Bone Marrow From Cadaver Donors for Transplantation

By Hideo Mugishima, Paul Terasaki, and Akihiro Sueyoshi

To determine the feasibility of obtaining bone marrow cells from cadaver donors for transplantation, marrow cells were prepared from 17 cadaver donors. After surgical removal of the iliac crest, as many as $2 \times 10^8$ cells were isolated. Cadaver marrow had a lower percentage of T cells (mean of 10%) than did marrow from living donors. The T cells were lysed by a monoclonal antibody and human complement to a point at which no sheep red blood cell-rosetting cells were detected. Low levels of T colonies, however, grew out from the monoclonal antibody-treated cells. Although cell loss inevitably occurs from purification, antibody treatment, freezing, and thawing, sufficient numbers can be recovered for transplantation. The yield of stem cells was 84% for CFU-C, 39% for CFU-E, 81% for BFU-E, and 48% for CFU-GEMM. We suggest that T cell-depleted marrow cells from cadaver donors could be used for transplantation. Improved immunosuppressive therapy may be required, however, to prevent graft rejection of allogeneic marrow that may have minor histocompatibility differences.

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Bone Marrow Transplantation, conventionally done from HLA-identical sibling donors, has recently been done from one-haplotype different related donors and from HLA-matched unrelated donors. Despite this recent advance, more than half the patients who could benefit from a bone marrow transplant are not treated because they lack suitable donors. Because kidneys are obtained from more than 2,000 cadaveric donors yearly in the United States, the possibility that bone marrow harvested from these same donors might be a useful source of transplantable bone marrow was re-investigated. As early as 1958, Ferreebee et al explored the possibility of obtaining bone marrow from cadavers. With the present ability to match HLA antigens and to treat marrow with monoclonal antibodies, the approach becomes practical. Recently, Maples et al reported that bone marrow cells have been harvested from 70 cadaveric donors who had died five or more hours previously. The first problem encountered was how best to obtain marrow cells from cadaver donors. After testing various methods, we describe a method whereby the operation can be completed in 30 minutes. The yield of various stem cells after freezing in liquid nitrogen and thawing is documented. In addition, the effectiveness of a monoclonal antibody in killing T cells in the marrow was examined.

MATERIALS AND METHODS

Bone marrow was harvested from 17 cadaver kidney donors, 12 males and five females, whose families had given permission to remove all organs. Their ages ranged from 5 to 51, and of the 17, 12 deaths were accidental, four deaths were from intracranial bleeding, and one death was caused by anesthetic shock.

Bone Marrow Preparation

After removal of the kidneys for transplantation, the outer cortex of both iliac bones was removed aseptically with an electric bone saw, and the trabecular bone containing red marrow was extracted using a chisel. Both cortical and trabecular samples were cut into small pieces and stirred with heparinized RPMI 1640 medium for 30 minutes to release bone marrow cells from the spongy matrix. The cell suspension was passed through a sterile No. 20 stainless steel sieve (Van Waters and Rogers, Los Angeles), washed, and layered over a Ficoll-Hypaque gradient. After centrifuging at 1,500 rpm for 30 minutes, the interface containing mononuclear cells was collected. The isolated mononuclear cells were incubated with mouse monoclonal antibody CT-2 (final concentration 1:100), which is cytotoxic to T lymphocytes, and with human complement (final concentration 1:2) for 45 minutes at room temperature with intermittent agitation. The cell suspension was then layered over a 40% Percoll gradient to remove dead cells. Using 20% fetal calf serum (FCS) and 10% dimethyl sulfoxide (DMSO) in RPMI 1640 medium, a 4-mL aliquot of viable cells at 1 to 3 $\times 10^7$/mL was put in 5-mL tubes and frozen in a program freezer (Cryo Med model 801, Mt Clemens, Mich). Frozen cells were stored in liquid nitrogen. A few aliquots at 1.5 mL of the above concentration of viable cells were also frozen in 2-mL tubes to be used for hematopoietic stem cell assays and bacterial contamination tests. The donor lymphocytes were also stored for mixed leukocyte culture (MLC) testing.

