Combined Transplantation of Allogeneic Bone Marrow and CD34⁺ Blood Cells

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Allogeneic peripheral blood progenitor cells (PBPCs) were transplanted after immunoselection of CD34⁺ cells. Two patient groups were studied: group I patients received immunoselected blood CD34⁺ cells and unmanipulated marrow cells from the same donor. Group II patients were given immunoselected blood and bone marrow (BM) CD34⁺ cells. One to 6 weeks before bone marrow transplantation (BMT), PBPCs from HLA-identical and MLC-sibling donors were mobilized with granulocyte colony-stimulating factor (G-CSF) (5 μg/kg twice daily subcutaneously) for 5 days. Aphereses were performed at days 4 and 5 of G-CSF application. CD34⁺ cells were separated from the pooled PBPC concentrates by immunoabsorption onto avidin with the biotinylated anti-CD34 monoclonal antibody 12.8 and then stored in liquid nitrogen. BM was procured on the day of transplantation. Patients were conditioned with either busulfan (16 mg/kg) or total body irradiation (12 Gy) followed by cyclophosphamide (120 mg/kg). Cyclosporin A and short methotrexate were used for graft-versus-host disease (GVHD) prophylaxis. After transplantation, all patients received 5 μg G-CSF/kg/d from day 1 until greater than 500 neutrophils/μL were reached and 150 U erythropoietin/kg/d from day 7 until erythrocyte transfusion independence for 7 days. Group I consisted of patients with acute myeloid leukemia (AML) (n = 2), chronic myeloid leukemia (CML) (n = 2), and T-γ-lymphoproliferative syndrome and BM aplasia (n = 1). The patients received a mean of 3.3 x 10⁶ CD34⁺ and 3.7 x 10⁶ CD3⁺ cells/kg body weight of PBPC origin and 4.5 x 10⁶ CD34⁺ and 172 x 10⁶ CD3⁺ cells/kg body weight of BM origin. Group II consisted of five patients (two AML, two CML, one non-Hodgkin’s lymphoma). They received a mean of 3.3 x 10⁶ CD34⁺ and 3.2 x 10⁶ CD3⁺ cells/kg from PBPC and 1.4 x 10⁶ CD34⁺ and 0.6 x 10⁶ CD3⁺ cells from BM. A matched historical control group (n = 12) transplanted with a mean of 5.2 x 10⁶ CD34⁺ and 156 x 10⁶ CD3⁺ cells/kg from BM alone was assembled for comparison. In group I, the median time to neutrophil recovery to >100, >500, and >1000/μL was 12, 15, and 17 days, respectively. Patients from group II reached these neutrophil levels at days 13, 15 and 17 post BMT. Neutrophil recovery in the control patient group occurred at days 17, 18, and 20 respectively. Group I patients were given platelet transfusions within 18 days and red blood cells within 10 days, whereas for group II patients, these time points were 26 and 17 days, respectively. These transfusions could be ceased within 38 and 24 days, respectively, in control patients. The addition of about 2% more peripheral blood CD3⁺ cells (group I patients) did not result in higher grades of acute GVHD (median grade II) as compared with the controls (median grade II). Four of five group II patients showed no signs of acute GVHD. These data suggest that the addition of immunoselected allogeneic CD34⁺ progenitor cells to BM cells may accelerate hematopoietic recovery.

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Transplantation of autologous peripheral blood progenitor cells (PBPCs) after myeloablative chemotherapy has shown advantages in comparison with bone marrow transplantation (BMT) alone. An accelerated hematopoietic recovery and a significant shortening of the thrombocytopenic period have been observed.1,2 Furthermore, the use of autologous PBPCs instead of autologous BMT resulted in a reduction in morbidity, supportive care, and period of hospitalization, as well as no need for general anesthesia. These advantages for PBPC transplantation may also be relevant in allogeneic BMT. Transplantation of syngeneic PBPCs,3 some reports of allogeneic PBPC transplantation,4,12 and treatment of poor marrow graft function after allogeneic BMT with PBPCs13 suggest that allogeneic PBPCs could provide durable hematopoietic engraftment. In healthy bone marrow (BM) donors, large numbers of PBPCs can be mobilized with hematopoietic growth factors.3,6,13-17 However, the high content of immunocompetent T cells in apheresis products may expose recipients of allogeneic PBPCs to an elevated risk of acute graft-versus-host disease (aGVHD).3,18,19 Thus, the use of an appropriate T-cell reduction technique, but not depletion technique, might reduce this risk.19,20 The hazards of rejection and of a higher relapse rate should be avoided by maintaining a portion of the T-cells in the graft.20,21 The positive selection of CD34⁺ cells from peripheral blood preparations simultaneously provides an approximately 2- to 3-log reduction of T cells.12,22 Purified CD34⁺ cells containing committed and pluripotent stem cells25-26 may be sufficient for allogeneic transplantation.27

The aim of this study was to determine whether allogeneic CD34⁺ blood progenitor cells may accelerate hematopoietic recovery. Granulocyte colony-stimulating factor (G-CSF) and mobilized and immunoselected CD34⁺ blood cells were given as an adjunct to allogeneic BM in 10 patients. Hematopoietic recovery was analyzed in comparison with a historical control group.

