Primary Transplantation of Allogeneic Peripheral Blood Progenitor Cells Mobilized by Filgrastim (Granulocyte Colony-Stimulating Factor)

By Norbert Schmitz, Peter Dreger, Meinolf Suttorp, Ernst B. Rohwedder, Torsten Haferlach, Helmut Löffler, Ann Hunter, and Nigel H. Russell

Transplantation of allogeneic peripheral blood progenitor cells (PBPCs) may have advantages over bone marrow transplantation (BMT) with regard to the speed of hematopoietic and immunologic recovery, which may then shorten the time spent in hospital and decrease costs. The recipient might also profit by an enhanced graft-versus-leukemia reaction exerted by the high number of natural killer cells contained in such grafts. The donor could be spared the discomfort and risks of general anesthesia and marrow harvesting. Primary transplantation of unmanipulated allogeneic PBPCs has not been reported so far because the vast amount of T cells contained in the collection product was thought to cause severe graft-versus-host disease. We present preliminary data on primary transplantation of allogeneic PBPCs in patients who either suffered from advanced leukemia or had a donor unable to undergo general anesthesia. Eight patients with a median age of 42 years suffering from acute myelogenous leukemia (AML) in first remission (n = 2), AML in second remission, AML in relapse (n = 2), acute lymphoblastic leukemia in second remission, or chronic myelogenous leukemia in accelerated phase received myeloablative therapy followed by transplantation of unmanipulated allogeneic PBPCs mobilized with granulocyte colony-stimulating factor (5 to 10 µg/kg of body weight of filgrastim administered for 5 to 6 days) in their HLA-identical donors. Hematopoietic reconstitution was achieved in all patients with a median of 15.5 (18.5) days after transplant needed to surpass an absolute neutrophil count of 0.5 (1.0) x 10⁹/L. The median time to an unsupported platelet count greater than 20 (>50) x 10⁹/L was 19.5 (41) days after grafting. Three patients did not exhibit signs of acute graft-versus-host disease (GVHD), grade I disease was seen in one patient, and three patients experienced grade II disease limited to the skin. The only patient with severe acute GVHD (grade III) refused to take his oral cyclosporin regularly and had ineffective serum levels for most of the time until relapse. Six of eight patients are currently alive without evidence of disease between 61 and 533 days after grafting; two patients grafted for AML in relapse achieved a complete remission after transplantation but relapsed again and died of leukemia on days +48 and +70, respectively. Primary transplantation of unmanipulated allogeneic PBPCs is feasible and results in long-term engraftment without causing detrimental GVHD.

© 1995 by The American Society of Hematology.

From the Departments of Internal Medicine II and Pediatrics, University of Kiel, Kiel, Germany; and the Department of Haematology, Nottingham City Hospital, Nottingham, UK.

Submitted September 10, 1994; accepted November 10, 1994.

Address reprint requests to Norbert Schmitz, MD, Department of Internal Medicine II, Christian-Albrechts-Universität Kiel, Chemnitzstr. 33, 24116 Kiel, Germany.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C section 1754 solely to indicate this fact.

© 1995 by The American Society of Hematology.

to both recipients and donors. In particular, the possibility of using BM instead of PBPCs was discussed with those four patients having a donor able to undergo general anesthesia. Written informed consent was obtained from the patients and their donors according to institutional guidelines.

Mobilization, collection, and transplantation of PBPCs. Mobilization and harvesting of PBPCs in healthy donors of syngeneic and allogeneic grafts has recently been described in detail. Briefly, 5 to 10 μg/kg of G-CSF (filgrastim; Amgen, Munich, Germany) was administered to the donors by subcutaneous injection for 5 or 6 consecutive days (Table 2). Leukapheresis was started on day 5 or 6 of filgrastim administration using a Baxter (Munich, Germany) CS-3000 cell separator. Nine to ten liters of blood was processed daily at flow rates of 30 to 60 mL/min using cubital veins in three donors and central-venous access in the other five. At the University of Kiel, collection products were cryopreserved and stored in liquid nitrogen until used.

