Engraftment After Infusion of CD34+ Marrow Cells in Patients With Breast Cancer or Neuroblastoma


The CD34 antigen is expressed by 1% to 4% of human and baboon marrow cells, including virtually all hematopoietic progenitors detectable by in vitro assays. Previous work from our laboratory has shown that CD34+ marrow cells can engraft lethally irradiated baboons. Because the CD34 antigen has not been detected on most solid tumors, positive selection of CD34+ cells may be used to provide marrow cells capable of engraftment, but depleted of tumor cells. In seven patients with stage IV breast cancer and two patients with stage IV neuroblastoma, 2.5 to 17.5 x 10^9 marrow cells were separated by immunoadsorption with the anti-CD34 antibody 12-8 and 50 to 260 x 10^6 positively selected cells were recovered that were 64 ± 16% (range 35% to 92%) CD34+.

We found that CD34+ marrow cells can engraft lethally irradiated baboons. The CD34 antigen is not detected on tumor cells from patients with most solid tumors including breast cancer and neuroblastoma. In vitro studies have demonstrated that immunoadsorption with antibody 12-8 can be used to separate normal marrow progenitors from tumor cells. In the present study, we asked if populations of enriched CD34+ cells isolated from the marrow of patients with metastatic breast cancer or neuroblastoma were capable of reconstituting hematopoiesis in vivo.

MATERIALS AND METHODS

Antibodies and immunofluorescence staining reagent. The production and characterization of the anti-CD34 antibody 12-8 has been described previously. This murine IgM antibody was partially purified from ascites fluid using boric acid precipitation. The biotin conjugate of affinity-purified goat anti-mouse IgM antisera was obtained from Southern Biotechnology Associates (Birmingham, AL). The fluorescein isothiocyanate conjugate of avidin (avidin-FITC) was obtained from Vector Laboratories (Burlingame, CA). The fluorescein isothiocyanate conjugate of goat anti-mouse IgM antisera (GAM-FITC) was obtained from Tago (Burlingame, CA).

Production of avidin-Biogel. Avidin obtained from Intenzymes, Falbrook, CA was conjugated to Biogel P-30 (Bio-Rad Laboratories, Richmond, CA) using a previously described procedure. Each mL of gel contained 100 µg of avidin.

Antibody treatment. Nucleated marrow cells (100 x 10^6/mL) obtained from a buffy coat preparation were incubated with antibody 12-8 (50 µg/mL in phosphate-buffered saline with 1% bovine serum albumin (PBS/BSA) for 30 minutes at 4°C. The cells were washed twice and then incubated at the same cell concentration in PBS/BSA with 0.5 µg/mL of biotinylated goat anti-mouse IgM antisera for an additional 30 minutes at 4°C. The cells were again washed twice and adjusted to a concentration of 100 x 10^6 cells/mL in PBS with 5% BSA for column treatment.

Column treatment. Antibody-treated cells were passed successively through a blood component recipient set (Fenwall Laboratories, Deerfield, IL) and Pharmacia K50/30FF column (Pharmacia, Piscataway, NJ) containing carboxylated Biogel P-30 gel (without avidin) to remove debris and cell aggregates. The cells were then passed directly over Chromaflex 15 x 2.5 cm columns (Kontes, Vineland, NJ) containing 20 mL avidin-Biogel at a flow rate of 5.5 mL/minute using an Ismatec Reglo 100 pump (Cole-Parmer, Chicago, IL) until the cells had passed through the column (approximately 100 mL volume). Marrows containing greater than 10^9 cells were divided in half and passed simultaneously over two Kontes columns each containing 20 mL avidin-Biogel. Approximately 100 mL of PBS was then passed through the avidin-Biogel at the same flow rate to wash out BSA. The adherent cells were dislodged by mechanical agitation with a 10 mL pipette until a total volume of 200 mL had been collected.