Furthermore, a protocol for stem cell mobilization in healthy BM donors should be developed. The present study should provide data necessary to begin a study with immunoselected CD34⁺ PBPCs for allogeneic transplantation as a substitute for BM.

Patients and METHODS

Informed consent was obtained from patients and donors using the protocols and forms approved by our institution’s ethical committee. Patients. Five patients with hematologic malignancies were con-
ALLOGENEIC BMT WITH BM AND CD34+ BLOOD CELLS

sidered for combined BMT with unmanipulated BM cells and purified blood CD34+ cells. The CD34+ cells were immunoselected from the G-CSF mobilized PBPC preparations of the corresponding BM donors (group I). Five other patients were transplanted with both immunoselection-needed BM and peripheral blood CD34+ cells (group II). A detailed description of the patients' characteristics at the time of BMT is provided in Table 1.

Mobilization and collection of PBPCs. All donors (9 men, 1 women, 28 to 45 years old) except two, were HLA-A, B, C, DR, ABO- and Rh completely identical siblings. Patient E and his donor had a minor ABO blood group incompatibility (donor, O; recipient, A1 [group II]). The donor of patient G (group II) exhibited a mismatch in one HLA-A locus as compared with the recipient (donor, HLA-A 2, 32; recipient, HLA-A 2). The mixed lymphocyte reactions between patients and their respective donors were negative in all cases. G-CSF (filgrastim; Amgen, Munich, Germany) was administered to the donors for PBPC mobilization as follows: 5 μg/kg twice daily subcutaneously for 5 days in 12-hour intervals. Only the morning G-CSF dose (5 μg/kg) was given on day 5. The daily dose was split according to the data of Sheridan et al,1 because of the clearance of G-CSF,28 on the corresponding mobilization schedule and in agreement with our own observations.13 Aphereses were performed via two peripheral blood accesses on days 4 and 5 days of G-CSF application with the Ceprate SC system (Cobe Laboratories, Haimstetten, Germany). The whole donor blood volume was processed three times with a flow of 50 dL/min per apheresis procedure.

BM harvest and transplantation. BM was procured 1 to 7 weeks after PBPC collection, and when indicated after conditioning of the recipient. Erythrocytes were separated from about 1,500 mL of marrow harvest using the COBE 2991 device (COBE Laboratories) and infused into the donor. The BM-nucleated cells were then washed in M199 (Sigma Chemical Co, Deisenhofen, Germany), concentrated (375 mL) and transfused into the recipients via a central venous catheter (group I). Group II patients received noncryopreserved BM-CD34+ cells after selection with the Ceprate SC system (CellPro Inc, Bothell, WA) as described below.

CD34+ blood cell transplantation. Concomitantly with the marrow cells the immunoselected CD34+ PBPCs were thawed in a water bath at 38°C for 5 minutes, diluted slowly to 30 mL with phosphate-buffered saline (PBS) (Dulbecco's without Ca2+ and Mg2+; Gibco-BRL, Eggenstein, Germany) supplemented with 10 U/mL heparin (Heparin novo, Nordisk, Mainz, Germany), and subsequently infused into the recipient.

Table 1. Patients' Characteristics

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<th>Patient</th>
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Patient characteristics at transplantation of hematopoietic stem cells.

Abbreviations: CP, chronic phase; CR, complete remission; PR, partial remission; TBI, 12 Gy fractionated total body irradiation; Bu, busulfan 16 mg/kg; Cy, cyclophosphamide 120 mg/kg.

Selection was performed on all PBPC preparations (patient groups I and II) and on the BM harvest of group II patients. The first PBPC apheresis product was stored at room temperature overnight and pooled with the second apheresis product before further processing. The pooled PBPC concentrates or the BM harvest were washed with PBS. All PBS wash steps were performed at 400g for 10 minutes, with the brake switched off (GS-6KR Beckman centrifuge, Beckman, Munich, Germany). CD34+ cell selections were performed according to the manufacturer's instructions. In brief, the washed cell pellet was resuspended into 100 to 150 mL PBS. Biotinylated IgM anti-CD34 monoclonal antibody (MoAb) (3-M1; vials; CellPro) and 0.7 mL 20% human serum albumin (HSA; DRK - Blutspendedienst, Springe, Germany) were added to the cell suspension. The uncoupled MoAbs were washed out after an incubation at 22°C for 30 minutes. The cells were resuspended gently in about 300 mL PBS and processed through the primed avidin-coated column of the Ceprate SC device. Five milliliters of HSA was added each to one priming-washing PBS bag and to the CD34+ cell container. Finally, the blood CD34+ cells were washed in PBS and concentrated to a density of between 10 and 50 x 10^6 cells/mL in a final solution of 7.5% dimethylsulfoxide (DMSO) and 4% HSA in PBS supplemented with 10 U/mL heparin. The cells were then transferred into 5 mL cryovials (Nunc, Wiesbaden, Germany), controlled frozen (Planer Kryo 10; SI; Messer Griesheim, Krefeld, Germany), and stored in liquid nitrogen until transplantation.

