Sustained Long-Term Hematopoiesis After Myeloablative Therapy With Peripheral Blood Progenitor Cell Support


A retrospective analysis of long-term hematopoiesis was performed in a group of 145 consecutive patients who had received high-dose therapy with peripheral blood progenitor cell (PBPC) support between May 1985 and December 1993. Twenty-two patients had acute myelogenous leukemia, nine had acute lymphoblastic leukemia, 43 had Hodgkin's disease, 57 had non-Hodgkin's lymphoma, and 14 patients had multiple myeloma. Eighty-four patients were male and 61 female, with a median age of 37 years (range, 16 to 58 years). In 46 patients, PBPC were collected after cytotoxic chemotherapy alone, while 99 patients received cytokines either during steady-state hematopoiesis or post-chemotherapy. Sixty patients were treated with dose-escalated polychemo therapy, and 85 patients had a conditioning therapy including hyperfractionated total body irradiation at a total dose of 14.4 Gy. The duration of severe pancytopenia posttransplantation was inversely related to the number of reinfused granulocyte-macrophage colony-forming units (CFU-GM) and CD34+ cells. Threshold quantities of 2.5 x 10^6 CD34+ cells per kilogram or 12.0 x 10^4 CFU-GM per kilogram became evident and were associated with rapid neutrophil and platelet recovery within less than 18 and 14 days, respectively. These numbers were also predictive for long-term reconstitution, indicating that normal blood counts are likely to be achieved within less than 10 months after transplantation. Conversely, 12 patients were autografted with a median of 1.75 x 10^6 CFU-GM per kilogram resulting in delayed recovery to platelet counts of greater than 150 x 10^9/L between 1 and 6 years. Our study includes bone marrow examinations in 59 patients performed at a median follow-up time of 10 months (range, 1 to 85 months) posttransplantation. A comparison with normal volunteers showed a 3.2-fold smaller proportion of bone marrow CD34+ cells, which was paralleled by an even more pronounced reduction in the plating efficiency of CFU-GM and burst-forming unit-erythroid. No secondary graft failure was observed, even in patients autografted with relatively low numbers of progenitor cells. This suggests that either the pretransplant regimens were not myeloablative, allowing autochthonous recovery, or that a small number of cells capable of perpetual self-renewal were included in the autograft products.

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The capacity and reserve of the mammalian hematopoietic system are remarkable. This has been shown in animal studies by sequential transplantsations of bone marrow-derived stem cells into lethally irradiated recipients. There is also a plentiful supply of pluripotent stem cells and lineage-determined progenitor cells in humans to maintain adequate blood counts under normal conditions, but hematopoiesis can be severely compromised by cytotoxic chemotherapy or irradiation. High-dose conditioning therapies that include total body irradiation (TBI) are notably myelotoxic and require the transplantation of hematopoietic progenitor cells. For the support of dose-escalated radiochemotherapy in cancer patients, bone-derived progenitor cells are increasingly used as a substitute for bone marrow. Hematologic reconstitution in mice after lethal irradiation and bone marrow grafting was shown to evolve in several phases that are mediated by different progenitor cell subsets. The ability of human blood-borne progenitor cells to reconstitute long-term hematopoiesis has been often questioned, as it was not clear whether peripheral blood contains early pluripotent stem cells, and if so, whether their numbers would be sufficient.

To address the question of hematopoietic recovery and long-term reconstitution after peripheral blood progenitor cell (PBPC)-supported high-dose therapy, we examined 145 patients with hematologic malignancies who were autografted at our center within the last 9 years. PBPC were mobilized using conventional cytotoxic chemotherapy or cytokine-based regimens. Follow-up examinations of bone marrow in 50 patients showed that posttransplant hematopoiesis is characterized by a significantly reduced number of hematopoietic progenitor cells when compared with healthy volunteers. Normal peripheral blood counts are maintained, and no graft failure has been observed in disease-free patients.