Immunophenotyping. Immunophenotypical analysis of blood and apheresis samples has been described elsewhere. Briefly, mononuclear cells (MNCs) were suspended with phycoerythrin (PE)- and fluorescein isothiocyanate (FITC)-conjugated specific antibodies (Dako, Hamburg, Germany) in phosphate-buffered saline containing 0.28% sodium azide. After 30 minutes of incubation and fixation with 1% formaldehyde, flow cytometry was performed with a FACScan flow cytometer (Becton Dickinson, Heidelberg, Germany). The antibodies used were 8G12-PE (HPCA-II), HLe-1-FITC (CD45), Leu-19-PE (CD56; all from Becton Dickinson), and UCHT1-FITC (CD3; Dako). For defining nucleated mononuclear cells (MNCs), a gate was established using a forward scatter/FITC dot plot. CD34+ cells were measured in a right-angle light scatter/CD34-FITC dot plot. Absolute numbers of lymphocyte subpopulations were determined by flow cytometry.

**Table 1. Clinical Characteristics and Outcome of Patients Grafted With Allogeneic PBPCs**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/Sex</th>
<th>Diagnosis</th>
<th>Regimen</th>
<th>Prophylaxis</th>
<th>GVHD Prophylaxis</th>
<th>ANC &gt;0.5 × 10^9/L</th>
<th>Platelets &gt;20 × 10^9/L</th>
<th>Maximum aGVHD</th>
<th>Last Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>No RC</td>
<td>45/M</td>
<td>ALL CR2</td>
<td>TBI (12 Gy)</td>
<td>CyA</td>
<td>MTX (d 1-11)</td>
<td>21/23</td>
<td>39/49</td>
<td>0</td>
<td>A &amp; W, no chronic GVHD d = 533</td>
</tr>
<tr>
<td>Ki SH</td>
<td>41/M</td>
<td>CML AP</td>
<td>BUS (16)</td>
<td>CyA</td>
<td>MTX (d 1-6)</td>
<td>25/31</td>
<td>20/28</td>
<td>II (skin only)</td>
<td>Alive with limited chronic GVHD d = 289</td>
</tr>
<tr>
<td>Ki BR</td>
<td>27/M</td>
<td>AML CR3</td>
<td>BUS (16)</td>
<td>CyA</td>
<td>MTX (d 1-11)</td>
<td>12/13</td>
<td>11/16</td>
<td>I</td>
<td>Alive with hepatitis C, no chronic GVHD d = 243</td>
</tr>
<tr>
<td>Ki DN</td>
<td>18/F</td>
<td>AML CR1</td>
<td>BUS (16)</td>
<td>CyA</td>
<td>MTX (d 1-11)</td>
<td>14/16</td>
<td>19/46</td>
<td>0</td>
<td>A &amp; W, no chronic GVHD d = 207</td>
</tr>
<tr>
<td>Ki PR</td>
<td>33/M</td>
<td>AML REL</td>
<td>BUS (16)</td>
<td>CyA</td>
<td>MTX (d 1-6)</td>
<td>15/16</td>
<td>14/57</td>
<td>II (skin only)</td>
<td>Relapsed d +42, died d +73</td>
</tr>
<tr>
<td>No SM</td>
<td>44/M</td>
<td>AML REL</td>
<td>BUS (16)</td>
<td>CyA</td>
<td>MTX (d 1-11)</td>
<td>23/25</td>
<td>28/34</td>
<td>III (skin &amp; gut)</td>
<td>Relapsed d +45, died d +48</td>
</tr>
<tr>
<td>Ki DG</td>
<td>46/F</td>
<td>AML CR1</td>
<td>BUS (16)</td>
<td>CyA</td>
<td>MTX (d 1-6)</td>
<td>15/15</td>
<td>16/23</td>
<td>II (skin only)</td>
<td>A &amp; W, no chronic GVHD d = 194</td>
</tr>
<tr>
<td>Ki GW</td>
<td>40/M</td>
<td>AML CR1</td>
<td>BUS (16)</td>
<td>CyA</td>
<td>MTX (d 1-6)</td>
<td>16/17</td>
<td>39/16</td>
<td>0</td>
<td>A &amp; W, no GVHD d = 61</td>
</tr>
</tbody>
</table>

