Allogeneic Peripheral Blood Progenitor Cell Transplantation in a Murine Model: Evidence for an Improved Graft-Versus-Leukemia Effect

By Bertram Glass, Lutz Uharek, Matthias Zeis, Peter Dreger, Helmut Löffler, Jörg Steinmann, and Norbert Schmitz

Peripheral blood progenitor cells (PBPCs) are increasingly being used to replace bone marrow cells (BMCs) as a source of hematopoietic stem cells also in the field of allogeneic transplantation. Whereas it is well known that PBPC grafts and BM differ significantly in progenitor cell content and lymphocyte dose, the clinical consequences of these differences with respect to engraftment, graft-versus-host disease (GVHD), and the graft-versus-leukemia (GVL) effect are more difficult to assess. We present a murine model that allows us to evaluate engraftment, GVHD, and GVL effect of allogeneic PBPC transplantation (PBPCT). Balb/c mice (H-2d) served as recipients. Donors were major histocompatibility complex-matched DBA/2 mice or syngeneic Balb/c mice, respectively. Experiments with increasing numbers of BMCs or Filgastrim-mobilized PBPCs showed that the number of progenitor cells in the graft was correlated with the probability to engraft, irrespective of the graft type. With identically

ALLOGENEIC bone marrow transplantation (BMT) is the treatment of choice for a variety of hematologic malignancies. Animal experiments performed as early as 1957 suggested that the strong antileukemic effect of BMT resulted not only from the high-dose chemoradiotherapy preceding the transplantation but also was a consequence of adoptive immunotherapy exerted by the graft itself. Numerous experimental and clinical reports have confirmed these findings and underscored our view that the superior antitumor effect of BMT is primarily due to the destruction of leukemic target cells by an allogeneic immune response mediated by T lymphocytes and natural killer (NK) cells contained in the transplant.

Recently, granulocyte colony-stimulating factor (G-CSF)–mobilized peripheral blood progenitor cells (PBPCs) have been introduced as an alternative source of hematopoietic stem cells for allogeneic transplantation. Harvesting PBPCs instead of BM avoids the discomfort of marrow harvesting and the risks of general anesthesia in the donor while the high numbers of progenitor cells contained in such a graft lead to rapid and reliable engraftment in the recipient. The large numbers of progenitor cells that can be harvested from the PB 12,13 also allow for extensive ex vivo manipulations and make PBPC attractive as target cells for gene therapeutic maneuvers and immunotherapy.

Important questions related to PBPCT remain unanswered at this time. Whereas reports from single institutions 14 and the European Group for Blood and Marrow Transplantation 15 suggest that the transplantation of allogeneic PBPC does not cause devastating graft-versus-host disease (GVHD), a more precise comparison of the incidence and severity of GVHD after PBPCT as opposed to BMT must await the results of prospective clinical trials. There is even more uncertainty if the high numbers of T cells and NK cells contained in a typical PBPC collection product exert a more vigorous graft-versus-leukemia (GVL) effect, reduce the risk of relapse, and can thus improve disease-free survival after allogeneic PBPCT.

To address these latter questions, we developed a murine model that allows us to investigate the engraftment potential, graft-versus-host reactivity, and GVL activity of allogeneic PBPCs. Experiments presented here show a superior antileukemic activity of allogeneic, major histocompatibility complex (MHC)-matched PBPCs over BM cells (BMCs) in leukemia-bearing mice.

MATERIALS AND METHODS

Animals. Balb/c (H-2b) and DBA/2 (H-2d) mice were bred and kept at the animal facilities of our institution. All animals were housed in conventional cages, 7 to 10 animals to a cage, and received nonsterilized food and water ad libitum. Cotrimoxazole was added to the water for 40 days after BMT or PBPCT.

Hematopoietic stem cell transplantation. A Cs137 source was used for total body irradiation (TBI) of the recipients. A lethal dose of 7.5 Gy TBI was chosen for conditioning. BM grafts were prepared according to standard procedures as previously described.7 Mice intended to serve as donors of PBPCs had their spleens removed under general anesthesia at least 14 days before the donation of PBPCs. Starting 5 days before the collection of PBPC, 5 Î¿ of rhu-met-G-CSF (Filgastrim; Amgen, Thousand Oaks, CA) was administered 2 hours before harvesting of PBPC. The mice were then anticoagulated with heparin, anesthetized, and killed by cervical dislocation. The PB was collected under sterile conditions by dissection of both Aa carotidea. Erythrocytes were lysed by incubation of PB in 0.15 mol/L ammoniumchloride buffer at 20°C for 5 minutes. The cell number was adjusted to 6 × 107 nucleated cells (NC)/mL.