MATERIALS AND METHODS

Patients

Between May 1985 and December 1993, a total of 145 patients with hematologic malignancies were included in the study. The majority of patients autografted during the first 3 years of the observation period had acute myelogenous leukemia (AML), while the present transplantation protocols predominantly include patients with malignant lymphoma and multiple myeloma. Eighty-four patients were male and 61 female, with a median age of 37 years (range, 16 to 58 years). At the time of PBPC-supported high-dose therapy, 140 patients were in complete or partial remission, whereas five patients had active disease. The patient characteristics, including information on the high-dose conditioning regimens, are summarized in Table 1. All patients were treated at the Departments of Internal Medicine V and Radiology of the University of Heidelberg (Heidelberg, Germany). Informed consent was obtained from each patient before therapy. The study was conducted under the guidelines of the Joint Ethical Committee of the University of Heidelberg. The cut-off date of this report is June 30, 1994.

PBPCs were collected either after cytotoxic chemotherapy, in the course of steady-state administration of hematopoietic growth factors, or during cytokine-enhanced marrow recovery postchemotherapy. Twenty-nine patients had bone marrow involvement by histopathologic examination before the start of PBPC mobilization. The
mobilization regimens and corresponding patient groups are shown in Table 2.

Hematologic short-term reconstitution, as mostly observed during the patient's stay in the hospital, was defined as the time required to achieve a white blood cell (WBC) count of ≥1.0 × 10^9/L, an absolute neutrophil count of ≥0.5 × 10^9/L, and a platelet count of ≥100 × 10^9/L. Patients were considered to have reached long-term reconstitution as soon as a hemoglobin level of ≥12.0 g/dL, a WBC count of ≥4.0 × 10^9/L, an ANC of ≥1.5 × 10^9/L, and a platelet count of ≥150 × 10^9/L were observed. As the majority of our patients were referred from other hospitals, follow-up examinations were also performed in those centers. For regular staging examinations, most of the patients were seen in our hospital at intervals of 3 to 6 months. In a group of 50 patients, we performed bone marrow examinations that included a clonogenic culture assay and immunophenotyping of hematopoietic progenitor cells. Samples of bone marrow and peripheral blood were obtained from 10 consenting normal volunteers for comparison.

Table 1. Patient Characteristics

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Melphalan dose was 200 mg/m²; TBI/melphalan, 140 mg/m².

Abbreviations: ALL, acute lymphoblastic leukemia; HD, Hodgkin's disease; CY, cyclophosphamide (200 mg/kg); BEAM, carmustine 300 mg/m², etoposide 1,200 mg/m², cytarabine 800 mg/m², and melphalan 140 mg/m²; CBV, cyclophosphamide 6.8 g/m², etoposide 1,600 mg/m², and carmustine 450 mg/m²; for the different dose-escalated regimens, see also Korbling et al.18 and Haas et al.19

Collection of PBPCs and Cryopreservation

Harvesting was performed with a Fenwal CS 3000 (Baxter Deutschland GmbH, München, Germany). For 75 patients, a total nucleated cells (TNC) target quantity of 0.4 × 10^9/kg was envisaged. PBPC collection was thereafter monitored by CD34+ cell assessment. In general, 10 L of blood was processed at a flow rate of 50 to 70 mL/min during each leukapheresis. The apheresis product was mixed with the same volume of minimal essential medium (MEM) containing 20% dimethylsulf oxide (DMSO; Merck, Darmstadt, Germany). The final cell suspension was transferred into freezing bags (Delmed Inc, New Brunswick, NJ) and frozen to −190°C with a computer-controlled cryopreservation device (Cryocon BV-6, Cryoson Deutschland GmbH, Schöllkrippen, Germany). The frozen cells were transferred into the liquid phase of nitrogen and stored at −196°C.