Cryopreservation, reconstitution, and infusion of cells. The washed adherent cells were resuspended to a final volume of 20 mL in a mixture of 16 mL of autologous plasma previously irradiated with...
30. 0 Gy from a cesium source and 4 mL of medium 199 with Hanks' salts and L-glutamine (TC199; GIBCO, Grand Island, NY). For cryopreservation, the cell mixture was placed into a DF200 Gambro Hemofreeze bag (Gambro, Dialsatoren, Germany), previously transversely heat-sealed 2 inches from the top of the bag and then rapidly mixed with an equal volume of a 20% DMSO solution in TC199 previously precooled in ice, to provide a final bag volume of 40 mL. For freezing, the Gambro bag was sealed, clamped between precooled copper plates, placed in a controlled rate freezer previously precooled to approximately 2°C as previously described and cooled at 2 to 2.5°C/min to −60°C. The cells were then removed from the freezer and placed for storage in the gas phase of liquid nitrogen at −135°C.

For reconstitution the bag was removed from the freezer and rapidly thawed in a water bath at 40°C with constant agitation. Eight milliliters of acid-citrate-dextrose (ACD-A) solution was then added to the bag through the infusion port, gently mixed, and the cells rapidly infused intravenously through a central Hickman line using an intravenous administration set without a filter (2C0001s Soln set; Fenwall, Round Lake, IL).

Immunofluorescence studies. The percentage of cells reactive with antibody 12-8 was determined by immunofluorescence staining and flow microfluorimetric analysis by using an indirect immunofluorescence procedure. Briefly, 5 × 10⁶ cells were incubated with a mixture of 1:100 dilution of avidin-FITC and 1:20 dilution of GAM-FITC in PBSBSA with 0.1% sodium azide for 20 minutes at 4°C. After exposure to hemolytic buffer the stained cells were analyzed with a modified FACS II with data collection and analysis performed using a Consort 40 computer software program (Becton Dickinson Immunocytometry Systems, Mountain View, CA). The unseparated and adsorbed cells labeled with antibody 12-8 and biotinylated goat anti-mouse IgM antisera were stained with avidin-FITC and GAM-FITC. As a negative control, the unlabeled marrow cells were stained with GAM-FITC and avidin-FITC or incubated successively with a control IgM antibody of irrelevant specificity and biotinylated goat anti-mouse IgM antisera and stained with GAM-FITC and avidin-FITC. The percentage of CD34⁺ cells was determined by subtracting from the percentage of cells positively stained with antibody 12-8 the percentage of fluorescent cells in the control.

Long-term marrow culture (LTMC). Two-stage LTMCs were performed using a modification of previously described methods. Briefly, 4 × 10⁶ marrow mononuclear cells were established in 2 mL cultures in McCoy's 5a modified medium (GIBCO) supplemented with sodium pyruvate, essential and nonessential amino acids, vitamins, L-glutamine, sodium bicarbonate, penicillin, streptomycin, Fungizone, 12.5% horse serum, 12.5% fetal calf serum, and 10⁻⁴ mol/L hydrocortisone succinate. The cell suspension was placed into Lux tissue culture amphotubes (Miles Scientific, Naperville, IL) and incubated at 33°C in a 5% CO₂ atmosphere.

Cultures were maintained by weekly demidepopulation with the addition of fresh medium. Under these conditions a uniform adherent stromal layer developed within 3 to 4 weeks. The primary culture was then irradiated with 10 Gy of γ irradiation using a Cesium source. Between 0.09 and 0.7 × 10⁶ test cells were overlayed on to irradiated allogeneic adherent stromal layers in LTMC. One amnibus from the allogeneic cultures was analyzed weekly. The adherent stromal layers were detached using Type 1 collagenase (240 units/mL, Sigma Chemical Company, St Louis, MO) and granulocyte-macrophage progenitor (CFU-GM) assays were performed in semisolid agar on supernatant and adherent layers separately using placent conditioned medium as a source of colony stimulating activity.

Patients. All patients or their legal guardians gave informed consent for treatment on protocols that had been approved by the Institutional Review Board where they received treatment. Patients eligible for transplantation with autologous CD34⁺ enriched marrow cells included individuals with stage IV breast carcinoma, with bone or marrow involvement, whose disease was unresponsive to conventional chemotherapy and/or hormonal therapy, and patients with stage IV neuroblastoma with a history of marrow involvement.