The BM CD34+ cells were washed, resuspended in 50 mL PBS supplemented with 10 U/mL heparin, and injected immediately into the recipient.

Quality control. Nucleated cell and platelet counts, the numbers of CD34+, CD34+, CD44+, CD8, and CD14+ cells, as well as the number of granulocyte-macrophage colony-forming units (CFU-GM) and burst-forming units-erythroid (BFU-E) were monitored in the peripheral blood of all donors as follows: before the start of G-CSF mobilization, on the evening of day 1 (after the second subcutaneous G-CSF injection), each morning on days 2 through 5 immediately before G-CSF application, and on the day of BM harvest. The same parameters were measured in the apheresis products and in the BM harvest, before and after positive selection of CD34+ cells.

Nucleated cells were counted manually in a Neubauer chamber and automatically using a Coulter counter (S Plus IV; Coulter Electronics, Munich, Germany).

Dual-color flow cytometry of CD34+/CD45+ and CD34+/CD3+ events was used to estimate the CD34+ cells with an anti-CD34 phycoerythrin (PE)-conjugated MoAb (QBend 10; Immunotech,
Hamburg, Germany) and fluorescein-isothiocyanate (FITC)-conjugated anti-CD45 (anti-H-Leu-1) or anti-CD3 (anti-H-Leu-4) MoAbs. There was no cross-binding between the QBend 10 (IgG) and the 12.8 (IgM) anti-CD34 MoAbs (data not shown). Samples incubated with anti-CD34 FITC (anti-H-Leu-3a) and anti-CD8 PE (anti-H-Leu-2a) MoAbs were used for compensation on the flow cytometer and for estimation of these T-lymphocyte subsets. The CD4+ cells were detected after incubation with PE-conjugated anti-H-Leu-M3 (Becton Dickinson, Heidelberg, Germany). Subset cell contents of the blood and preparations were assessed on the fluorescence-activated cell scanner (FACScan, Becton Dickinson) as previously described.\textsuperscript{15-17} Briefly, 20,000 to 50,000 events were acquired from cell suspensions that had been preincubated with the corresponding MoAbs, lysed (FACS-Lysing solution; Becton Dickinson) and washed twice in PBS. PC-LYSYS software (Becton Dickinson) was used for dot-plot analysis of the list mode data.

Colony assays for CFU-GM and BFU-E were used as a control for the clonogenic capacity of the CD34+ cells in vitro.\textsuperscript{12,13} The cells were prepared for plating in different ways: 2.5 and 5.0 × 10\(^6\) density-gradient separated mononuclear cells (MNCs) were taken from the samples of the peripheral blood, unmanipulated BM or PBPC concentrates (Ficol-Faque, density 1.077 g/mL, Pharmacia, Uppsala, Sweden), and only 1.0 or 2.0 × 10\(^6\) washed cells were used in the assay with positively selected CD34+ cell suspensions. The samples were suspended in quadruplicate in 1 mL methyl cellulose Progenitor Cell Growth Medium (GIBCO-BRL) supplemented with 2 U/mL erythropoietin (Boehringer Mannheim, Mannheim, Germany) and 5% placenta-conditioned medium in Iscove’s modified Dulbecco’s medium (GIBCO-BRL). Cultures were incubated at 37°C in 100% humidified air with 5% CO\(_2\). Formation of the CFU-GM and BFU-E containing more than 50 cells per colony or burst was enumerated at day 14.

Postthawing recovery and viability of CD34+ were controlled by cell counting and trypan blue dye exclusion. In all instances, the recovery rates were at least 90%.

**Clinical transplantation protocol.** The day of BMT was standardized as day 0. After transplantation, all patients received G-CSF and erythropoietin as follows: G-CSF (filgrastim), 5 μg/kg as short intravenous daily infusion, started at day 1 and continued until more than 500/μL neutrophils were detected for 3 consecutive days; and recombinant human erythropoietin (Recormon, Boehringer, Mannheim, Germany) and 5% placenta-conditioned medium in Iscove’s modified Dulbecco’s medium (GIBCO-BRL). Cultures were incubated at 37°C in 100% humidified air with 5% CO\(_2\). Formation of the CFU-GM and BFU-E containing more than 50 cells per colony or burst was enumerated at day 14.