Clonogenic Assay for Hematopoietic Progenitor Cells

The concentration of hematopoietic progenitor cells in bone marrow, leukapheresis products, and peripheral blood was assessed using a semisolid clonogenic culture assay. In October 1990, a commercially available assay was introduced that was also used for the normal volunteers (StemCell Technologies Inc, Vancouver, Canada). The culture medium consisted of 30% fetal calf serum, 10% medium conditioned by phytohemagglutinin-stimulated leukocytes (PHA-LCM), 1 IU recombinant human erythropoietin, 5 × 10^-5 mol/L 2-mercaptoethanol, and 0.9% methicellulose. The mononuclear cells (MNC) were plated in duplicate and incubated at 37°C and 5% CO₂ in humidified atmosphere. After 14 days, colonies were scored using an inverted microscope.

Immunofluorescence Staining and Flow Cytometry

For immunofluorescence analysis, 1 × 10^5 MNC of bone marrow, leukapheresis products, and peripheral blood were incubated for 30 minutes at 4°C with the fluorescein isothiocyanate (FITC)-or phycoerythrin (PE)-conjugated monoclonal antibody (MoAb) HPCA-2 (CD34; Becton Dickinson, Heidelberg, Germany). After the MoAb incubation, the cells were washed twice with phosphate-buffered saline and resuspended in a solution containing 1% BSA in PBS. For immunofluorescence analysis, the cells were incubated with MoAb directed against CD34, CD117, and CD45.

Table 2. PBPC Mobilization Regimens

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<td>13</td>
<td>9</td>
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Abbreviations: IL-3, interleukin-3; G-CSF, granulocyte colony-stimulating factor.

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saline (PBS), and red blood cells were removed using a FACS lysing solution (Becton Dickinson). For single-color analysis, a total of 10,000 cells was acquired. Immunofluorescence analysis was performed using a five-parameter FACScan (Becton Dickinson) equipped with an argon-ion laser tuned at 488 nm and 0.3 W. Emission from FITC and PE was measured using filters of 530 nm and 585 nm, respectively.

A side scatter characteristics (SSC) versus CD45 fluorescence dot plot was used to discriminate between the smallest lymphohematopoietic cell population and erythrocytes or debris. The CD34+ cells were analyzed in a fluorescence versus SSC plot. Thus, only cells with lymphoid or lymphoblastoid appearance (low SSC) were counted as CD34+ cells. The percentage of false-positive events accepted in the isotype-specific controls was 0.05% and was subtracted from the percentage of CD34+ cells that were then determined in relation to the percentage of CD45+ cells. The concentration of CD34+ cells per milliliter of bone marrow was measured as follows: the volume of the bone marrow aspirate was recorded, and the MNC were tracted from the percentage of CD34+ cells that were then determined by simple linear regression and correlation analysis. To assess for statistical significance between grouped data of patients and normal volunteers, the Mann-Whitney U test was applied. A significance level of 0.05 was chosen.

**Statistical Analysis**

The clinical and laboratory data of the patients were analyzed according to standard statistical methods using a commercially available computer program (Statworks; Cricket Software, Philadelphia, PA). The relationship between the content of the autografts (TNC per kilogram, granulocyte-macrophage colony-forming units (CFU-GM) per kilogram, and CD34+ cells per kilogram) and the duration of short-term and long-term reconstitution was determined by simple linear regression and correlation analysis. To assess for statistical significance between grouped data of patients and normal volunteers, the Mann-Whitney U test was applied. A significance level of $P < 0.05$ was chosen.

**RESULTS**

**Hematologic Reconstitution After PBPC-Supported High-Dose Therapy**

**Short-term recovery.** The group of 145 patients presented here is heterogeneous except that all patients had hematologic malignancies and received blood-derived hematopoietic progenitor cells for the support of dose-escalated cytotoxic therapy. The autografts also add to the diversity, as PBPC were collected using different mobilization regimens.

Seven patients died of early transplantation-related complications, which translates into a mortality rate of 4.8%. The autografts of these patients contained a median 7.8-fold smaller number of CFU-GM per kilogram compared with the other 138 patients. Eleven patients relapsed between 1 and 8 months posttransplantation without having reconstituted. Interestingly, eight of these early relapses were observed in patients with AML autografted in first or second remission. It is questionable whether the term graft failure is appropriate for these cases, as recurrence of disease was interfering with the engraftment of reinforced normal progenitor cells.