**Table 2. Donor and Allograft Characteristics**

<table>
<thead>
<tr>
<th>Donor for Patient</th>
<th>Donor Age/Sex</th>
<th>G-CSF Dose (μg/kg)*</th>
<th>No. of Apheresis Procedures</th>
<th>Total Apheresis Volume (L)</th>
<th>MNC ×10^6/kg*</th>
<th>CFU-GM ×10^3/kg*</th>
<th>BFU-E ×10^3/kg*</th>
<th>CD34+ ×10^3/kg*</th>
<th>CD3+ ×10^3/kg*</th>
<th>CD56+CD3+ ×10^3/kg*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No RC</td>
<td>52/M</td>
<td>10</td>
<td>2</td>
<td>18</td>
<td>7.6</td>
<td>36.8</td>
<td>ND</td>
<td>7.5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ki SH</td>
<td>67/M</td>
<td>10</td>
<td>3</td>
<td>30</td>
<td>12.7</td>
<td>11.5</td>
<td>30.0</td>
<td>2.7</td>
<td>317</td>
<td>127</td>
</tr>
<tr>
<td>Ki BR</td>
<td>18/F</td>
<td>5†</td>
<td>3</td>
<td>30</td>
<td>7.2</td>
<td>19.5</td>
<td>53.7</td>
<td>3.0</td>
<td>418</td>
<td>62</td>
</tr>
<tr>
<td>Ki DN</td>
<td>20/M</td>
<td>7.5</td>
<td>2</td>
<td>20</td>
<td>14.3</td>
<td>46.3</td>
<td>28.8</td>
<td>7.7</td>
<td>362</td>
<td>143</td>
</tr>
<tr>
<td>Ki PR</td>
<td>28/M</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>6.0</td>
<td>47.4</td>
<td>63.3</td>
<td>6.9</td>
<td>137</td>
<td>23</td>
</tr>
<tr>
<td>No SM</td>
<td>44/M</td>
<td>10</td>
<td>2</td>
<td>20</td>
<td>9.6</td>
<td>30.0</td>
<td>ND</td>
<td>8.1</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ki DG</td>
<td>48/M</td>
<td>10</td>
<td>2</td>
<td>20</td>
<td>11.6</td>
<td>ND</td>
<td>ND</td>
<td>6.5</td>
<td>541</td>
<td>126</td>
</tr>
<tr>
<td>Ki GW</td>
<td>44/M</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>4.3</td>
<td>9.8</td>
<td>13.2</td>
<td>2.2</td>
<td>195</td>
<td>30</td>
</tr>
</tbody>
</table>

Abbreviations: AP, accelerated phase; REL, relapse; A & W, alive and well; NR, not reached; R, recipient; D, donor.

* Values for CY and BUS are in milligrams per kilogram of body weight.

† No regular oral intake.

Abbreviation: ND, not determined.

* Body weight.

† Ten micrograms per kilogram was administered on day 6 (after the second leukapheresis).
The analysis of chimerism in the PB and BM after transplantation of allogeneic PBPCs by DNA fingerprinting. D, full donor chimerism; D/R, donor and recipient cells detectable.

calculated from the percentage of positive cells in a gate that contained virtually all CD3+, CD19+, and CD56+ lymphocytes using the corresponding differential blood count.

Progenitor cell assays. CFU-GM were grown by plating 1 × 10^5 MNCs in 0.3% agar culture medium containing 20% fetal calf serum and 5% human placenta-conditioned medium in supplemented Iscove's modified Dulbecco's medium (IMDM). Colonies were counted after 14 days of incubation in a humidified atmosphere of 5% CO_2 at 37°C. For burst-forming units-erythroid (BFU-E), MNCs were plated in methylcellulose containing 30% FCS, purified human erythropoietin (Terry Fox Laboratories, Vancouver, British Columbia, Canada), and PHA-leukocyte-conditioned medium as supplements.