GVHD prophylaxis consisted of 3 to 5 mg/kg cyclosporin A (CsA; continuous intravenous infusion) adapted to a target whole blood level of 200 ng/mL and methotrexate (MTX): 15 mg/m\(^2\) intravenously at day 1 and 10 mg/m\(^2\) on days 3, 6, and 11 for group I patients. CsA was given orally, as soon as oral mucositis had disappeared, and was stopped by day 180 after transplantation. MTX application for group II patients was reduced to days 1, 3, and 6. Patient G was treated additionally with 1 mg/kg prednisolone, because of the HLA-A mismatch. Supportive care included isolation in laminar air-flow rooms and total antimicrobial decontamination. Pentamidine-isethionate (Pentacarinat; Rhöne-Poulenc-Rorer, Cologne, Germany) inhalations were used as prophylaxis for *Pneumocystis carinii* pneumonia instead of trimethoprim/sulfamethoxazole as published previously.\textsuperscript{18} Five hundred milligrams acyclovir per square meter intravenously three times daily (Zovirax Wellcome, Großburgwedel, Germany) was given prophylactically between day 1 and 30. Red blood cell (RBC) transfusions were given to maintain a hemoglobin level above 80 g/L and platelet transfusions were used to keep platelet counts above 20,000/μL. Blood cells were counted daily.

Time to engraftment was assessed by determining the number of days after transplantation for patients to achieve 100, 500, and 1,000 neutrophils/μL, 50,000 platelets/μL and 10,000 reticulocytes/μL. The numbers of RBC and platelet transfusions and the time to transfusion-independence were also evaluated. Control BM aspirations were routinely performed between days 8 and 28 posttransplant. The CD34+ cell content of BM was investigated in the BM aspirates of seven patients. Restriction fragment length polymorphism (RFLP) analyses of patients’ BM and peripheral blood nucleated cells were performed to prove successful engraftment more than 3 weeks post-BMT.\textsuperscript{19}

**Control group.** An appropriate historical control group of 12 patients (median age, 35 years; 8 males, 4 females) with similar diagnoses and BM transplantation regimens was assembled for comparison. Patients were selected for having received the same protocol for stimulation of hematopoiesis with G-CSF and erythropoietin and for the availability of data of the CD34+ cell content in the BM. They had been treated consecutively before the start of this study. Thus, the number of control patients is low. According to the underlying disease, the BMT preparative regimens were as follows: busulfan, 16 mg/kg, and cyclophosphamide, 120 mg/kg, for three patients with acute myeloid leukemia (AML), four patients with chronic myeloid leukemia (CML), and one patient with paroxysmal nocturnal hemoglobinuria; 12 Gy fractionated total body irradiation (TBI) and cyclophosphamide 120 mg/kg for one patient with multiple myeloma; TBI and VP16 60 mg/kg for one patient with non-Hodgkin’s lymphoma and one patient with acute lymphoblastic leukemia; and total nodal irradiation (8 Gy) and cyclophosphamide 200 mg/kg for one patient with severe aplastic anemia. With regard to CsA and MTX for GVHD prophylaxis the same protocols were used as for group I. G-CSF and erythropoietin therapy (see above), supportive care and clinical transplantation protocols were identical for the control group as for groups I and II.

**RESULTS**

**PBPC mobilization, BM harvest, and CD34+ cell immuno-selection.** G-CSF application produced bone pain and headache in seven donors that was relieved with analgesics. Cell separation was well tolerated. The blood leukocyte counts of the donors increased from a mean of 6.4 × 10\(^9\) μL before the onset of mobilization to 47.9 and 44.7 × 10\(^9\) μL at days 4 and 5 of G-CSF application (7.5- and 5-fold increase). Simultaneously, the MNC counts increased from a mean basal level of 2.2 × 10\(^9\) μL to 6.9 × 10\(^9\) μL (threefold increase). No significant changes in platelets counts were obtained under G-CSF treatment and before completing the first apheresis procedure at day 4. Platelet counts were below 200 × 10\(^3\)μL after both aphereses, but did not decrease under 150 × 10\(^3\)μL. The exact kinetics of CFU-GM, BFU-E, and CD34+ cell numbers during and after mobilization are depicted in Fig 1. The number of circulating CD34+ cells increased about 13.5- and 18-fold on mobilization days 4 and 5, respectively. The measured increase was 43- and 61-fold for CFU-GM and 22.5- and 24.5-fold for BFU-E on the same days.

BM was harvested at a median of 39 days (range, 10 to 57) after the start of G-CSF mobilization. The interval between the last G-CSF injection and the marrow harvest was 10 to 12 days, respectively, in only two donors. In all other donors, the interval was longer (range, 23 to 51 days). The counts of the monitored blood cell subgroups were normalized to the reference ranges for healthy individuals during...
that time: mean, $8.9 \pm 3.3 \times 10^3$ leukocytes/$\mu$L; mean, $2.6 \pm 1.3 \times 10^4$ MNCs/$\mu$L; and mean $298 \pm 60 \times 10^3$ platelets/$\mu$L.