RESULTS

Nine patients were treated with myeloablative therapy and then given autologous CD34⁺ marrow cells. The patients' prior history and therapy are summarized in Table 1.

Marrow treatment. Table 2 summarizes data from the marrow treatment for the nine patients. A total of 2.5 to 17.5 × 10⁶ nucleated marrow cells were placed on the column, 0.7% to 4.0% of which were CD34⁺. We recovered 50 to 260 × 10⁶ adsorbed cells from the columns that were 35% to 92% CD34⁺. The mean recovery of CD34⁺ cells was 42 ± 13% (range 25% to 58%) in the adsorbed cell fractions (n = 7). The adsorbed cell fractions were highly enriched for precursors of colony-forming cells that grow in LTMC (Table 2). Isolated CD34⁺ cells from each patient that were cultured over irradiated marrow stroma formed

Table 1. Pretransplant Clinical Data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Disease*</th>
<th>Age/Sex</th>
<th>Metastatic Sites of Disease†</th>
<th>Previous Therapy‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Breast cancer</td>
<td>43/F</td>
<td>L,H,B,M</td>
<td>CY,AD,5-FU</td>
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<td>2</td>
<td>Breast cancer</td>
<td>36/F</td>
<td>B,M</td>
<td>CY,MTX,5-FU,VB,AD</td>
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<td>3</td>
<td>Breast cancer</td>
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<td>H,B,M</td>
<td>CY,AD,5-FU,Tam,Pred</td>
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<td>Neuroblastoma</td>
<td>2/M</td>
<td>M,B</td>
<td>Unknown</td>
</tr>
<tr>
<td>5</td>
<td>Breast cancer</td>
<td>32/F</td>
<td>M</td>
<td>CY,AD,5-FU,VCR,Pred</td>
</tr>
<tr>
<td>6</td>
<td>Breast cancer</td>
<td>35/F</td>
<td>L,H,B,CNS,M</td>
<td>CY,AD,5-FU,VCR,MP</td>
</tr>
<tr>
<td>7</td>
<td>Breast cancer</td>
<td>50/F</td>
<td>H,B,M</td>
<td>CY,AD,5-FU,VCR,Pred</td>
</tr>
<tr>
<td>8</td>
<td>Neuroblastoma</td>
<td>5/M</td>
<td>M,LN,L,B</td>
<td>CY,AD,VCR,DOP</td>
</tr>
<tr>
<td>9</td>
<td>Breast cancer</td>
<td>44/F</td>
<td>H,LN,B</td>
<td>CY,MTX,5-FU,VB,MP</td>
</tr>
</tbody>
</table>

*All breast cancer patients had estrogen receptor negative infiltrating ductal breast carcinoma except patient 9, who was estrogen receptor positive.
†Metastatic sites at time of transplant: lung (L), liver (H), bone (B), marrow (M), CNS (brain), lymph node (LN).
‡Therapy before transplant: CY, cyclophosphamide; AD, Adriamycin; 5-FU, 5-Fluorouracil; MTX, methotrexate; VB, vinblastine; Tam, Tamoxifen; Pred, prednisone; VCR, vincristine; DOP, cis-platinum; MP, methylprednisolone.
were treated with cyclophosphamide, 60 mg/kg daily for 2

ENGRAFTMENT OF CD34+ MARROW CELLS

marrow with the presence of all lineages. Subsequently, five

30 to 60 days posttransplant demonstrated normocellular

granulocyte count over 500/mm³ ranged from 21 to 47 days

normal peripheral blood counts at the time of death.

patients died but had normocellular marrow at autopsy and

One breast cancer patient received mitoxantrone, 50 mg/m²

and received busulfan, 16 mg/kg in 16 doses over 4 days

(mean, 34 days, followed by 8 Gy total body irradiation given at a dose

rate of 40 cGy per minute by a linear accelerator (Table 3).

four patients with breast cancer had a history of irradiation

leukocyte engraftment with 20% and 40% of normocellular-

erythrocyte and platelet transfusions. Follow-up studies in

all of these patients except patient 8 (see below) performed

30 to 60 days posttransplant demonstrated normocellular

marrow with the presence of all lineages. Subsequently, five

patients died but had normocellular marrow at autopsy and

normal peripheral blood counts at the time of death.

