. Immunoperoxidase stains and viral cultures were negative for viruses.

Treatment for GVHD was initiated with 2 mg/kg/d of methylprednisolone and escalated to 88 mg/kg/d before improvement of the skin lesions and partial resolution of diarrhea were noted. During this period, increased abdominal distress, hyperamylasemia, renal failure requiring dialysis, antibody-negative hemolytic anemia, disseminated intravascular coagulation, and pneumonia with progressive cardiorespiratory deterioration developed. Fiber-optic bronchoscopy was significant for single septate hyphae, and urinalysis was positive for Aspergillus fumigatus and a Kloeckera species. He died on the 67th day post-BMT of cardiorespiratory failure. Permission for an autopsy was denied.

**Typing for HLA-DR, -DP, -DQ.** Typing for class I (HLA-A, -B, -C) and class II (HLA-DR, -DP, -DQ) antigens was performed using a two-stage microcytotoxicity assay as previously described.³⁰³¹ Class 1 antigen typing was performed on mononuclear cells, whereas class 2 antigen typing was accomplished using nylon wool–purified B lymphocytes.

**Bone marrow processing.** Bone marrow was aspirated from the posterior iliac crest and mixed with Hanks’ balanced salt solution, 5% human serum albumin, and 100 U heparin/mL. The mixture was filtered and centrifuged at 4640 g for ten minutes. The buffy coat cells were resuspended in Hanks’ balanced salt solution with 10% dimethylsulfoxide and 5% human serum albumin before cryopreservation. The methods for cryopreservation and thawing have been previously described.²⁹ No modifications of the marrow processing procedure were made. Bone marrow cryopreservation was started seven hours postmortem. Routine bacterial culture and marrow cell viability (as assessed by trypan blue exclusion) were performed.

**Cell culture techniques.** Techniques for bone marrow multipotent CFU-MIX (CFU-GEMM) and committed myeloid (CFU-GM) and erythroid (BFU-E and CFU-E) in vitro progenitor culture have been previously described.²³²⁴ CFU-E assays were performed in a plasma clot assay as previously described.³⁵

**Engraftment studies.** DNA genotypic analysis was accomplished post-BMT by identifying donor-specific restriction fragment length polymorphisms.³⁶³⁷ Peripheral blood mononuclear cells and granulocytes were isolated by differential gradient centrifugation (Ficoll-Hypaque; density, 1.077) as previously described.³⁷ Methods for the isolation of high–molecular weight DNA, restriction enzyme digestion, gel electrophoresis, capillary transfer of digested fragments onto nylon membranes, hybridization of the radiolabeled probes, and stringency washes of the filter membrane have been described in detail.³⁷

Probes D9S1, D14S1, D15S1, D17S1, and D20S1 were generously provided by Dr Ray White (University of Utah and the Howard Hughes Institute). Probes D12S4 and D13S2 were kindly provided by Dr Webster Cavenee (University of Cincinnati). Description of these probes, alleles detected, and hybridization conditions have been previously reported.¹⁹⁻²⁰ Probes binding to informative alleles from Eco R1–generated polymorphisms are D12S4 and D14S1, whereas D13S2, D15S1, D17S1, and D20S1 are informative with Msp 1, and D9S1 and D13S2 are informative with Taq 1 digestion of genomic DNA.

Chromosomal analysis was performed on the donor and recipient pre-BMT using quinacrine-bandng techniques.³⁸ No donor-recipient polymorphisms were identified, and therefore, no post-BMT studies were obtained.

**RESULTS**

A total of 1.94 × 10⁶ nucleated marrow cells were obtained 40 minutes postmortem. Lymphocytes comprised 11.3% of the original marrow specimen, monocytes 4.6%, neutrophil and neutrophil precursors comprised 62.3%, and erythroblasts were 15.4% of the total marrow cell population. Viability was noted to be greater than 99% by trypan blue dye exclusion. Buffy coat isolation resulted in a 27% loss of cells. At the time of the second thawing and infusion into the recipient, the marrow consisted of 81% mononuclear cells,
7% neutrophil and neutrophil precursors, and 9% normoblasts. Routine bacterial cultures were negative.

Fresh progenitor cell cultures all yielded values that were greater than or equal to median values for our laboratory. Preservation of CFU-MIX, BFU-E, CFU-E, and CFU-GM were present at levels of 15% to 20% of the fresh marrow sample at the time of BMT. The total number of progenitor cells in the infusion was 0.2, 3.7, 11.8, and 3.8 x 10^7/kg for CFU-MIX, BFU-E, CFU-E, and CFU-GM, respectively.

Engraftment studies. Pre-BMT, the donor and recipient were studied for polymorphic differences of red blood cell phenotypes and genotypic polymorphisms by cytogentic techniques and by Southern blotting analysis. Red cell phenotyping revealed a difference in Rhesus blood typing of the donor (D-) and the recipient (D+). However, post-BMT specimens were not obtained, and therefore, red cell engraftment cannot be evaluated. Cytogenetic analysis with Q-banding techniques demonstrated no donor-recipient polymorphisms and, therefore, was not a useful marker for engraftment in this patient.

Southern blotting analysis of donor and recipient was performed using three different restriction endonucleases—Eco R1, Msp 1, and Taq 1. These were selected since there is a higher likelihood of informative alleles detectable with a variety of radiolabeled probes and initially noninformative blots could be reported with one or more radiolabeled probes. Screening of the donor and recipient identified a donor-specific polymorphism with D13S2 and D15S1. All other probes failed to identify unique donor or host fragments that could be used to monitor engraftment. Post-BMT, as illustrated in Fig 2, mononuclear cells and granulocytes were found to have the genotypic pattern of the donor.