In the blood, the mean values of $6.4 \pm 1.3$ CD34$^+$ cells/$\mu$L, 0.4 CFU-GM/$\mu$L, and 0.8 BFU-E/$\mu$L were still about two times higher than before application of G-CSF, but lower than the peak mobilization levels ($P = .01, .13$ and .038, see Fig 1). The BM samples from the two individuals in whom the harvest was performed shortly after the start of mobilization (10 and 12 days) contained more granulocytes than the others (89% v 67.5%). The total number of nucleated cells procured was approximately three times that of others, and thus, the CD34$^+$ cell, CFU-GM, and BFU-E yields were similar.

The mean body weight of the 10 recipients was 73.5 kg (70.6 kg for group I patients and 76.4 kg for group II patients). The single aphereses PBPC ($n = 20$) contained a mean (±SD) of $4.0 \pm 1.6 \times 10^6$ nucleated blood cells, 89% of which were MNCs. The total numbers (mean ±SD) of MNC subsets were as follows: $3.9 \pm 3.1 \times 10^6$ CD34$^+$ cells, $3.2 \pm 2.2 \times 10^7$ CFU-GM, $4.6 \pm 3.2 \times 10^7$ BFU-E, $1.5 \pm 0.6 \times 10^9$ CD3$^+$ cells with a CD4 to CD8 cell ratio of 1.67, and $1.2 \pm 0.6 \times 10^9$ CD14$^+$ cells. A description of cells transplanted from blood and BM is given in Table 2.

The whole processing of the PBPC concentrates, including overnight storage, pooling, multiple washes, and immunoselection, resulted in an yield of CD34$^+$ cells of approximately 30% ($n = 10$). The PBPC preparations for transplantation contained a mean percentage of 70% CD34$^+$ cells (see Table 2). The yields for CFU-GM and BFU-E were 25% and 17%, respectively. The peripheral blood CD3$^+$ cells were reduced from $2.04 \times 10^6$ cells/kg body weight by 3 log to 3.7 to $3.2 \times 10^5$ cells/kg as well as the corresponding CD14$^+$ cells. The CD4$^+$ versus CD8$^+$ ratio was converted to 0.7. The transplanted, unmanipulated BM of group I had a CD4$^+$ to CD8$^+$ ratio of 0.8. In group I, about 42% of the transplanted CD34$^+$ cells and 2% of the transplanted CD3$^+$ cells were of peripheral blood origin (Table 2).

The harvested, unmanipulated BM for group II patients had a CD4$^+$ versus CD8$^+$ cell ratio of 1.26.

The immunoselection yielded 48% of the CD34$^+$ BM cells with a mean purity of 66%. These values for the yields of CFU-GM and BFU-E were 96% and 80%, respectively. The CD4$^+$ versus CD8$^+$ cell ratio was slightly elevated to 1.36. A CD3$^+$ and CD14$^+$ cell reduction of about 2 log was obtained. Group II patients received a mean of 70% CD34$^+$ cells and 84% CD3$^+$ cells from the PBPCs (Table 2).

**Patient data** The data on hematopoietic recovery for individual patients is displayed in Table 3.

All 10 patients who received additional CD34$^+$ PBPCs were compared with the control patients with respect to the time taken to reach 100, 500, and 1,000 neutrophils/$\mu$L ($P = .03, .09, and .52$, respectively).

The $P$ values for the time to reach 50,000 platelets/$\mu$L and to become transfusion independent were .5 and .08 respectively.

In both group I and II, two of five patients had major infections after BMT. Although patient A recovered promptly from myelopoesis, the patient developed *Toxoplasmosis* pneumonia and died of adult respiratory distress syndrome and multiorgan failure 45 days posttransplant. Patient E also...
had a complicated clinical course. Trilineage hematopoiesis developed veno-occlusive liver disease at day 30 post-BMT. GVHD of the skin, gut, and liver between days 17 and 45; recovered within controls.

Controls*  

| Group I    | A     | 9.38 | 7.13 | 5.81 | 49.53 | 49.53 | 744.0 | 8.18 | 230.6 | 6.73 | 15.10 |
|           | B     | 2.28 | 1.93 | 1.05 | 3.73 | 4.78 | 224.4 | 2.69 | 174.9 | 4.04 | 7.67 |
|           | C     | 6.08 | 4.93 | 4.56 | 31.14 | 18.98 | 142.9 | 3.86 | 84.26 | 4.72 | 8.20 |
|           | D     | 2.48 | 1.69 | 4.74 | 11.11 | 8.33 | 426.8 | 3.43 | 302.1 | 9.88 | 15.03 |
|           | E     | 1.58 | 0.77 | 2.38 | 8.32 | 1.26 | 461.5 | 4.15 | 69.23 | 7.08 | 13.15 |