Having excluded the patients with toxic death as well as those who did not engraft and who also experienced early relapse, a total of 127 patients were available for the evaluation of short-term reconstitution. The relationship between the number of hematopoietic progenitor cells reinfused and the time required for short-term reconstitution is best characterized by threshold numbers. Autografting of more than $2.5 \times 10^7$ CD34+ cells per kilogram or $1.0 \times 10^7$ CFU-GM per kilogram resulted in neutrophil and platelet recovery within less than 18 and 14 days, respectively (Fig 1). In contrast, the number of TNC transplanted per kilogram had no predictive quality.

There was a remarkable difference between the patient groups with regard to the amount of CFU-GM autografted per kilogram. For instance, maximum numbers were observed in patients with low-grade NHL whose transplants contained a median 15-fold greater quantity of CFU-GM per kilogram compared with patients with AML. As shown in Fig 2, patients with low-grade NHL had shorter times of neutrophil and platelet recovery.

Another point relates to the history of five patients with AML who received mafosfamide-purged bone marrow between 39 and 228 days (median, 98 days) after PBPC-supported high-dose therapy, because they showed no signs of reconstitution. Two patients achieved stable long-term reconstitution and are in complete remission, one patient died of a hemolytic-uremic syndrome, and two patients relapsed.

**Long-term recovery.** We continue with the long-term follow-up of the 127 patients who achieved early trilineage engraftment. While reconstitution data are not available for nine patients, one patient died of toxoplasma gondii encephalitis 97 days after PBPC autografting. We also observed one case of myelodysplasia 39 months posttransplantation in a patient with follicular lymphoma autografted in second remission with as few as $0.93 \times 10^6$ CFU-GM per kilogram.

Twenty-three patients relapsed between 1 and 25 months (median, 5 months) after PBPC-supported high-dose therapy without having reached normal blood counts. In the case of palliative chemotherapy, hematologic recovery was further compromised by tumor progression and treatment-related myelotoxicity. On the other hand, one relapsed patient with AML and one with Hodgkin's disease were successfully treated using second-line cytotoxic chemotherapy, which was accompanied by recovery to normal blood counts.
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Early Hematological Reconstitution

of ANC to $0.5 \times 10^9/l$

of Plt to $20 \times 10^9/l$

Fig 1. Comparison of CFU-GM and CD34$^+$ cells autografted per kilogram bodyweight with days to recovery of neutrophil counts to $0.5 \times 10^9/L$ and of platelet counts (Plt) to $20 \times 10^9/L$. For CFU-GM, the evaluation is based on a total of 123 patients autografted between May 1985 and December 1993, while the number of CD34$^+$ cells transplanted is available for 78 patients. As indicated by the shaded areas, threshold quantities of $2.5 \times 10^6$ CD34$^+$ cells per kilogram or $12.0 \times 10^4$ CFU-GM per kilogram became evident and were associated with rapid neutrophil and platelet recovery within less than 18 and 14 days, respectively. PBPC, peripheral blood progenitor cell transplantation.

Bone marrow progenitor cells after PBPC-supported high-dose therapy. The number of bone marrow (BM) progenitor cells was examined in 50 patients at a median follow-up time of 10 months (range, 1 to 85 months). The plating efficiency as well as the number of CFU-GM and burst-forming units-erythroid (BFU-E) were significantly diminished in comparison with 10 normal volunteers. Interestingly, the mean plating efficiency of CFU-GM and BFU-E posttransplantation was 6.0- and 4.3-fold smaller compared with healthy donors, whereas the patients’ mean proportion of BFU-E was only 3.2-fold reduced (Table 3).

The concentration of CD34$^+$ cells tended to be greater in BM samples of patients who had received large numbers of CD34$^+$ cells. However, the relationship between these two parameters was moderate at best, considering a correlation coefficient of only $R = .31$. It was interesting to find that the time elapsed between PBPC-supported high-dose therapy and the BM examination was not related to the number of CFU-GM, BFU-E, or CD34$^+$ cells in the BM samples.