Patient 8 had a granulocyte count of 220/mm³ at day 35. He was started the following day on a 10-day course of recombinant human GM-CSF (250 µg/m²/day). However, his granulocyte count rose and remained over 500/mm³ by day 37 where it remained and he became independent of platelet transfusions by day 66. He relapsed shortly thereafter and his bone marrow showed massive infiltration of neuroblastoma cells concomitant with a renewed requirement for platelet transfusions.

Three patients died at days 17, 14, and 14 posttransplant, two of infections and one of pulmonary hemorrhage. Two of these patients had rising granulocytes counts at the time of death. Additionally, marrow examinations performed at day 14 in these two patients showed evidence of bilineage or trilineage engraftment with 20% and 40% of normocellularity, respectively. A marrow sample was not obtained on the third patient.

Clinical outcome. Patients 1 and 2, who received total body irradiation, died of biopsy proven idiopathic interstitial pneumonitis 82 and 95 days posttransplant. Patient 3 developed a neurologic syndrome characterized by headaches, visual disturbances, and paresthesias. Neurologic workup including CT and MRI scans and spinal fluid analysis was unremarkable, and the patient was treated with dexamethasone. She subsequently died 306 days posttransplant of disseminated aspergillosis. Patient 4 with neuroblastoma relapsed in the marrow 150 days posttransplant. Marrow examinations continued to show normal hematopoiesis with a mixture of tumor cells up to the time of his death 386 days posttransplant. Patients 5 and 6 died of staphylococcus bacteremia and unidentified fungemia, at 17 and 14 days, posttransplant, respectively. Patient 7 died of a massive pulmonary hemorrhage on day 14. Patient 8 relapsed on day 115 in bone and retroperitoneal lymph nodes. Subsequent examination of bone marrow demon-

<table>
<thead>
<tr>
<th>Patient</th>
<th>No. x 10⁶</th>
<th>%CD34+</th>
<th>No. x 10⁶</th>
<th>%CD34+</th>
<th>%CD34+ Yield</th>
<th>Start#</th>
<th>Adsorbed*</th>
<th>Unadsorbed**</th>
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<tr>
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<td>2.3</td>
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<td>67</td>
<td>46</td>
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<td>21,369</td>
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<td>—</td>
<td>110</td>
<td>65</td>
<td>—</td>
<td>429</td>
<td>44,640</td>
<td>12</td>
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<td>16,500</td>
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</tr>
<tr>
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<td>—</td>
<td>56</td>
<td>92</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>13,100</td>
<td>1.0</td>
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<td>62</td>
<td>25</td>
<td>132</td>
<td>2,948</td>
<td>22</td>
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<tr>
<td>6</td>
<td>17,500</td>
<td>2.0</td>
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<td>62</td>
<td>25</td>
<td>180</td>
<td>27,148</td>
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<tr>
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<td>38</td>
<td>—</td>
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<td>—</td>
</tr>
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<td>8</td>
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<td>85</td>
<td>53</td>
<td>—</td>
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<td>9</td>
<td>15,000</td>
<td>1.0</td>
<td>160</td>
<td>54</td>
<td>58</td>
<td>862</td>
<td>31,694</td>
<td>496</td>
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</table>

*Number of cells before treatment.
†Percentage of CD34+ cells in unseparated marrow as determined by FACS analysis. Data were not available for patients 2 and 4.
‡Number of adsorbed cells recovered after immunoadsorption.
§Percentage of CD34+ cells in adsorbed cell fraction.

\[
\frac{\text{Number of adsorbed cells} \times 100}{\text{Number of starting cells} \times \%CD34^+}
\]

Cumulative CFU-GM per 4 x 10⁶ cells used to initiate LTMC. Data were not available for patients 4, 7 and 8.