Assay for Hemopoietic Precursors

Preparation of PHA-LCM. The medium was conditioned for six days by human peripheral blood leukocytes of normal individuals in the presence of 1% phytohemagglutinin (PHA: Wellcome HA-15) and 10% heat-inactivated FCS (MA Bioproducts, Walkersville, Md).

CFU-C assay. A modification of the agar assay described by Robinson and Pike was used for CFU-C. A 1-mL aliquot of $2 \times 10^5$ marrow cells was cultured in triplicate in 35-mm culture dishes containing 0.3% agar, 10% FCS, 10% PHA-LCM, and MEM-alpha medium ( Irvine Scientific, Calif). The plates were incubated at 37 °C in an atmosphere of 5% CO2 and high humidity. On day ten the number of colonies with greater than 40 cells was counted using an inverted microscope.

CFU-E and BFU-E assays. Prepared bone marrow mononuclear cells were suspended in enriched MEM-alpha medium containing 0.8% methylcellulose (Dow Chemical Co, Midland, Mich), 30%
colonies were counted. The colonies with more than 40 cells were observed as a whole plate preparation after staining cytochemical-analysis. Chloroacetate esterase and nonspecific esterase were recognized by their distinct color and morphology.

Table 1 shows the yield of bone marrow cells after each step in the isolation and thawing of cells. In cases 1 through 8, the cell suspension was obtained by perfusion of the bone marrow with media. Before freezing, the yield of mononuclear cells using Ficoll-Hypaque separation was 72%; after thawing, the yield was 53%.

In cases 9 through 17, the cell suspension was obtained using a bone cutter. By this procedure, the yield of bone marrow cells after Ficoll-Hypaque separa-
Fig 1. The cells' viability after thawing was approximately 80% if the entire procedure took less than ten hours.

ration was 42%, which was lower than that obtained by using the perfusion procedure. However, the yield after thawing these samples (86%) was better because it required less time to isolate the cell suspension and dead cells were removed by Percoll gradient. The mean number of bone marrow cells harvested was $13.6 \times 10^6$. The mean number after Ficoll-Hypaque separation was $5.4 \times 10^6$. The mean number after treatment with anti-T cell monoclonal antibody and complement was $3.2 \times 10^6$. Finally, after freezing and thawing the bone marrow, the mean was $2.2 \times 10^6$. The yield of bone marrow mononuclear cells after thawing was 16%.

Figure 1 shows the relationship between cell viability and isolation time (time between the bone marrow removal until freezing the samples). The cells' viability after thawing was about 80% if the entire procedure took less than ten hours. Samples that required more time, especially early in our experience, had about 50% viability.

Bone marrow samples from five living donors were contaminated with 20% to 34% E rosetting cells, and samples from 16 cadaver donors were contaminated with 2.5% to 21.5% E rosetting cells. Thus, the number of T lymphocytes in bone marrow suspensions from cadaver donors was significantly lower than the number of T lymphocytes in bone marrow cells from living donors ($P < .005$).

Nine bone marrow samples were monitored for T cell contamination before and after treatment using E rosette formation and indirect immunofluorescence. Almost all T lymphocytes were removed after treatment, as shown in Table 2 (E rosette-positive cells > 99%; Leu-1-positive cells > 95%; OKT-3-positive cells > 90%). However, a few T cell colonies were detected after the treatment (% removal > 94%). The treatment did not suppress any hematopoietic stem cell precursors in the nine samples (Table 2).

We studied the yield of hematopoietic stem cells after freezing for one month and then thawing. Table 3 shows that the yield of CFU-C was 84%, CFU-E was 39%, BFU-E was 81%, and CFU-GEMM was 48%. The total number of CFU-C and CFU-GEMM after thawing was as shown in Table 4. The mean number of CFU-C was $1.7 \times 10^6$ and CFU-GEMM was $1.6 \times 10^5$.

