Improved Immune Reconstitution After Allotransplantation of Peripheral Blood Stem Cells Instead of Bone Marrow

By H.D. Ottinger, D.W. Beelen, B. Scheulen, U.W. Schaefer, and H. Grosse-Wilde

Clinical studies are evaluating possible advantages of allogeneic peripheral blood stem cell transplantation (PBSCT) over bone marrow transplantation (BMT). We compared immune reconstitution after PBSCT (n = 20) and BMT (n = 20) in terms of lymphocyte subset counts and proliferative in vitro responses to mitogens and recall antigens (follow-up: 5 to 11 months posttransplant). Additionally, 10 PBSCT harvests and 10 marrow harvests were analyzed for their composition of immunocompetent cells. Compared with BMT patients, the PBSCT recipients had PB counts of naive (CD4+CD45RA+ and memory (CD4+CD45R0+) helper T cells and of B cells (CD19+) that were elevated (P < .003, P < .001, and P < .004, respectively) and proliferative responses to phytohemagglutinin (P < .0001), pokeweed mitogen (P < .02), Tetanus toxoid (P < .0005), and Candida (P < .004) that were increased. PBSCT recipients received a mean of 188 (range, 44 to 280) \( \times 10^6 \) naive helper T cells and 169 (range, 18 to 296) \( \times 10^6 \) memory helper T cells per kilogram; the corresponding numbers for BMT recipients were 11 (range, 4 to 24) and 10 (range, 1 to 22) \( \times 10^6 \) cells per kilogram, respectively. The question of whether the documented improved in vitro immune competence after PBSCT is associated with a lower incidence of infectious complications in vivo still needs further study.

The data on immune reconstitution presented here might aid in developing a recommendation.

PATIENTS AND METHODS

Patients and controls. The present prospective study enrolled the first 20 white adult patients undergoing PBSCT from related donors (HLA-identical, n = 12; partially HLA mismatched, n = 8) at our center from October 1994 to August 1995. The three main reasons to perform PBSCT instead of BMT were: (1) the presence of one or more known risk factors for graft failure in the patient, such as donorrecipient HLA-A, B, or DR disparities or prolonged (>12 months) pretreatment of chronic myelogenous leukemia (CML) patients with interferon-\( \alpha \); (2) a serious infectious disease documented in the patient within the last 4 months before transplantation (eg, aspergillosis, active tuberculosis); and (3) donor at increased risk for general anesthesia. Clinical characteristics of the study group are summarized in Table 1. The mean age of the study group was 39 years (range, 18 to 58).

Twenty consecutive white adults (10 male, 10 female) who received unmodified allogeneic marrow from HLA identical siblings during the same period served as controls. Eighteen controls were transplanted for hematologic malignancies (chronic myelogenous leukemia, acute myelogenous or lymphatic leukemia, myeloproliferative syndromes) and two for severe aplastic anemia. The mean age of control patients was 34 years (range, 18 to 57).

Identical regimens for conditioning (4 x 2.5 Gy fractionated total body irradiation followed by 60 mg/kg intravenous [IV] cyclophosphamide on each of 2 consecutive days) and GVHD prophylaxis (cyclosporin 3 mg/kg/d IV and a short course of methotrexate IV, 15 mg/m\(^2\)/d on day 1 and 10 mg/m\(^2\)/d on days 3, 6, and 11 posttransplant) were used in both groups. After PBSCT, brain not after BMT, G-CSF (5 /\( \mu \)g/kg/d) was administered until leukocyte recovery (>1.000/\( \mu \)L, on 3 consecutive days), which occurred in all but one patient within 20 days. For stem cell mobilization, donors received G-CSF (10 to 16 /\( \mu \)g/kg/d on days 0 through 5). Leukaphereses were performed on days 5 and 6.

Quantitation of PBSC subsets. Total white blood cell (WBC) counts and WBC differential counts were determined by an electronic, automatic counter (STKS; Coulter, Krefeld, Germany). For evaluation of mononuclear cell subsets, two- and three-color (FITC, PE, PerCP) flow cytometric immunophenotyping was performed as described previously. T cells (CD3+), helper/inducer T cells (CD3+CD4+), naive (CD3+CD4+CD45RA+) and memory (CD3+CD4+CD45R0+) helper/inducer T cells, suppressor/cytotoxic T cells (CD3+CD8+), B cells (CD19+), monocytes (CD45+CD14+), and natural killer (NK) cells (CD3-CD16+ and/or CD56+) were analyzed on an EPICS XL3 (Coulter) cell sorter. Mouse monoclonal antibodies (MoAbs) directly conjugated to fluorochromes were used. CD3, CD4, CD45RA, CD8, and CD19 MoAbs were purchased from Coulter, and all other MoAbs were from Becton Dickinson (Heidelberg, Germany). In brief, the appropriate amounts and combinations of undiluted MoAbs were added to 100 /\( \mu \)L of whole blood (EDTA collection tubes; Sarstedt, Essen, Germany) followed by an incubation of 15 minutes at room temperature. Red blood cells were then lysed (Immunoprep; Coulter). Small lymphocytes, monocytes, and granulocytes were gated on using log side scatter (SSC) and linear forward scatter (FSC) characteristics. Mono-
cyte contamination of the gated lymphoid cells to be analyzed was ruled out by their lack of reactivity (<1%) with anti-CD14 antibodies. Absolute counts for each cellular subset were calculated as the product of the absolute WBC count and lymphocyte differential percentage (derived from the electronic, automatic counter) and the cellular subset percentage (as evaluated by flow cytometry).