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For smaller grafts, a cell concentration of $1 \times 10^7$ NC/mL was used. BM or PBPC grafts were injected intravenously 2 hours after conditioning of the recipients by TBI.

T-cell depletion of PBPC grafts. Immunomagnetic depletion of T cells from the PBPC grafts was used in some experiments. Immunomagnetic beads (Dyna, Oslo, Norway), coated with goat-anti-mouse Ig, were coupled to an antimouse CD3 monoclonal antibody (MoAb; KT-3; Serotec, Wiesbaden, Germany). The CD3 MoAb was used at a concentration of 11 $\mu$g per 4 $\times 10^8$ beads. Coupling of MoAb and magnetic beads was performed at 4°C for 30 minutes. The ratio of beads/target cell was 6:1. The magnetic beads were coincubated with the PBPC graft for 1 hour at 21°C, and the cells were then removed with a strong permanent-magnetic device (Dynal, OSLO, Norway).

Engraftment. A20 is a B-cell leukemia/lymphoma of Balb/c origin that occurred spontaneously in a 15-month-old mouse. The cells were maintained in culture with RPMI 1640 + 5% fetal calf serum at 37°C and 5% CO₂. We performed in vivo passages of these cells by intravenous injection into Balb/c animals. After the mice had developed hepatosplenomegaly, they were killed and their spleens were removed. Spleen cell suspensions containing close to 100% of these in vivo passaged leukemia/lymphoma cells were stored in liquid nitrogen and used for further experiments.

RESULTS

Mobilization of PB progenitor cells and composition of the transplants. To avoid pooling of PBPCs in the spleen, mice were splenectomized before G-CSF administration. This led to an increase of white blood cells from 6.4/nL to 9.9/nL (range, 9.6 to 10.8 cells/nL) in DBA/2 mice. Differential counts were not altered after splenectomy. G-CSF administration induced a substantial increase of the leukocyte count up to 59.6/nL (range, 42.9 to 73.3/nL) in Balb/c mice or 72.9/nL (range, 51.8 to 85.8/nL) in DBA mice. In steady state, blood of DBA mice contained 0.80 CFU-GM/10⁴ NC. After mobilization, the PBPC collection product contained 1.13 CFU-GM/10⁴ NC, resulting in a substantially higher concentration of progenitor cells in PB (8.23 CFU-GM/µL after and 0.79 CFU-GM/µL blood before G-CSF administration). BM of DBA mice gave rise to 4.83 CFU-GM/10⁴ NC. Balb/c mice were shown to have lower concentrations of progenitor cells in BM or PB, but the relationship between the PBPC graft and BM was identical (Table 1).

Engraftment. In experiments with nonleukemic animals, three parameters possibly determining engraftment were varied: the cell dose of the transplant, the immunogenetic difference between donor and recipient, and the source of hematopoietic stem cells. With PBPCs, the number of nucleated cells transferred had a major impact on engraftment as measured by the rate of graft failure (Fig 1) or the kinetics of hematopoietic recovery in the surviving animals (Fig 2).
Table 1. Cellular Composition of Stem Cell Transplants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>n</th>
<th>Leukocytes (x10⁶)</th>
<th>Neutrophils (%)</th>
<th>MNC</th>
<th>T Cells CD3⁺ (%)</th>
<th>NK Cells CD56⁺ (%)</th>
<th>B Cells CD19⁺ (%)</th>
<th>CFU-GM (x10⁴ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balb/c</td>
<td>PB</td>
<td>4</td>
<td>4.9 (3.4-8.2)</td>
<td>10 (10-50)</td>
<td>90</td>
<td>21 (11-32)</td>
<td>7 (4-11)</td>
<td>ND</td>
<td>0.13 (0.06-0.2)</td>
</tr>
<tr>
<td></td>
<td>PBPC</td>
<td>6</td>
<td>59.6 (42.6-73.3)</td>
<td>50 (20-80)</td>
<td>50</td>
<td>32 (15-35)</td>
<td>4 (2-5)</td>
<td>11 (4-13)</td>
<td>0.53 (0.2-0.87)</td>
</tr>
<tr>
<td>DBA/2</td>
<td>PB</td>
<td>6</td>
<td>9.9 (9.4-10.8)</td>
<td>50 (10-60)</td>
<td>50</td>
<td>20 (12-27)</td>
<td>11 (8-13)</td>
<td>ND</td>
<td>0.80 (0.67-3.0)</td>
</tr>
<tr>
<td></td>
<td>PBPC</td>
<td>6</td>
<td>72.9 (51.8-85.8)</td>
<td>40 (15-60)</td>
<td>60</td>
<td>62 (50-71)</td>
<td>5 (2-7)</td>
<td>20 (11-24)</td>
<td>1.13 (0.07-3.6)</td>
</tr>
<tr>
<td></td>
<td>BM</td>
<td>6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>14 (6-30)</td>
<td>ND</td>
<td>ND</td>
<td>4.83 (1.4-11.47)</td>
</tr>
</tbody>
</table>

Characterization of stem cell transplants as administered to leukemia-bearing Balb/c recipients. Medians and ranges are given for data obtained from the pooled grafts derived from 1 or 2 donors. The flow cytometry analysis was performed in parallel in 6 of 10 single transplantation experiments. The percentage of lymphocytes is given as the percentage of nucleated cells. Nucleated cells were also the basis for the calculation of graft size in all experiments. The MoAb 5E6 labels a subpopulation of NK cells. CFU-GM were counted on day +12 after seeding.