Lymphohematopoietic chimerism. Tests for chimerism were performed in seven patients. No samples were available from patient NoSM. Ten milliliters of peripheral blood (PB) or 3 to 5 mL of BM aspirate were collected between 12 and 489 days after grafting (Fig 1). Genomic DNA was isolated from unfractionated nucleated cells by phenol-chloroform extraction. Ten micrograms of DNA was digested with restriction enzyme HindII, subjected to agarose gel electrophoresis, and hybridized in the gel with either probe OA-Y-22 or (GTG)_5. The sequences of the microsatellite multilocus probe (GTG)_5 and the minisatellite single-locus probe OA-Y-22 have been published.16 Probes were synthesized by commercial suppliers. Radioactive 5'-end labeling of probes with [γ-32P]-ATP (Amersham, Braunschweig, Germany) was performed using a T4-polynucleotide kinase reaction kit (Boehringer, Mannheim, Germany). After hybridization, the gel was washed at 42°C in 6× SSC buffer for 2 minutes in the case of probe (GTG)_5 and at 60°C when probe OA-Y-22 was used. Bands were visualized by autoradiography. Mixing experiments with varying concentrations of donor and recipient DNA had shown that the sensitivity for detection of a minor DNA fraction was less than 10% with probe (GTG)_5 and less than 0.5% with probe OA-Y-22.16 Except in patient NoRC, for whom no pretransplant sample was available, multilocus probe (GTG)_5 gave distinct donor and recipient bands in all cases. Monolocus probe OA-Y-22 was fully informative in patients KISH, KiBR, and KiDN, but gave only a recipient-specific band in patient KiPR and a donor-specific band in patient KiDG. This probe was not used in patient KIGW. Engraftment could be evaluated by probe OA-Y-22 in all patients except patient KiPR. Residual host cells after grafting would have been detected by this probe in all patients except KIGW.

Conditioning regimens. Patient NoRC received a combination of total body irradiation (6× 2 Gy, 2 fractions per day, total dose 12 Gy) followed by cyclophosphamide (60 mg/kg on 2 consecutive days). Patient KISM received busulfan (1 mg/kg every 4 hours for 4 days, total dose 16 mg/kg) and cyclophosphamide (50 mg/kg for 4 consecutive days, total dose 200 mg/kg). Patient NoSM received the BAC regimen (busulfan, cytosine-arabinoside, cyclophosphamide).17 In the remaining five patients, the preparatory regimen consisted of busulfan (16 mg/kg) followed by cyclophosphamide (120 mg/kg).18

GVHD prophylaxis. Prevention of GVHD was attempted by intravenous infusion of cyclosporin A (CyA) starting on day -1 and continued until oral medication was possible. As of September 1, 1994, patients KISH, KiDG, and KIGW are the only ones remaining on CyA. Two patients received four doses of methotrexate: 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6, and +11. Three MTX injections (days +1, +3, and +6) were administered to three patients (KISH, KiDG, and KIGW). If acute GVHD developed, prednisolone
was administered intravenously starting at a dose of 1 mg/kg and then increased or decreased depending on clinical response. Acute GVHD was evaluated using the criteria of Glucksberg et al. Chronic GVHD was classified as proposed by Shulman et al. Hematopoietic recovery. Engraftment was monitored by daily differential blood counts for the time of the patients' initial hospital stay. Thereafter, complete blood counts were performed at least twice weekly or more often if indicated. Granulocyte engraftment is described by the number of days after transplant needed to achieve an absolute neutrophil count (ANC) ≥0.5 and ≥1.0 × 10^9/L, respectively. The numbers of days to achieve an unsupported platelet count ≥20 and ≥50 × 10^9/L, respectively, are given to describe engraftment of the megakaryocytic lineage. Achievement of an unsupported platelet count ≥20 (50) × 10^9/L was defined as the first day after grafting with a platelet count ≥20 (50) × 10^9/L but without any platelet transfusion on this and the following 6 days. All patients had BM biopsies performed between days 12 and 14 after transplantation; further biopsies and/or aspirations were planned on days +28, +56, +100, and +365.

RESULTS

The donors. Filgrastim administration and leukapheresis procedures were generally tolerated well. Mild bone pain occurred in four of six donors treated with 10 μg/kg of filgrastim, whereas no side effects were observed in the donors receiving 5 and 7.5 μg/kg, respectively. Although it was intended to use peripheral veins for the apheresis procedure in all donors, this turned out to be difficult in five of them, who therefore needed placement of a central-venous catheter, which was achieved without complications. The kinetics of the filgrastim-induced increase of CD34+ cells, colony-forming units granulocyte-macrophage (CFU-GM), and BFU-E in the peripheral blood of healthy volunteers, including most of the donors reported here, has recently been published. In brief, a steep increase of CD34+ cells as well as of colony-forming cells was first noticed on day 4 of treatment with filgrastim, resulting in peak values of 33.1 CD34+ cells (range, 12.2 to 152.8) × 10^6/mL, 6.851 CFU-GM (range, 891 to 10,890)/mL, and 5,401 BFU-E (range, 3,321 to 12,365)/mL of blood on day 5. These figures represented a 16- (range, 9 to 23), 38- (range, 13 to 88), and 6- (range, 6 to 22) fold increase over pretreatment values for CD34+ cells, CFU-GM, and BFU-E, respectively.