Lymphocyte transformation test (LTT)-mitogens and LTT-antigens. In parallel, cellular in vitro responses to mitogens (phytohemagglutinin [PHA] and pokeweed mitogen [PWM]) and recall antigens (Candida, herpes simplex virus [HSV-1], Tetanus toxoid) were quantified using standardized assay formats (LTT-mitogen and -antigen; BAG, Lich, Germany). In brief, 20 mL of heparinized mononuclear cells (PBMCs) were collected after centrifugation on Ficoll-Paque density gradients (Pharmacia, Uppsala, Sweden).12

PB mononuclear cells (PBMCs) were collected after centrifugation on Ficoll-Paque density gradients (Pharmacia, Uppsala, Sweden).12 The lyophilized mitogens and antigens on BAG microtiter plates were reconstituted by adding 200 μL of cell culture medium per well (180 μL RPMI 1640; GibCO, Life Technologies, Paisley, UK, plus 20 μL of inactivated pooled human serum). Fifty thousand PBMCs suspended in 10 μL RPMI were added per well. Mitogen and antigen cultures were grown in quadruplicates for 3 days and in sextuplicates for 5 days, respectively (37°C, 5% CO₂). For the following 16 hours the cultures were labeled with 2 pCi H'-thymidine (Amersham Buchler, Braunschweig, Germany). At the end of incubation cultures were harvested (96 well automatic harvester; Skatrol, Lier, Norway) onto glass fiber filtermats (Wallac, Turku, Finland) without delay. For the determination of incorporated radioactivity a liquid scintillation β-counter was used (1205 Betaplate; Wallac). For evaluation, the mean activity of the mitogen and of the antigen cultures was calculated. Hereby, for correction of outliers, the two mitogen cultures and the two antigen cultures exhibiting lowest and highest activity were disregarded. “Background” activity was determined as the mean activity of 4 (mitogen plate) and of 6 (antigen plate) autologous controls, respectively. Results were expressed as mean counts per minute (cpm) “increment,” ie, the mean of the measured activities (cpm) minus “background” (cpm).

In situ hybridization. To identify the origin of proliferating cells, x/y chromosome analysis was performed in case of donor/recipient sex mismatch (Spectrum CEP; Vysis, Stuttgart, Germany).

Quantitation of immune cells in PBSC and marrow harvests. Additionally, the diverse subsets of immunocompetent cells were also quantified in 10 consecutive donor stem cell harvests and in 10 marrow grafts using flow cytometry as outlined above. Results were expressed as number of cells infused per kilogram of body weight of the recipient.

Statistical analysis. Repeated measures analysis of variance was used. 15 Cut-off for evaluation was January 15, 1996.

RESULTS

Levels of BPC subsets. CD3+CD4+ T-cell counts were significantly higher after allogeneic PBSC compared with BMT (P < .004, Fig 1A). Interestingly, this difference persisted long term (factor 2.9, 2.2, and still 1.2 at 1, 9, and 11 months after transplant, respectively) and was seen for both the naive and the memory CD3+CD4+ T-cell subsets (Fig 1B, P < .003 and Fig 1C, P < .001, respectively). In contrast, the levels of CD8+ T cells did not differ significantly after PBSC and BMT (Fig 1D). Consequently, the reversal of the CD4:CD8 ratio characteristic for allogeneic BMT could not be observed after allogeneic BMT (Fig 1E, P < .0001).

The CD19+ B-cell levels were also found to be clearly higher after PBSC compared with BMT (Fig 1F, P < .004). Mean monocyte counts rapidly reached the reference range after PBSC and BMT as well and even exceeded the upper limit of normal early after PBSC (Fig 1G, P < .02 for the
Fig 1. Phenotypic reconstitution of mononuclear cell subsets after allogeneic transplantation of PBSCs (n = 20, □) and of BM (n = 20, □). PBC counts (mean ± SEM) of helper (CD4+ T cells, naive (CD4+CD45RA+) and experienced (CD4+CD45RO+) helper T cells as well as CD8+ T cells, CD19+ B cells, NK cells, and monocytes are shown (A through D and F through H, respectively). Additionally, our data on CD4:CD8 ratios are summarized in (E). Shaded area: Reference range, ie, 5th to 95th percentile of 60 healthy age-matched white donors. Three patients of the PBSCT group died within 2 months after transplant and 1 patient at 4 months after PBSCT. Among BMT patients, 4 died at month 5, 6, 7, and 9 post-transplant, respectively. According to the limited periods of follow-up the number of observations decreased from n = 20 (1 month posttransplant) to n = 5 (11 months posttransplant).

interval months 0 to 2). Among the 10 documented PBSCT patients with monocyte counts >10,000/µL, 5 were on G-CSF 3 to 5 days before the monocyte count determination. In the other 5 cases, however, monocytosis was persisting for more than 5 days after discontinuation of G-CSF, namely for 6, 18, 26 (2 cases), and 30 days, respectively. Monocyte counts after PBSCT and after BMT (0 to 2 months) remained significantly different (P = .044) even after exclusion of the 5 patients mentioned above who were on G-CSF 3 to 5 days before monocyte counting. For completeness, NK cell levels were similar after PBSCT and BMT (Fig 1H).