**DISCUSSION**

With the increasing use of cadaver donors for multiple organ harvests of kidneys, livers, hearts, and corneas, it could be asked why bone marrow is also not routinely salvaged from cadaver donors. The following are among the reasons for this lack of use: (a) difficulty in obtaining the marrow; (b) difficulty in matching for HLA antigens, since only about one in 10,000 recipients would be compatible with a given donor; and (c) the cost of preparing and storing marrow in liquid nitrogen is extremely high.

In the present study, we addressed mainly the first question. Although the surgical removal of the iliac crest requires procedures that are different from those used in nephrectomy and requires specialized orthopedic instruments, once incorporated into the

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**Table 2. % T Cells Pretreatment and Posttreatment of MAB CT-2 With Human Complement**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>SRBC Rosette (%)</th>
<th>Indirect Leu-1</th>
<th>Immuno-fluorescence</th>
<th>CT-2 (%)</th>
<th>CFU-TL/1 x 10^8</th>
<th>Toxicity of MAB CT-2 Against Stem Cells*</th>
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<td>8.5/2.0</td>
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<tr>
<td>17</td>
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<td>10.0/0</td>
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% Removal 99.5 ± 1.7 96.6 ± 6.7 92.6 ± 12.0 99.7 ± 1.0 94.7 ± 8.2

ND - not done.

All assays were done in triplicate (per 2 x 10^6 cells).
routine, we have shown that it is possible. The extra team that is required is no different than if livers are harvested in addition to the routine kidneys. Studies on marrow cell viability indicate that these cells should be harvested immediately, thus necessitating an on-call group of technicians who would isolate cells at night. The number of cells, and particularly the number of hemopoietic blast cells as opposed to peripheral blood, is obtained in fairly high yields from cadaver marrow. Of course, some loss in viability and numbers is introduced by the freezing and thawing procedures, as well as treatment of the marrow for the T cell deletion. The yields after all these procedures are documented here. We conclude that it is feasible to obtain sufficient numbers of viable cells from cadaver marrow after treatment with monoclonal antibody and freezing and thawing.

With respect to histocompatibility matching, considerable advances have been made recently in reducing the graft-versus-host (GVH) reaction by the use of lectins8 or monoclonal antibodies.19 With both these techniques, it appears possible to eliminate immuno-competent T cells from the bone marrow suspension, leaving only the hemopoietic blood cells. As T cell depletion is improved, the GVH problem would probably not be the primary one in preventing the use of cadaver marrow. It is more likely that the host-versus-graft (HVG) reaction, even against minor compatibilities, will continue to be difficult to manage. There are indications that the HVG problem is aggravated with T cell-depleted marrow.20 For patients who have no HLA-identical donors and who currently are rejected for transplantation, future efforts probably should be directed to perfecting immunosuppressive regimens capable of surmounting minor incompatibilities. Because of a decreasing birthrate, in the future the current dependence on HLA-identical sibling donors must be broadened to other sources. The fact that one-haplotype-different donor transplants can successfully be performed indicates that complete matching is not necessarily required and that one-haplotype matched cadaver donors can be similarly used. With the storage of frozen aliquots of marrow, it is possible to perform multiple MLC tests before use of the marrow in transplants, permitting wider-scale checking for the degree of incompatibility between donor and host.

The cost problem, particularly in bone marrow storage, may appear at first glance to be prohibitive. If, however, it could be established that the GVH reaction could be eliminated by various methods, and that the HVG reaction can be controlled in a reasonable percentage of patients, the cost benefit calculation can be made. If bone marrow transplantation achieves the success rate of kidney transplants, to pay $10,000 for a marrow graft is not excessive, for a marrow graft should not be any less valuable to a patient than a kidney transplant.

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