Abbreviations: MNC, mononuclear cells; ND, not determined.

after allogeneic MHC-matched PBPCCT than after syngeneic PBPCCT. Complete engraftment was achieved with a minimum cell dose of 5 x 10⁸ cells/kg BW after syngeneic PBPCCT; the same cell dose caused a graft failure rate of 19% after allogeneic PBPCCT. Thus, the immunogenetic difference was another parameter determining engraftment after PBPCCT. On the basis of fixed numbers of nucleated cells transferred, allogeneic BMT was followed by lower rejection rates (Fig 1) than allogeneic PBPCCT. The source of hematopoietic stem cells also influenced engraftment kinetics: a cell dose of 5 x 10⁷ cells/kg BW gave a substantial slower engraftment after PBPCCT (>2 leukocytes/mL on day 17) as compared with BMT (>2 leukocytes/mL on day 11). Long-term engraftment was investigated by immunofluorescence staining of recipient splenocytes with an anti-Lyt 1.1 antibody (CD5) and subsequent FACS analysis (Fig 3). Staining of the spleen cells of allo-PBPCCT recipients 100 days after transplantation showed the majority of T lymphocytes to be of donor origin in all surviving recipients tested (n = 10). High cell numbers of either origin, PBPC and BM, uniformly resulted in complete and very fast engraftment (>2 leukocytes/mL on day 7, Fig 2).

**GVHD.** The transplantation of 1 x 10⁸ cells/kg BW allogeneic MHC-matched PBPCs resulted in stable engraftment, but 4 of 16 (25%) nonleukemic animals died with signs of GVHD. Histological examination showed GVHD-typical infiltration in the liver and skin in these animals. After allogeneic BMT with 1 x 10⁷ cells/kg BW, 2 of 8 animals died on days 43 and 93 with signs of GVHD (Fig 4). This type of mortality was not observed after syngeneic PBPCCT. After depletion of the CD3⁺ cells from allogeneic PBPC transplants, only 1 of 17 animals (6%) died with macroscopic signs of GVHD. One of the animals died during the period of TBI-induced aplasia.

**GVL effect.** To evaluate the GVL effects after transplanting PBPCs or BM cells, the relapse risk of Balb/c mice bearing the lymphoid leukemia A20 and transplanted with PBPCs or BM cells was assessed. After intravenous injection of 1 x 10⁵ A20 cells, all untreated animals died after a median of 28 days. TBI with a dose of 7.5 Gy and subsequent syngeneic PBPCCT with 1 x 10⁶ cells/kg BW 2 days after leukemia cell injection resulted in a prolongation of the time to relapse to a median of 43 days; 20% of the animals remained free from leukemia. Transfer of 1 x 10⁶ cells/kg BW allogeneic MHC-matched PBPCs after identical pre-treatment of the recipients caused a significantly lower relapse rate with freedom from leukemia at 66% (P = .0045). Freedom from leukemia is 40% (median time to relapse, 58 days) after allogeneic BMT and thus significantly lower than after allogeneic PBPCCT (P = .032; Fig 5).

**DISCUSSION**

The animal data presented here address some of the important questions related to transplantation of allogeneic PBPCs. In particular, and in contrast to other murine21-23 and dog experiments,24 our model allows the investigation of the GVL activity of PBPCs as opposed to BM grafts. Results of animal experiments can seldom be directly translated into
the clinic because of inherent differences between the experimental setting and the clinical situation. In our model, recipients (Balb/c) and donors (DBA/2) of hematopoietic stem cells share the MHC complex but differ with respect to the rest of their genetic background. This situation most closely resembles the transplantation of hematopoietic stem cells between HLA-identical donor/recipient pairs, more so in the experimental setting than in the HLA-identical sibling setting. There are other limitations of the model pertaining to the composition of both marrow and PBPC grafts in mice and humans and their clinical implications. Although other parameters may be more important with respect to certain experimental or clinical endpoints, we chose to primarily characterize marrow and PBPC grafts by their content of nucleated cells and relate comparisons between BMT and PBCT to fixed numbers of nucleated cells present in either type of graft. This is practical and matches the way how clinical BM and PBPC grafts have been characterized in many instances.\textsuperscript{10,12,13} We additionally determined the progenitor cell and lymphocyte content of the grafts to enable a comparison of these parameters and their possible relevance with respect to engraftment potential, graft-versus-host reactivity, and GVL effect observed after BMT or PBCT.