Group II  

| F         | 3.27 | 1.91 | 2.81 | 16.76 | 15.45 | 3.09 | 1.70 | 0.77 | 7.05 | 10.88 |
| G         | 3.26 | 1.19 | 6.07 | 19.83 | 13.04 | 2.22 | 1.58 | 1.02 | NA   | NA   |
| H         | 5.62 | 4.89 | 1.01 | 27.01 | 23.40 | 1.63 | 1.12 | 0.10 | 7.81 | 3.25 |
| I         | 2.91 | 2.38 | 2.39 | 23.77 | 19.34 | 2.38 | 1.78 | 0.50 | 5.14 | 6.47 |
| J         | 7.52 | 6.28 | 3.5  | 27.66 | 52.91 | 1.69 | 0.99 | 0.49 | 2.59 | 3.45 |

| Controls* | Mean ± SD | 4.38 ± 3.3 | 3.29 ± 2.6 | 3.71 ± 1.9 | 20.8 ± 19.2 | 16.7 ± 19.5 | 403.3 ± 235 | 4.46 ± 2.2 | 172.3 ± 98 | 6.5 ± 2.3 | 11.8 ± 3.6 |

Abbreviation: NA, not available.

*Corresponding control group data (n = 12).

Table 2. Characterization of Blood and BM Cells

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<td>23.77</td>
<td>19.34</td>
</tr>
<tr>
<td>J</td>
<td>7.52</td>
<td>6.28</td>
<td>3.5</td>
<td>27.66</td>
<td>52.91</td>
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Table 3. Hematopoietic Recovery, Transfusions, and Acute GVHD

<table>
<thead>
<tr>
<th>Days to Reach (per μL)</th>
<th>Neutrophils</th>
<th>Platelets</th>
<th>Reticulocytes</th>
<th>Days to Last Transfusion</th>
<th>Transfusions</th>
<th>Acute GVHD, Grade</th>
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<tr>
<td>Patients</td>
<td>100</td>
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<td>Group I</td>
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<tr>
<td>A</td>
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</tr>
<tr>
<td>E*</td>
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<td>14</td>
<td>32</td>
<td>23</td>
<td>13</td>
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</tr>
<tr>
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<td>15</td>
<td>17</td>
<td>24</td>
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<td>12-33</td>
<td>20-112</td>
<td>15-35</td>
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</table>

Engraftment and GVHD characteristics of the patients and the corresponding control data (n = 12). The numbers of patients with each GVHD grade (0-IV) were as follows: 0, no patients; 1, five patients; 2, three patients; 3, three patients; IV, one patient. Compared with the historical controls, the severity of acute GVHD is reduced in the 10 patients with CD34⁺ PBPCs (P = .02).

Abbreviation: NR, not reached (early death).

* All patients received single-donor apheresis platelet concentrates, each of them containing approximately 8 U of 0.5 × 10¹¹ platelets.

† Patient E developed CMV infection and VOD and became platelet transfusion dependent between days 30 and 62.
The diagnostic BM aspirations proved trilineage repopulation of the BM in all group I patients at days 14 and 28. The CD34+ cell percentages of the BM of patient C at day 9 (1.8%), patient D at day 28 (0.85%), and patient E at days 20 (0.81%) and 32 (2.73%) showed almost normal values. Complete engraftment was also proved by RFLP-analysis with donor DNA pattern of BM and blood nucleated cells. As of April 25, 1995, the four living patients are in complete remission and have stable hematologic parameters between 10 and 13 months posttransplant.

In group II, the hematopoietic recovery of patient G probably was impaired by preemptive ganciclovir therapy for CMV antigenemia from day 24 to day 37. His transplanted hematopoiesis eventually recovered 4 months after transplant to normal and stable blood values.

In group II, patient I did not receive MTX because of the increase in his liver enzymes after conditioning at day 1.

Only one patient from group II (patient H) developed grade II acute GVHD of the skin, gut, and liver between days 22 and 25.

The diagnostic BM aspirations proved trilineage repopulation of the BM in patients F, H, and J after day 14. Patient F had 0.33% and 0.75% BM CD34+ cells at days 14 and 34, and patient H had 0.65% at day 14. The CD34+ cells in the BM of patient I were evaluated only at day 9 (4.73%). Engraftment in all group II patients was proven by RFLP analysis. They showed the donor DNA pattern of BM and blood nucleated cells. As of April 25, 1995, the five patients are in complete remission with stable hematologic parameters, 6 to 9 months posttransplant.