The leukapheresis products. One to three 9- to 10-L leukapheresis procedures yielded a median of 8.6 × 10^6 MNCs/kg of recipient weight containing between 2.2 and 8.1 × 10^6 CD34+ cells/kg (median, 6.7 × 10^6/kg), between 9.8 and 48.3 × 10^6 CFU-GM/kg (median, 30.0 × 10^6/kg), and 13.2 to 63.3 × 10^6 BFU-E/kg (median, 30.0 × 10^6/kg), respectively (Table 2). Immunophenotypical analysis of lymphocytes contained in the leukapheresis products showed that between 1.37 and 5.41 × 10^9 CD3+ T cells/kg (median, 3.40 × 10^9/kg) and between 0.23 and 1.43 × 10^5 CD56+ CD3- NK cells/kg (median, 0.94 × 10^5/kg) were present in the grafts.

The recipients' survival. The clinical outcome of the eight patients who have been grafted with allogeneic PBPCs is summarized in Table 1. Six of eight patients are currently alive with Karnofsky scores ≥80% between 61 and 533 days after grafting. Most recent marrow biopsies confirmed that the underlying disease was in remission. No bcr/abl rearrangement was detectable in the PB of patient KiSH. Two patients grafted for AML in relapse achieved a complete remission after transplantation but relapsed again 42 and 45 days after grafting and died of leukemia shortly thereafter.

Engraftment. Patients KiDG and KiGW received filgrastim at a dose of 5 μg/kg per day as a continuous infusion starting 24 hours after transplantation. Treatment was stopped on days +18 and +19, respectively, when the ANC had been greater than 1.0 × 10^9/L for 3 consecutive days. All other patients had no hematopoietic growth factors infused after grafting. As shown in Table 1, the kinetics of neutrophil recovery after transplantation of allogeneic PBPCs were variable, with a minimum of 12 days (13 days) and a maximum of 25 days (31 days) needed to achieve an ANC ≥0.5 (≥1.0) × 10^9/L. Recovery to an unsupported platelet count ≥20 (≥50) × 10^9/L varied even more, with 11 (16) to 39 (37) days after grafting needed to surpass these thresholds. Patient GW, who, at the time of this writing, was 61 days after grafting, still had not reached 50 × 10^9/L platelets (latest platelet count, 38 × 10^9/L). BM biopsies performed between 12 and 14 days posttransplant showed variable degrees of hypocellularity, with the lowest cellularity reported in those patients with the lowest cell counts at this time. The most recent BM biopsy confirmed trilineage engraftment in all surviving patients with cellularity between 50% and 100%. Patients KiBR and KiDN were grafted from donors with major ABO blood group antigen disparities. Patient KiBR developed a massive delayed alloimmune hemolytic anemia with overt intravascular hemolysis (fragmentocytes in PB, hemoglobinuria, hyperbilirubinemia, and imminent renal failure). Having had his last platelet and red blood cell (RBC) transfusions on day +10 and day +17, respectively, his hematocrit and platelet count dropped again and he needed a total of 16 RBC and 19 platelet transfusions between days 42 and 87 after grafting. Along with a gradual decrease of the anti-A titer, his clinical condition improved and the transfusion intervals widened. The last RBC and platelet transfusions were administered on days +81 and +87, respectively. Patient KiDN, being independent of platelet transfusions since day +19, experienced an isolated decrease of her platelet count on day +26 that necessitated the transfusion of 9 thrombapheresis products until day +42. Hematocrit and neutrophil count of this patient remained stable and no clinical or laboratory signs of hemolysis were found. None of the patients without ABO-incompatibility to the donor needed RBC or platelet support once the level of 20 × 10^9/L platelets had been reached.