Cellular in vitro responses to mitogens and recall antigens. Responses to PHA and PWM were clearly enhanced after PBSCT compared with BMT (Fig 2A, P < .0001 and Fig 2B, P < .02, respectively). The same was true for Tetanus toxoid and Candida (Fig 2C, P < .0005 and Fig 2D, P < .0001).
Furthermore, we documented a switch from negative to positive in vitro response against HSV-1 in 3 PBSCT patients at month 4 (1 case) and at month 7 (2 cases) after transplantation from HSV-1 nonreactive donors. In 2 of these patients an acute HSV infection posttransplant had been diagnosed on clinical grounds. Comparable cases were not seen among BMT controls. Sex chromosome analysis in the responding cells of 5 study patients (including 2 patients nonresponsive to Tetanus toxoid before transplantation, but grafted with a Tetanus toxoid reactive donor and 1 of the above cases with switch of response to HSV-1) showed donor origin in all cases.

**Numbers of immunocompetent cells in donor stem cell and in marrow harvests.** Finally, 10 PBSC harvests and 10 marrow harvests were analyzed for their composition of immunocompetent cells. Results of the different cellular subsets were expressed as absolute numbers of cells in the harvest divided by the body weight (kilograms) of the recipient. Total cell numbers transplanted were consistently higher in PBSCT recipients. As illustrated in Table 2, this involved all lymphocyte subsets studied. For most subsets analyzed, the number of transfused cells per kilogram was more than one order of magnitude higher in PBSCT than in BMT recipients. For monocytes and naive as well as memory helper T cells the differences were most pronounced with ratios of 24, 17, and 17, respectively.

**DISCUSSION**

In this study we analyzed immunologic recovery in allogeneic transplant recipients given either BM or G-CSF-stimulated PBSCs from a related donor.

In controls (after BMT) we found the PB counts of T helper cells to be long-term low posttransplant, whereas the levels of CD8+ cells, monocytes, and NK cells returned quickly to the reference ranges (5th to 95th percentile of 60

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age-matched healthy donors), confirming the data reported previously by Atkinson. However, in contrast to the Sydney group, we and others documented the B-cell levels to be decreased for a period clearly exceeding 1 month post BMT.

An important finding of this study was that after PBSCT the PBC counts of naive and of memory helper T cells significantly exceeded those observed after BMT (Fig 1B and C). At present, we have no direct experimental evidence of whether the augmented pool of T cells circulating in PBSCT recipients is derived from mature T cells infused with the graft or from transplanted blood stem cells that have differentiated. However, two findings of this study point to a role of infused mature cells. Firstly, the mean quantity of T-helper cells in a PBSCT graft is 17 times higher than in a conventional BMT harvest (Table 2). Secondly, the observed sustained in vitro responses of donor T cells to Tetanus toxoid after PBSCT support the view that infused mature, namely memory T cells, do survive long-term in the recipient. The documented proliferative response to Tetanus toxoid could hardly be mounted by donor T cells derived from transfused stem cells because a new encounter of Tetanus antigen in vivo posttransplant appears highly improbable in the clinical setting used. The monocyte peak observed early after PBSCT (Fig 1G) might be caused by the combination of high numbers of infused monocytes and stimulation of myelopoiesis by G-CSF. Monocytosis early after PBSCT could hardly be attributed to G-CSF alone, because it persisted in 5 of 10 PBSCT patients for 6 to 30 days after discontinuation of the drug.

Another aspect was the enhanced (and sustained) in vitro responses to the mitogen PHA and PWM as well as to the recall antigens Candida, HSV-1, and Tetanus toxoid after PBSCT compared with BMT.

The elevated cellular responses of PBSCT patients to PHA and PWM might be explained simply by their higher levels of circulating memory helper T cells, because lectins were shown to stimulate preferentially this particular cellular subset. However, the specific cellular in vitro responses toward recall antigens in most of the study patients point to an adoptive immune transfer. The observed in vitro responses to Tetanus toxoid post PBSCT can be explained by memory T cells transfused from donors who have received tetanus vaccine. Furthermore, the documented switch of donor cells posttransplant from a negative to a positive in vitro response to HSV-1 after an acute HSV infection of the recipient suggests that naive HSV-1 specific donor T cells have differentiated into memory T cells after antigen encounter in vivo. These findings suggest that the immune repertoire of the donor is transferred earlier to the recipient by PBSCT than by BMT. In conclusion, PBSCT may accelerate immunologic reconstitution. However, the question of whether the improved in vitro immune competence after PBSCT is associated with a lower incidence of infectious complications in vivo still needs further study.

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