#Unseparated marrow cells.
**Column-bound cells.
††Cells passing through column.

substantial numbers of colonies. In contrast, the unadsorbed cells formed few colonies in LTMC.

Conditioning regimen. Two patients with breast cancer were treated with cyclophosphamide, 60 mg/kg daily for 2 days, followed by 8 Gy total body irradiation given at a dose rate of 40 cGy per minute by a linear accelerator (Table 3). Four patients with breast cancer had a history of irradiation and received busulfan, 16 mg/kg in 16 doses over 4 days followed by cyclophosphamide, 60 mg/kg daily for 2 days. One breast cancer patient received mitoxantrone, 50 mg/m² for 1 day, and N,N',N"-Triethylenethiophosphoramide (Thiotepa), 300 mg/m² daily for 3 days. One patient with neuroblastoma was conditioned with single doses of carmustine (BCNU), 300 mg/m² and melphalan, 200 mg/m² in addition to vincristine, 1 mg/m² for 1 day followed by 0.5 mg/m² daily for 3 additional days. A second patient with neuroblastoma received cyclophosphamide 60 mg/kg daily for 2 days, L-phenylalanine mustard, 220 mg/m², and fractionated total body irradiation, 200 cGy per day for 6 days.

Engraftment. Following conditioning, patients received 1.0 to 5.2 x 10⁶ CD34+ enriched cells/kg (Table 3). Six patients survived more than 17 days and achieved trilineage engraftment as documented by marrow examination as well as recovery of peripheral blood counts. The days to reach a granulocyte count over 500/mm³ ranged from 21 to 47 days (mean, 34 ± 10 days), while the time to recovery of platelets to greater than 20,000/mm³ was 28 to 66 days (mean, 46 ± 14 days). All six patients became independent of erythrocyte and platelet transfusions. Follow-up studies in all of these patients except patient 8 (see below) performed 30 to 60 days posttransplant demonstrated normocellular marrow with the presence of all lineages. Subsequently, five patients died but had normocellular marrow at autopsy and normal peripheral blood counts at the time of death.

Table 2. CD34+Enrichment and Detection of Hematopoietic Progenitors After Immunoadsorption With Antibody 12-8

<table>
<thead>
<tr>
<th>Patient</th>
<th>Starting Cell</th>
<th>Adsorbed Cell</th>
<th>Cumulative CFU-GM (weeks 2-6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. x 10⁶</td>
<td>%CD34+†</td>
<td>No. x 10⁶</td>
</tr>
<tr>
<td>1</td>
<td>15,000</td>
<td>2.3</td>
<td>260</td>
</tr>
<tr>
<td>2</td>
<td>11,000</td>
<td>—</td>
<td>110</td>
</tr>
<tr>
<td>3</td>
<td>16,500</td>
<td>0.7</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>2,500</td>
<td>—</td>
<td>56</td>
</tr>
<tr>
<td>5</td>
<td>13,100</td>
<td>1.0</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>17,500</td>
<td>2.0</td>
<td>135</td>
</tr>
<tr>
<td>7</td>
<td>16,000</td>
<td>1.4</td>
<td>240</td>
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<td>8</td>
<td>4,200</td>
<td>4.0</td>
<td>104</td>
</tr>
<tr>
<td>9</td>
<td>15,000</td>
<td>1.0</td>
<td>160</td>
</tr>
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</table>

*Number of cells before treatment.
†Percentage of CD34+ cells in unseparated marrow as determined by FACS analysis. Data were not available for patients 2 and 4.
‡Number of adsorbed cells recovered after immunoadsorption.
§Percentage of CD34+ cells in adsorbed cell fraction.

\[
\frac{\text{Number of adsorbed cells} \times 100}{\text{Number of starting cells} \times \%CD34^+}
\]

Cumulative CFU-GM per 4 x 10⁶ cells used to initiate LTMC. Data were not available for patients 4, 7 and 8.

