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.

Bone Marrow Transplantation

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 reinvestigated. As early as 1958, Ferrebee 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 2% fetal calf serum (FCS) and 10% dimethyl sulfoxide (DMSO) in RPMI 1640 medium, a 4-mL aliquot of viable cells at 1 to $3 \times 10^7$ 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 hemopoietic 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 $2 \times 10^8$ 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%

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FCS, 1% deionized bovine serum albumin (BSA, Sigma Chemical Co, St Louis), and 2 IU/mL erythropoietin (Connaught Laboratory, Canada, or Green Cross Corp, Tokyo) to a final cell concentration of 2 x 10^6 cells per milliliter. The colonies were scored on day seven for CFU-E and on day 14 for BFU-E according to Gregory's and Eaves' criteria.

**CFU-GEMM assay**. Modified CFU-GEMM assay was done as described by Fauser and Messner. Bone marrow cells (2 x 10^6) were plated in quadruplicate in 35-mm culture dishes containing a 1-mL mixture of MEM-alpha medium, 1% methylcellulose, 30% FCS, 10% PHA-LECM, 2 IU/mL erythropoietin, 1% BSA, and 5 x 10^-3 mol/L 2-mercaptoethanol. After 16 days' incubation at 37 °C with 5% CO2 and high humidity, the colonies were enumerated. For this assay, several types of colonies were recognized by their distinct color and morphology using an inverted microscope. The CFU-GEMM colony usually consists of central compact hemoglobinized cells with peripheral small and large nonhemoglobinized cells in a flatter arrangement.

**CFU-TL Assay**

Bone marrow cells (1 x 10^6) were suspended in 1 mL of RPMI 1640 containing 1% methylcellulose, 20% FCS, 5 x 10^-3 mol/L 2-mercaptoethanol, and 15 to 30 μg PHA-P (DIFCO Lab, Chicago) and plated in 35-mm culture dishes in quadruplicate. After seven days' incubation under the same conditions as mentioned earlier, colonies were counted. The colonies with more than 40 cells were removed from the plate to evaluate for E rosette formation.

**Cytochemical Examination of Individual Colonies**

Individual colonies were removed from the methylcellulose layer by a micropipette and centrifuged (Shaden Cytospin 2, Shandon, Runcorn, Cheshire, England). Colonies in the agar dishes were observed as a whole plate preparation after staining cytochemically. Chloroacetate esterase and nonspecific esterase stainings were also used to confirm their cytologic composition.

**Immunological Cell Marker Analysis**

Treated and nontreated cells were assayed by two methods. In the first method, indirect immunofluorescence, 1 x 10^6 cells were incubated for 30 minutes at 4 °C with mouse monoclonal antibodies CT-2, OKT-3 (Ortho Pharmaceutical Corp, Raritan, NJ), and Leu-1 (Becton Dickinson, Oxnard, Calif). The cells were then washed in phosphate-buffered saline (PBS), incubated for an additional 30 minutes with fluorescein-conjugated goat anti-mouse IgG or IgM (Meloy Laboratories, Springfield Va), and washed and resuspended in PBS glycerol. Using a vertical fluorescence light microscope, 200 cells were examined per slide. The second method used was E rosette formation using 2% neuraminidase-treated sheep red blood cells.

**Typing for HLA-A, -B, -C, and -DR**

Typing for HLA-A, -B, and -C antigens was performed by using the microcytotoxicity assay. HLA-DR typing was done by using a modified microcytotoxicity assay using nylon wool-purified B cells.

**RESULTS**

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 sepa-

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Nucleated Cells (x10^6)</th>
<th>Ficoll-Hypaque Isolated Cells (x10^6)</th>
<th>Yield (%)</th>
<th>CT-2 and C* Treated Cells (x10^6)</th>
<th>Yield (%)</th>
<th>After Thawing (x10^6)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ND</td>
<td>1.5</td>
<td>1.2</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>ND</td>
<td>1.8</td>
<td>1.0</td>
<td>56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8.3</td>
<td>6.3</td>
<td>3.7</td>
<td>59</td>
<td></td>
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<td></td>
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<tr>
<td>4</td>
<td>61.7</td>
<td>45.7</td>
<td>21.9</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>47.1</td>
<td>19.8</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>81.8</td>
<td>59.4</td>
<td>28.5</td>
<td>48</td>
<td></td>
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<td>47.6</td>
<td>26.7</td>
<td>11.7</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>44.1</td>
<td>36.0</td>
<td>16.2</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>48.7 ± 27.0</td>
<td>28.1 ± 22.6</td>
<td>72.2 ± 9.7%</td>
<td>13.0 ± 10.3</td>
<td>52.8 ± 12.5%</td>
<td></td>
<td></td>
</tr>
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</table>