Using both probes, (GTG)_5 and OA-Y-22 nucleated PB cells and BM aspirates from 7 of 8 patients have been analyzed at least twice between day +12 and day +489 to determine the type of lymphohematopoietic chimerism. In all instances, exclusively donor bands were detected, except for patient KiPR, who exhibited a mixture of donor and host cells at the time of relapse after grafting (Fig 1).

GVHD. Three patients did not develop acute GVHD and one patient developed grade I disease that resolved after brief treatment with systemic corticosteroids. Three patients developed acute GVHD of grade II limited to the skin. Although patients KiPR and KiDG responded to corticosteroid treatment and were off any steroids before relapse (KiPR)
or at the time of last follow-up, patient KiSH continues on oral prednisolone. This patient has also been treated with psoralen plus UV A (PUVA)\textsuperscript{31} with a complete response of skin GVHD. Patient NoSM was the only one to exhibit grade III disease involving both the skin and the gut. GVHD developed at day +30 posttransplant after discharge of the patient from the BMT unit. Of note, this patient refused to take oral CyA regularly and had suboptimal CyA levels (<40 ng/mL) most of the time after grafting until he relapsed on day +45 and it was decided to withdraw all treatment. Five patients are beyond 100 days after grafting and are thus evaluable for the development of chronic GVHD. Patient KiSH grafted from his 67-year-old phenotypically identical father is the only one showing chronic GVHD limited to the liver with minor skin involvement. This patient currently receives CyA and prednisolone (8 mg daily). Thalidomide has recently been added with minor improvement.

**DISCUSSION**

Until recently, transplantation of allogeneic PBPCs has largely been restricted to patients with failure of a marrow graft\textsuperscript{13} or a donor unable to undergo general anesthesia.\textsuperscript{12} Few patients with advanced leukemia have simultaneously been grafted with BM plus PBPCs.\textsuperscript{22} Fears to administer G-CSF to healthy individuals, the lack of reliable data on the long-term engraftment potential of PBPC, and concerns that transplantation of allogeneic PBPCs might cause devastating GVHD may all have contributed to this situation. We show that PBPCs can be safely mobilized in healthy donors and, if administered to the recipient as the only source of hematopoietic progenitor cells without further manipulation, do reliably engraft and remain detectable in the recipient’s BM and PB more than 1 year after grafting.

The administration of filgrastim and the harvest procedure were tolerated without significant side effects except for mild bone pain in those donors who received 10 \( \mu \text{g/kg} \) of the drug. Lower doses of filgrastim were tolerated without side effects. However, for the time being, we prefer to use 10 \( \mu \text{g/kg} \) because this dose results in more predictable yields of progenitor cells with one or two leukapheresis procedures.\textsuperscript{9} Administering even higher doses of filgrastim may be considered to find out if higher numbers of CD34\textsuperscript{+} cells in the graft can accelerate hematopoietic recovery. Although the long-term toxicities of filgrastim cannot be freely assessed at present, its short-term administration to healthy individuals is deemed acceptable especially if the avoidance of the risks of general anesthesia and the marrow harvest procedure are taken into account. It was quite disturbing to realize that five of the eight donors needed a central venous line to achieve adequate venous flow for the harvest procedure. Placing a central venous access is not a trivial procedure and further attempts are necessary to avoid this manoeuvre whenever possible.

Although transplant experiments in mice\textsuperscript{21} and dogs\textsuperscript{22} as well as the detection of long-term culture-initiating cells in human PBPC harvests\textsuperscript{23} gave circumspect evidence that growth factor-mobilized PBPCs should be able to sustain hematopoiesis for prolonged periods of time, the present study formally proves that long-term engraftment is achieved with allogeneic PBPC. Excluding the analysis performed at the time of relapse in patient KiPR exclusively, donor cells were detected in all seven patients investigated at time periods between 12 and 489 days after grafting.