DISCUSSION

This study showed that the addition of allogeneic purified CD34+ cells from peripheral blood to BM cells accelerates hematopoietic recovery. This is in line with the experience in autologous transplantation, where the time to recovery of hematopoiesis is reduced if autologous PBPCs are used. One case report on graft failure treatment43 strongly suggested that allogeneic PBPCs could restore BM function. Another report presented data on a patient who was transplanted successfully with allogeneic G-CSF mobilized PBPCs instead of marrow.44 No signs of severe GVHD were observed, although the allogeneic PBPCs transplanted in both cases were not additionally manipulated. In a recent report, we were able to show that immunoaffinity-selected CD34+ blood cells were also able to restore hematopoiesis in two patients with poor marrow graft function, without aggravating GVHD.13

Three recent papers described the successful engraftment of allogeneic PBPCs alone.10-12

In our present study, more than 2 × 10^6 CD34+ cells and more than 15 × 10^6 CFU-GM and BFU-E/kg body weight of the recipients were harvested with each PBPC apheresis. Some reports suggest that 4 × 10^6 CD34+ cells/kg body weight are necessary for durable hematopoietic engraftment.35

The critical number of allogeneic CD34+ cells from peripheral blood for durable engraftment is not yet known. However, if the cell numbers from BM are calculated for comparison, then 2 × 10^6 nucleated cells/kg body weight are considered to be sufficient.35 In our study, the marrow preparations contained approximately 1% CD34+ cells, corresponding to 2 × 10^6 CD34+ cells/kg. In autologous stem cell transplantation, the standard number of CD34+ cells for reliable hematopoietic recovery is 2.0 to 5.0 × 10^6/kg.36-38 If one accepts the CD34+ marker as an indicator for the stem cell and progenitor cell fraction, then this number of CD34+ blood stem cells would be sufficient.39 However, it is not clear yet whether this figure is also valid for allogeneic PBPC transplantation, and others assume 5 to 15 × 10^6 CD34+ cells/kg to be valid.12

Without mobilization by growth factors, the levels of nonmobilized circulating CFU-GM, BFU-E, and CD34+ cells are low and collection of adequate quantities of progenitor cells takes a long time with multiple apheresis procedures being necessary. Only 1 to 5 CD34+ cells/μL peripheral blood of healthy individuals are present at steady state hematopoiesis. If an average male adult is considered to have a whole blood volume of about 6 L and an average female adult about 5 L, then the total number of circulating CD34+ cells can be extrapolated. Thus, between 4 and 7 × 10^6 CD34+ cells (about 0.7 × 10^6 CD34+ cells/kg of a recipient weighing 70 kg) should be collected by apheresis; the assumptions being that three times the whole blood volume is processed, the efficacy of the collection is 100%, and the levels of the circulating CD34+ cells remain unchanged or even increase. If such ideal conditions were possible, a minimum of six aphereses would have to be performed to procure the required number of PBPCs. Taking into account that the further processing of such a preparation would be associated with a certain loss of progenitors, more than 10 collection procedures would theoretically have to be performed to achieve results similar to those suggested by our data. This hypothesis was proven practically by Kessinger et al who performed 10 aphereses to collect enough PBPCs for allogeneic transplantation.3 Since then, more data have been gathered on G-CSF mobilization of PBPCs. With respect to the definition of the progenitor cells, Tjønnfjord et al9 have analyzed six healthy volunteers treated with 10 μg/kg/d G-CSF using a large panel of lymphohematopoietic markers. The peak number of circulating CD34+ cells, CFU-GM, and BFU-E were detected at days 4 and 5 of G-CSF mobilization. Another recent study showed the effectiveness of G-CSF for 4 days in the mobilization of CD34+ PBPC in normal subjects.17 Sufficient quantities of PBPCs for allogeneic transplantation could be collected by leukapheresis.17 Our data on total leukocyte, CFU-GM, BFU-E, and CD34+ cell counts in the peripheral blood and on the yields of PBPC aphereses in our donors were concordant with published data.1,3,4,15,17,34 It was also proven that the hematopoiesis parameters studied normalized within 2 to 3 weeks of completing G-CSF mobilization.

Bone pain and myalgia were the major and often only side effects of G-CSF application both in patients and healthy individuals.3,4,15,17,34 In this study, in addition to headache and mouth dryness, bone pain was also a leading side effect of G-CSF application in the donors. Our results confirm the suitability of G-CSF for PBPC mobilization, but the lack of acute toxicities of G-CSF cannot yet exclude unknown long-term or late complications.

Processing the whole blood volume of a G-CSF mobilized
individual three times usually produced a greater than $2 \times 10^{10}$ leukocyte yield with a mean CD3$^+$ cell percentage of 37%. The procured BM contained about 5% CD3$^+$ cells by similar cell yield of $2.7 \times 10^{10}$ nucleated cells. In addition to the data presented here, we have monitored more than 300 autologous apheresis products and each BM harvest performed at our institution for the purposes of other studies. The mean number of PBPC concentrates was greater than $2 \times 10^{10}$ nucleated cells per apheresis. Approximately 30% of them were CD3$^+$ cells. The CD3$^+$ cell proportion in the BM harvests was always lower than 7% from a mean of 1.5 $\times 10^6$ cells (own unpublished data). Gathering two PBPC apheresis products resulted in approximately 10 times more CD3$^+$ cells compared with a standard BM harvest. Thus, for a 75-kg adult patient, a graft composed of two such PBPC concentrates would contain roughly more than $2 \times 10^6$ CD3$^+$ cells/kg, which could increase the incidence of GVHD. Moreover, several authors suggested that the infusion of BM cell preparations containing more than $1 \times 10^6$ T cells/kg increases the risk of GVHD. The mean number of PBPC concentrates would be more than 2 $\times 10^6$ CD3$^+$ cells/kg, which could increase the incidence of GVHD. Moreover, several authors suggested that the infusion of BM cell preparations containing more than $1 \times 10^6$ T cells/kg increases the risk of GVHD. The mean number of PBPC concentrates would be more than 2 $\times 10^6$ CD3$^+$ cells/kg, which could increase the incidence of GVHD.