DISCUSSION

We present data on the hematopoietic recovery of 145 patients with hematologic malignancies who received PBPC-supported high-dose therapy between May 1985 and December 1993. An important finding relates to the restorative capacity of the PBPCs, considering that their ability to main-
Cumulative Frequency of Recovery

of ANC to $0.5 \times 10^9/l$

of Plt to $20 \times 10^9/l$

Cumulative Frequency of Recovery

Fig 2. Cumulative frequency of days to recovery of neutrophil counts to $0.5 \times 10^9/L$ and of platelet counts (Plt) to $20 \times 10^9/L$ after PBPC-supported high-dose therapy. Data are given for each patient group separately. AL, acute leukemia; HD, Hodgkin’s disease; NHL, non-Hodgkin’s lymphoma; MM, multiple myeloma; PBPC, peripheral blood progenitor cell transplantation.

The data presented here show how the hematopoietic content of the autografts affects the rapidity of marrow recovery. Short-term reconstitution was clearly related to the number of progenitor cells transplanted. Both CFU-GM and CD34 cells were predictive for rapid neutrophil and platelet recovery, with threshold quantities of $2.5 \times 10^9$ CD34 cells and $12.0 \times 10^9$ CFU-GM per kilogram, respectively. These figures are consistent with the data of other centers. Reinforcing more progenitor cells did not result in faster recovery, suggesting that marrow sites for homing and proliferation are limited. To further shorten the period of severe aplasia, precursor cells of later developmental stages would be needed. The seven patients who died of early transplantation-related complications received significantly fewer CFU-GM per kilogram compared with the other 138 patients. At first glance, this finding provides a strong argument to aim at harvests containing at least the threshold numbers. However, only one patient with severe lung bleeding died of a complication precipitated by prolonged pancytopenia. The other patients died of toxic organ failures apparently not related to bleeding or infections. A low yield of hematopoietic progenitor cells may be, therefore, considered as a more general risk factor of early death.

The threshold numbers of progenitor cells derived from the analysis of early reconstitution were also valid for late reconstitution. All patients autografted with at least $2.5 \times 10^9$ CD34 cells per kilogram and $12.0 \times 10^9$ CFU-GM per kilogram, respectively, developed normal blood counts within less than 10 months after transplantation. Except for one patient, delayed recovery was confined to patients with acute leukemia and Hodgkin’s disease, reflecting the fact that...
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Fig 3. As demonstrated by the Kaplan-Meier plots, the probabilities of achieving long-term reconstitution of the erythroid, neutrophil, and megakaryocytic lineages were closely related to each other. A patient was considered to have reached long-term reconstitution as soon as a hemoglobin level (Hb) of \( \geq 12.0 \) g/dl, an ANC of \( \geq 1.5 \times 10^9/\text{l} \), and a platelet count (Plt) of \( \geq 150 \times 10^9/\text{l} \) were observed.

Fig 4. Comparison of CFU-GM and CD34\(^+\) cells autografted per kilogram of bodyweight with days to recovery of platelet counts to \( 150.0 \times 10^9/\text{l} \). For CFU-GM, the evaluation is based on a total of 69 patients, while the number of CD34\(^+\) cells transplanted is available for 43 patients. As for early hematologic reconstitution, the shaded areas indicate threshold quantities of \( 2.5 \times 10^6 \) CD34\(^+\) cells per kilogram and \( 12.0 \times 10^6 \) CFU-GM per kilogram to be associated with platelet recovery within less than 10 months.

the autografts of these patients contained smaller numbers of CFU-GM compared with the other patient groups. In a previous study of patients with malignant lymphoma, we showed that the amount of previous cytotoxic chemotherapy and irradiation are the major factors adversely affecting the yield of PBPCs.\(^{19}\) In addition, the patient groups presented here vary considerably with regard to the PBPC mobilization treatment. For instance, in the majority of patients with acute leukemia, PBPCs were harvested postchemotherapy without cytokine support, whereas mobilization with interleukin-3/GM-CSF after cytarabine/mitoxantrone was only used in patients with high-grade NHL. Moreover, mutual dependence exists not only between diagnosis, the cytotoxic pre-treatment, and the PBPC mobilization modality, but includes also the pretransplant conditioning therapy. All patients with Hodgkin’s disease, for example, received the CBV regimen, while patients with acute leukemias underwent high-dose therapy with cyclophosphamide and TBI.