#Unseparated marrow cells.
**Column-bound cells.
††Cells passing through column.
Table 3. Engraftment of Patients Receiving CD34+ Enriched Marrow Cells

<table>
<thead>
<tr>
<th>Patient</th>
<th>Conditioning Regimen</th>
<th>Cell No.1 × 10^6/kg</th>
<th>Marrow Engraftment#</th>
<th>Day &gt; 500 gran./mm3**</th>
<th>Day &gt; 20,000 platelets/mm3††</th>
<th>Survival‡‡ (d)</th>
<th>Cause§§ of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CY/TBI*</td>
<td>4.0</td>
<td>E,G,P (100%)</td>
<td>45</td>
<td>52</td>
<td>95</td>
<td>Pneumonitis</td>
</tr>
<tr>
<td>2</td>
<td>CY/TBI</td>
<td>1.5</td>
<td>E,G,P (100%)</td>
<td>47</td>
<td>55</td>
<td>82</td>
<td>Pneumonitis</td>
</tr>
<tr>
<td>3</td>
<td>BU/CY1</td>
<td>1.2</td>
<td>E,G,P (100%)</td>
<td>21</td>
<td>35</td>
<td>306</td>
<td>Aspergillosis</td>
</tr>
<tr>
<td>4</td>
<td>BCNU/Melph/VCR§</td>
<td>4.5</td>
<td>E,G,P (100%)</td>
<td>28</td>
<td>40</td>
<td>386</td>
<td>Relapse neuroblast</td>
</tr>
<tr>
<td>5</td>
<td>BU/CY</td>
<td>1.0</td>
<td>E,G (20%)</td>
<td>(120)†††</td>
<td>NR</td>
<td>17</td>
<td>Bacteremia</td>
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<tr>
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<td>BU/CY</td>
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<td>E,G,P (40%)</td>
<td>(360)</td>
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<td>BU/CY</td>
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<td>ND</td>
<td>NR</td>
<td>NR</td>
<td>14</td>
<td>Hemorrhage</td>
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<tr>
<td>8</td>
<td>CY/TBI/L-PAM§</td>
<td>5.2</td>
<td>E,G,P (100%)</td>
<td>37</td>
<td>66</td>
<td>267</td>
<td>Relapse neuroblast</td>
</tr>
<tr>
<td>9</td>
<td>Mito/ThioI</td>
<td>2.5</td>
<td>E,G,P (100%)</td>
<td>29</td>
<td>28</td>
<td>105</td>
<td>Relapse breast cancer</td>
</tr>
</tbody>
</table>

Abbreviations: NR, not reached; ND, not done.
* Cyclophosphamide, 120 mg/kg and cyclophosphamide—120 mg/kg.
† Busulfan, 16 mg/kg and cyclophosphamide—120 mg/kg.
‡ BCNU, 300 mg/m², melphalan—200 mg/m², and vincristine—2.5 mg/m².
§ Cyclophosphamide, 120 mg/kg, 12 Gy total body irradiation at 2 Gy/day, L-phenylalanine mustard—220 mg/m².
|| Mitoxanthrone, 50 mg/m², Thiotepa—900 mg/m².
# Adsorbed cells infused per kilogram.
# Hematopoietic lineages observed in marrow (days 30 through 60 posttransplant), erythroid (E), myeloid (G), or megakaryocytes (P) and percentage of normocellularity in parentheses. Patients 5 and 6 represent day 14 marrow results.
** Day posttransplant when granulocyte count greater than 500/mm³.
†† Day posttransplant when untransfused platelet count greater than 20,000/mm³.
‡‡ Number of days posttransplant survived.
§§ Primary cause of death.
††† Neutrophils/mm³ at time of death in parentheses.

stratified almost complete replacement by neuroblastoma and the patient eventually died 267 days posttransplant. Patient 9 died 105 days posttransplant of breast cancer metastatic to lung, pleura, pericardium, bone, and liver.

Patient 1 showed a complete response of tumor as determined by follow-up scans of liver and bone at 8 to 12 weeks. Patients 2 and 3 showed no evidence of tumor at autopsy.