The immunoselection of CD34$^+$ cells by the Ceprate SC procedure is a simple technique for the immunoaffinity selection of cells definitively expressing the CD34 antigen. This marker is predominantly presented on the cell membrane of hematopoietic progenitors, containing the pluripotent stem cell. The blood T cells were reduced to 0.1 to 0.5 $\times 10^6$ cells/kg recipient's body weight in 8 of 10 procedures, and below $1 \times 10^6$ in all procedures. Similar results have been published recently by another group. The CD3$^+$ cells were always reduced below $0.5 \times 10^6$ kg in the immunoaffinity selection procedures performed with the BM harvest. At 1 $\times 10^6$ T cells/kg body weight was found to be necessary for maintaining the graft versus leukemia (GVL) effect and for preventing graft failure or rejection. These data have been corroborated prospectively in a recent clinical trial. The positive selection of CD34$^+$ cells with a concomitant reduction of T cells to the number desired, may reduce both the GVHD and rejection risks and preserve the GVL effect. Further clinical trials are necessary to prove this hypothesis and the possible advantage of CD34$^+$-selected PBPC.

The combined transplantation of immunoselected CD34$^+$ blood cells with BM resulted in a prompt engraftment in 9 of 10 patients. It is not possible to determine definitively whether this acceleration of hematopoietic reconstitution was because of the CD34$^+$ blood cell transfusion, since no labeling of the CD34$^+$ cells was performed. However, there was a clear trend to shorten cytopenia and duration of transfusion dependence as compared with the control group. Others have shown that after syngeneic transplantation of PBPCs, the median hematopoietic engraftment time for neutrophil recovery to greater than 500/μL was 13 days. In patients with haploidentical BM transplants, the addition of PBPC resulted in a rapid hematopoietic recovery. In our 10 patients, recovery was achieved at 15 days as compared with 18 days for control patients. An acceleration of erythropoiesis and thrombopoiesis was also suggested. Thus, transplantation of allogeneic PBPCs may be even more advantageous for these cell lineages.

After termination of G-CSF support, leukocyte counts remained above $1 \times 10^9$/L. Control BM aspirations ascertained repopulation of the marrow and the trilineage engraftment. Furthermore, the circulating and BM nucleated cells were proven to be of donor origin. It is unlikely that the early death of the first patient (group I) was caused by pancytopenia or progenitor cell infusion. The other four group I patients are still in remission with stable hematologic parameters. No differences in GVHD incidence compared with the controls were seen in either case.

We conclude that the selection of hematopoietic progenitor cells from peripheral blood provides one approach to obtain engrafting hematopoietic cells. The presented data suggest that CD34$^+$ cells, immunoselected from the PBPCs of the allogeneic HLA-identical sibling donors, may be appropriate for transplantation in place of BM. However, the risk of severe acute or chronic GVHD by PBPC or immunoselected CD34$^+$ blood transplantation is unknown. We are now investigating whether immunoselected allogeneic CD34$^+$ PBPCs alone can engraft as BM and accelerate hematopoietic recovery as shown in autologous transplantation.

**ACKNOWLEDGMENT**

We thank Martin Zaki and the nursing staff of the Blood Bank of our institution for the excellent quality of the PBPC apheresis procedures, Dirk Löhmann and Dr Wolfram Ebell for their technical assistance, and the nursing staff of the BMT unit.

**REFERENCES**


ALLOGENEIC BMT WITH BM AND CD34+ BLOOD CELLS


39. To LB, Roberts MM, Haylock DN, Dyson PG, Branford AL, Thorp D, Ho JQ, Dart GW, Horvath N, Davy ML, Olweny CLM, Abdi E, Juttner CA: Comparison of haematological recovery times and supportive care requirements of autologous recovery phase peripheral blood stem cell transplants, autologous bone marrow transplants and allogeneic bone marrow transplants. Bone Marrow Transplant 8:277, 1992


Combined transplantation of allogeneic bone marrow and CD34+ blood cells

H Link, L Arseniev, O Bahre, RJ Berenson, K Battmer, JG Kadar, R Jacobs, J Casper, J Kuhl and J Schubert

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