Despite the lack of host-specific fragments, it is likely that if there were residual host cells present post-BMT, these cells would constitute <25% of the overall population based on the relative intensity of the different fragments in the post-BMT samples. In this instance, the use of two probes on the blot provides an internal control for the assessment of engraftment based on the relative intensities of the different alleles (Fig 2). Since the host is homozygous for the alleles to which these probes bind, an even lower percentage of host cells should be detectable in this system. We conclude from these studies that the host was repopulated with greater than 75% donor mononuclear and granulocytic precursor cells, although partial engraftment at a level of up to 25% cannot be discounted. Further screening for host-specific polymorphisms was not possible because of the small amount of recipient pre-BMT DNA available. Lastly, the presence of biopsy-proven multi-organ system GVHD, particularly in the absence of viral infection, is further suggestive (but indirect) evidence that donor cells were present in substantial numbers.

**DISCUSSION**

Engraftment of cells from at least two lineages occurred in this recipient of paternal, cadaveric marrow. Cadaveric marrow as a feasible source of donor marrow has been infused into animal models in vitro and in vivo since the availability of cryopreservation techniques in the 1950s. The first human cadaveric marrow infusion was performed by Thomas and colleagues in 1957. Cadaveric marrow obtained three hours postmortem was frozen for 3 weeks, and 3.17 x 10^9 cells were infused throughout a 3-day period into a 59-year-old man with chronic lymphocytic leukemia who was conditioned with 4.5 Gy TBI divided throughout eight days. The patient had an apparent temporary erythrocyte engraftment of less than 1-month duration. Ferrebee and co-workers compared the cell yields and viability of marrow obtained from the human fetus, the usual rib removed at surgery, or ilium aspiration from adult cadaveric donors whose marrow cells were removed and frozen up to six hours postmortem. Adequate numbers of viable cells were obtainable from each of these sources postmortem, especially if procured within four hours postmortem. The feasibility of marrow procurement at the time of cadaveric kidney harvesting was reported by Monaco and colleagues. These investigators infused cadaveric marrow following kidney transplantation as a means of inducing donor-specific tolerance in the setting of zero-antigen matching. In their patient, donor erythrocytes persisted up to 2 months postreinfusion, although there was no evidence of lymphoid engraftment despite the use of antilymphocyte serum, prednisone, and azathioprine as preinfusion immunosuppression. As suggested by the case descriptions of Thomas, et al and Monaco and colleagues, erythroid engraftment is possible following cadaveric marrow infusion.

The pluripotent capacity of cadaveric marrow was not
evident, however, in these preliminary studies. Liu et al documented the ability of murine cadaveric marrow obtained at varying times postmortem to produce day 7 (committed cell lineage) and day 10 (multilineage) splenic colonies in lethally irradiated mice. 7,44 Human cadaveric marrow 49 has been shown to be capable of generating erythroid and committed myeloid colonies up to 19 hours postmortem in colony assays (BFU-E and CFU-GM, respectively).

Leukocyte engraftment as denoted by a leukocyte count > 10⁹/µL occurred prior to day 28 post-BMT, which is within the normal range of age- and disease-matched controls in our institution (Filippovich, unpublished observations). Engraftment of mononuclear cells and granulocytes was demonstrated in our patient by Southern blotting techniques. The extent of engraftment of these two lineages was comparable. In other patients, we have observed differences in the degree of engraftment of mononuclear cells and granulocytic cells in cases in which the recipient was at higher risk for incomplete engraftment or graft rejection based on the pre-BMT cytoreductive regimen used or the use of T cell depletion of donor marrow for GVHD prophylaxis. 37

The availability and feasibility of using cadaveric marrow would eliminate the potential, albeit small, risk of marrow donation and hospitalization of volunteer unrelated donors. Several centers have already begun to store cadaveric marrow. 36,37 However, it should be emphasized that the use of cadaveric donors poses several difficulties, particularly on a national scale. The probability of matching for HLA antigens is low, approaching 1 in 10,000 to 30,000. The cadaveric donor pool size required to provide potential recipients with phenotypically compatible marrow can be estimated from the available experiences of living, volunteer, unrelated donor pools. Data from the Anthony Nolan Unrelated Donor Registry in England, which has had an accrual rate of 5,000 volunteer donors per year since 1975 and now contains over 50,000 names, is thought to be of sufficient size to provide HLA-A, -B, -D/DR-compatible, MLC nonreactive donors for approximately 30% of the patients for whom a search is requested. 30

In addition, the current complications of BMT from nonsibling donors would not be diminished with the use of cadaveric marrow and, therefore, would present formidable obstacles to the routine usage of this type of donor marrow. Of the less than 100 patients who have received transplants from unrelated donors, the overall survival rate is approximately 20%. 51 The major causes of mortality have been GVHD, infection, and leukemic relapse in this group of patients, many of whom had advanced disease. 51 Insufficient numbers of patients have received HLA phenotypically identical unmanipulated marrow from unrelated donors to permit definitive conclusions with respect to the morbidity and mortality for patients lacking histocompatible sibling donors. However, recent results of the Seattle group in their experience with related donors who were not HLA-identical siblings are encouraging. 53 Donor and recipient pairs who were phenotype identical but genotypically nonidentical had a similar incidence of acute GVHD as compared with controls who received genotypically identical marrow. The ultimate success of this procedure may rely, in part, on the development of more effective methods of GVHD prophylaxis and treatment and, perhaps, more sophisticated HLA typing. 53 Whether these problems can be solved to permit the routine collection of cadaveric marrow by institutions in the near future is not certain.

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