DISCUSSION

We have now shown that marrow preparations highly enriched for CD34+ cells obtained from patients with metastatic breast cancer or neuroblastoma are capable of reconstituting hematopoiesis in vivo after myeloablative therapy. Evidence that the radiation treatment regimens used in this study (patients 1, 2, and 8) were myeloablative comes from extensive experience in and in human19,20 that has demonstrated that total body irradiation exposures of 6 Gy and greater are myeloablative even at dose rates slower than those described in this study. Previous work in rats and clinical experience in humans suggest that the busulfan and cyclophosphamide regimen used for patient 3, 5, 6, and 7 was also myeloablative.21,22 Data supporting the myeloablative properties of the chemotherapy regimen used in patient 4 are less definitive, although clinical studies have demonstrated that patients treated with melphalan at doses greater than 140 mg/m² require bone marrow reinfusion.23,24 Clinical trials have shown that patients receiving Thiotepa at total doses in excess of 180 mg/m² require marrow transplant for hematologic recovery.25 Furthermore, mitoxantrone doses greater than 30 mg/m² have not been given to patients without a subsequent marrow transplant, because of severe and prolonged myelo-suppression.26,27 Therefore, it is probable that the high dose combination of Thiotepa and mitoxantrone given to patient 9 also had marrow ablative properties.

The rate of engraftment of patients 3, 4, and 9 was similar to that seen in patients receiving unmodified autologous marrow. However, patients 1, 2, and 8 showed delayed hematologic recovery. Possible explanations for this delay include adverse effects of the immunoadsorption procedure, cryopreservation damage of purified hematopoietic progenitors, or injury to hematopoietic progenitors caused from the immunoadsorption treatment.
by previous chemotherapy given to these patients. In patient 8, the presence of occult neuroblastoma in the marrow before frank relapse may have contributed to poor marrow function. Indeed, poor initial engraftment has been observed in patients undergoing autologous marrow transplantation who later relapsed.\textsuperscript{28} Studies of larger numbers of patients are needed to determine the factors that may influence rate of hematologic recovery in patients receiving CD34\textsuperscript{+} cells.

Although we saw no clear adverse effects of positive stem cell selection, it may have contributed to delay of engraftment in some patients. It is unknown whether immunologic reconstitution occurred in these patients. Two patients with metastatic breast cancer treated with total body irradiation died of idiopathic interstitial pneumonitis (IIP). IIP has also been observed in patients with breast cancer who received unmodified autologous marrow after conditioning with an identical chemoradiotherapy regimen and was felt to be secondary to the high radiation dose rate (40 cGy/min) (C. Collins, personal communication). Consequently, it is unlikely that the infusion of CD34\textsuperscript{+} cells contributed to the development of IIP in these two patients. Patient 3 died of aspergillosis at day 306 while on dexamethasone for an undiagnosed neurologic syndrome but had normal peripheral blood counts, marrow examination, and no evidence of recurrent disease at autopsy.

These studies provide evidence that CD34\textsuperscript{+} human marrow cells can reconstitute in vivo hematopoiesis after marrow ablative therapy. Conclusive proof that they account for long-term hematopoiesis await studies in which the infused cells can be distinguished from recipient cells that may have survived the myeloablativ treatment. Additionally, it is possible that contaminating non-CD34\textsuperscript{+} cells that were also infused may have played a role in engraftment. Work is in progress to improve the selectivity of CD34\textsuperscript{+} cells using the avidin-biotin immunoadsorption procedure.

Although the selection of stem cells may provide a generic approach to obtain engrafting hematopoietic cells, the efficacy of tumor cell depletion using this positive selection technique remains to be determined. Furthermore, the high relapse rates observed in patients receiving allogeneic transplants suggest that current conditioning regimens may not eradicate residual tumor in patients. Until more effective conditioning regimens have been developed, the contribution of residual tumor cells in infused marrow to relapse after autologous marrow transplantation will remain unclear. The clinical role of positive stem cell selection in autologous marrow transplantation therefore will require further study.

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Engraftment after infusion of CD34+ marrow cells in patients with breast cancer or neuroblastoma

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