The role of additional BM grafting in the setting of PBPC transplantation can be briefly addressed. From the observation in five patients, we conclude that reinfusion of BM after PBPC autografting had no influence on the kinetics...
of hematopoietic recovery. It is conceivable that either the number of viable progenitor cells reinfused was critically low or engraftment was insufficient due to the lack of an immediately preceding conditioning therapy. The latter explanation is less likely, as marrow cells could be successfully engrafted in syngeneic mice without cytotoxic pretreatment.

The fact that we have seen no graft failure even in patients transplanted with extremely low numbers of CFU-GM or CD34+ cells suggests a stem cell not extinguishable by the pretransplant conditioning regimens. Although the low PBPC yields in those patients already indicated a severely compromised hematopoiesis, long-term recovery was eventually achieved despite the substantial myelotoxicity added by the pretransplant therapy. Posttransplantation hematopoiesis may then result to some degree from reinfused progenitor cells as well as from surviving pluripotent stem cells. This view is consistent with the detection of chimerism after noninvasive PBPC grafting, a greater amount of progenitor cells might be also advantageous for the competition with eventually surviving stem cells of the recipient. This mechanism could be synergistically effective with a T-cell-mediated inhibition of host-derived hematopoiesis.

If autochthonous recovery is possible even after TBI-containing high-dose regimens, there would be no rationale for reinfusing higher amounts of progenitor cells than the threshold numbers. However, it is conceivable that an increased number of progenitor cells might reduce the risk of myelodysplastic syndrome. In the setting of syngeneic or allogeneic PBPC grafting, a greater amount of progenitor cells might be also advantageous for the competition with eventually surviving stem cells of the recipient. This mechanism could be synergistically effective with a T-cell-mediated inhibition of host-derived hematopoiesis.

Whether reinfused progenitor cells contribute to long-term hematopoiesis or not, there is no question that posttransplantation hematopoiesis is maintained by a significantly lower number of progenitor cells compared with normal donors. A similar observation was made by Messner et al in patients after allogeneic bone marrow transplantation. The investigators also observed an increased proportion of colony-forming cells in cell cycle to compensate for the smaller number of progenitor cells. It was interesting to note that the number of hematopoietic progenitor cells that we found in the BM samples posttransplantation was hardly related to the quantity of CD34+ cells and CFU-GM transplanted. There was also no relationship between the concentration of progenitor cells and the time that had elapsed after PBPC autografting. Although hematopoiesis may not be evenly distributed posttransplantation, the failure to establish a relationship between cellular input and progenitor cell concentration postgrafting is unlikely to result from sampling errors alone. The random distribution rather suggests that autochthonous regeneration from surviving pluripotent stem cells may contribute at least partially to long-term hematopoiesis.

Further implications of the results presented here concern the use of genetically altered CD34+ cells for molecular replacement therapy, as long as it is questionable to what extent the autografted progenitor cells would contribute to long-term hematopoiesis. Gene marking studies are, therefore, required to address this question prospectively.

ACKNOWLEDGMENT

We thank the nursing staff of the Departments of Internal Medicine V and Radiology for their outstanding care of these patients. We are grateful to Kirsten Flentje, Evi Holdermann, Magdalena Volk, and Margit Pforsich for excellent technical assistance, and to Ulla Scheidler for expert secretarial help.

REFERENCES


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![Fig. 5. Correlative display of days to platelet recovery (Plt) to 20 x 10^9/L and 150 x 10^9/L, demonstrating the close relationship between early and late reconstitution.](image)
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Sustained long-term hematopoiesis after myeloablative therapy with peripheral blood progenitor cell support

R Haas, B Witt, R Mohle, H Goldschmidt, S Hohaus, S Freuhauf, M Wannenmacher and W Hunstein