We and others have previously shown that the progenitor cell dose in a graft is the decisive parameter to predict the engraftment potential of a hematopoietic stem cell graft if pretransplantation immunosuppression and the immunoge-
cludes any conclusive answer to the question if the relatively low relapse rates observed after PBPCT so far are the consequence of a more vigorous GVL effect. Reliable clinical data on the GVL effect after transplantation of unmanipulated or T-cell–depleted allogeneic PB stem cells will not be available for years because this will need long-term follow-up of a homogeneous patient population grafted with either PBPCs or BM. The results presented here suggest an advantage of PBPC over BM grafts with respect to their antileukemic activity. What are the possible explanations for this finding? First, there have been reports that priming with G-CSF can alter T-cell phenotype and function. It cannot be excluded that these differences not only affect graft-versus-host reactivity but also GVL activity. Second, it has repeatedly been reported that human PBPC grafts contain 10-fold more T cells and 19- to 25-fold more NK cells as compared with BM grafts.11-13 In this experimental system, even with a fixed number of nucleated cells administered, we also transferred more T cells and NK cells with PBPCs than with BM grafts.

Fig 4. Survival of nonleukemic Balb/c mice after TBI only (n = 12), transfer of 1 x 10⁸ syngeneic PBPCs/kg BW (n = 10), and transfer of the same dose of unmanipulated allogeneic PBPC (n = 16) or CD3-depleted allogeneic PBPCs (n = 16). T-cell depletion was performed by immunomagnetic removal of these cells by anti-CD3-coated magnetic beads, resulting in less than 1% CD3⁺ cells as shown by flow cytometry.

Netic difference between donor and recipient remain unchanged. In this series of experiments we were able to confirm that the transfer of high numbers of marrow or PB cells improve engraftment and engraftment kinetics. However, when the engraftment potential of BM cells and PBPCs was compared with identical numbers of nucleated cells in the grafts, BM cells resulted in faster and more reliable engraftment, a situation contradictory to clinical experience.9 This seemingly surprising result can easily be explained if one keeps in mind that, in the clinic, we aim to transplant the highest possible number of cells and do not transfer fixed numbers of nucleated cells, CD34⁺ cells, or colony-forming cells. However, the average number of nucleated cells contained in a PBPC collection product is 3 to 4 times higher than in a typical BM graft and thus can more than compensate for the relative scarcity of colony-forming cells in PB.

Acute and chronic graft-versus-host disease are less well defined and may even have a partly different pathogenesis in mice as compared with humans or dogs. Therefore, sublethal forms of GVHD were not taken into account for this analysis. With these limitations in mind, we did not find substantial differences between BM or PBPC grafts with respect to their GVHD-inducing potential. Roughly the same rates of mortality due to GVHD (25%) were observed after allogeneic BMT and PBPC. With approximately three times the number of T cells in a PBPC graft than in marrow, this is a surprising finding that is in line, however, with clinical observations reported so far.14,15 The model of the leukemia-bearing mouse allows to investigate the GVL effect conferred by allogeneic PBPCs as compared with allogeneic BMCs. This feature of the model is especially valuable because the heterogeneity of human recipients of allogeneic PBPCs in terms of their underlying disease and stage pre-

Fig 5. Freedom from leukemia of Balb/c mice injected with 1 x 10⁶ cells of the B-lymphocytic leukemia cell line A20. Recipients received no further treatment (n = 10) or received TBI with a dose of 7.5 Gy followed by transplantation of hematopoietic stem cells 2 days after leukemia cell injection. The transplants were either syngeneic PBPCs (n = 22), allogeneic BM cells (n = 23), or allogeneic PBPCs (n = 26).
tive cells but could result in a substantial increase of GVL-reactive cells. Further experiments using the experimental model reported here are underway to further elucidate the relative roles of T cells and NK cells triggering graft-versus-host reactivity and GVL activity. These experiments will hopefully shed further light on the basic biology of graft-versus-host reactivity and GVL effects and give some indication how allogeneic PBPC grafts should best be manipulated to avoid GVHD but preserve their antileukemic potential. The experiments reported here have encouraged us to continue with the clinical evaluation of allogeneic PBPC.

ACKNOWLEDGMENT
The authors thank S. Schlemminger for expert technical assistance.

REFERENCES
20. van Bekkum DW: What is graft versus host disease? Bone Marrow Transplant 7:110, 1991 (suppl 2)


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