*The viability was poor because of a malfunction in the program, freezer.*

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all assays were done in triplicate (per 2 x 10⁶ cells).

![Graph](image_url)

**Fig 1.** The cells' viability after thawing was approximately 80% if the entire procedure took less than ten hours.

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 hemopoietic stem cell precursors in the nine samples (Table 2).

We studied the yield of hemopoietic 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-GMM 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 x 10⁶ and CFU-GEMM was 1.6 x 10⁵.

**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
Table 3. Yield of Hemopoietic Stem Cells After Freezing and Thawing in One Month

<table>
<thead>
<tr>
<th>Case No.</th>
<th>CFU-C</th>
<th>CFU-E</th>
<th>BFU-E</th>
<th>CFU-GEMM</th>
</tr>
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<tbody>
<tr>
<td>8</td>
<td>68.0</td>
<td>23.0</td>
<td>92.0</td>
<td>24.0</td>
</tr>
<tr>
<td>9</td>
<td>86.9</td>
<td>ND</td>
<td>102.0</td>
<td>90.0</td>
</tr>
<tr>
<td>10</td>
<td>87.0</td>
<td>ND</td>
<td>ND</td>
<td>30.0</td>
</tr>
<tr>
<td>11</td>
<td>ND</td>
<td>26.0</td>
<td>94.0</td>
<td>19.1</td>
</tr>
<tr>
<td>12</td>
<td>100.0</td>
<td>67.0</td>
<td>75.0</td>
<td>36.6</td>
</tr>
<tr>
<td>15</td>
<td>79.0</td>
<td>37.0</td>
<td>63.0</td>
<td>46.5</td>
</tr>
<tr>
<td>16</td>
<td>66.0</td>
<td>45.0</td>
<td>60.0</td>
<td>55.0</td>
</tr>
<tr>
<td>17</td>
<td>100.0</td>
<td>35.0</td>
<td>82.0</td>
<td>80.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>83.8% ± 13.7%</td>
<td>38.8% ± 15.9%</td>
<td>81.1% ± 16.0%</td>
<td>47.7% ± 25.9%</td>
</tr>
</tbody>
</table>

ND = not done.

Table 4. Total No. of CFU-C and CFU-GEMM After Thawing

<table>
<thead>
<tr>
<th>Case No.</th>
<th>CFU-C (x 10^3)</th>
<th>CFU-GEMM (x 10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>0.6</td>
<td>4.4</td>
</tr>
<tr>
<td>10</td>
<td>0.7</td>
<td>4.6</td>
</tr>
<tr>
<td>11</td>
<td>ND</td>
<td>7.2</td>
</tr>
<tr>
<td>12</td>
<td>1.3</td>
<td>7.0</td>
</tr>
<tr>
<td>15</td>
<td>1.8</td>
<td>6.6</td>
</tr>
<tr>
<td>16</td>
<td>2.3</td>
<td>23.0</td>
</tr>
<tr>
<td>17</td>
<td>3.6</td>
<td>62.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>((1.7 ± 1.1) \times 10^8)</td>
<td>((1.6 ± 2.1) \times 10^8)</td>
</tr>
</tbody>
</table>

ND = not done.

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, con-
siderable advances have been made recently in reduc-
ing the graft-versus-host (GVH) reaction by the use of
leptins or monoclonal antibodies. 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 prob-
ably 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 compatibili-
ties, will continue to be difficult to manage. There are
indications that the HVG problem is aggravated with
T cell–depleted marrow. 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 suc-
cessfully be performed indicates that complete match-
ing 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 check-
ing 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 per-
centage 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.

ACKNOWLEDGMENT

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