Looking at the kinetics of early hematopoietic engraftment it becomes obvious that the recovery of neutrophils and particularly of platelets after allogeneic PBPC is much more variable than might have been anticipated from the respective data in the autologous situation. Besides the fact that two of the patients received filgrastim after PBPC and therefore possibly experienced a faster recovery of their neutrophils, the wide range of the number of days needed to recover from pancytopenia probably mirrors the much more complex interactions between host and donor cells and the plethora of cytokines released into the circulation by different cell types of either origin after allografting.\textsuperscript{26} Minor HLA-disparities between recipient and donor, the quality of the graft as (incompletely) characterized by the number of hematopoietic and T cells contained, acute GVHD, and some of the typical early complications of allogeneic BMT such as viral infections or venoocclusive disease might all interfere with hematopoietic recovery. Even from the limited number of cases reported here it seems evident that the administration of methotrexate after grafting of PBPCs may cause a delay of hematopoietic reconstitution comparable to that seen after allogeneic BMT.\textsuperscript{27} AB0-incompatibility between recipient and donor may not only negatively influence engraftment of erythroid progenitors and cause hemolysis but also defer platelet recovery.\textsuperscript{28}

Serious concern with regard to transplantation of allogeneic PBPCs has been elicited by the presence of approximately 10 times more T cells in PBPC than in marrow harvests. More than 25 years ago, van Bekkum and de Vries\textsuperscript{29} had reported that in rodents the number of T cells in a marrow graft was correlated with the incidence and severity of GVHD, and this dogma has been held until recently.\textsuperscript{29,31} Furthermore, Kernan et al\textsuperscript{32} showed a clear-cut correlation between the number of T cells and the occurrence of acute GVHD when they calculated the number of residual clonable T cells in T-cell-depleted marrow grafts. On the other hand, Atkinson et al\textsuperscript{26} and Jansen et al\textsuperscript{33} had been unable to detect such a correlation when unmanipulated BM was used for BMT. These observations are not necessarily contradictory because neither experimental nor clinical data exist that show a correlation between the occurrence of acute GVHD and T-cell numbers in a dose range equal to or higher than that normally present in an unmanipulated marrow graft (>10\textsuperscript{5} T cells/kg). The data presented here document that the infusion of greater than 10\textsuperscript{5} T cells/kg into HLA-identical recipients need not result in life-threatening GVHD. Severe GVHD of grade III was seen in a single patient who refused regular CyA intake and had very low trough levels for most of the time after cessation of intravenous CyA on day +13 until relapse. Given the clinical characteristics of our patients (age distribution, sex match, disease status, and other variables known to influence the occurrence of GVHD),\textsuperscript{27,28} it seems unlikely that a lack of risk factors for GVHD was responsible for the low incidence of severe acute GVHD observed. Although it cannot be excluded that freezing and
thawing of the graft or keeping part of it at 4°C overnight selectively inactivated T lymphocytes, the most likely explanation for our findings with respect to GVHD may be that the specificity of donor T cells rather than T-cell number plays a pivotal role in triggering GVHD.

The number of NK cells that are considered to be important effector cells of GVL activity transplanted with allogeneic PBPCs is approximately 20 times greater than with a normal marrow graft. The question if this will translate into a more potent GVL effect in patients grafted with allogeneic PBPCs as compared with BM cannot be answered at this time but surely deserves further scrutiny.

Although the data presented here show that primary transplantation of unmanipulated allogeneic PBPCs mobilized by filgrastim is feasible, results in trilineage long-term engraftment, and does not cause detrimental GVHD, many theoretical and practical questions remain unanswered, the most obvious of which is how the speed of hematopoietic recovery, the incidence and severity of acute and chronic GVHD, and, most importantly, how long-term leukemia-free survival will ultimately compare with allogeneic BMT. Such complex and interacting issues can only be addressed in a prospective randomized trial. Therefore, a study comparing the results of myeloablative therapy followed by allogeneic PBPC or BMT in patients with good-risk leukemia has recently been launched by the European Group for Blood and Marrow Transplantation.

ACKNOWLEDGMENT

We are indebted to K. Viehmann, H. Bosse, and B. Petersen for skilful technical help and to Christel Diener for assistance in the preparation of the manuscript. The PCR analyses to detect bcr/abl rearrangement were performed by T. Lion (St Anna Kinderspital, Vienna, Austria).

REFERENCES


36. van Bekkum DW: The selective elimination of immunologically competent cells from bone marrow and lymphatic cell mixtures. I. Effect of storage at 4 C. Transplantation 2:393, 1964

Primary transplantation of allogeneic peripheral blood progenitor cells mobilized by filgrastim (granulocyte colony-stimulating factor) [see comments]

N Schmitz, P Dreger, M Suttorp, EB Rohwedder, T Haferlach, H Loffler, A Hunter and NH Russell