To the Editor:

Insufficient graft function is an infrequent but serious complication of unmanipulated HLA-matched bone marrow transplantation (BMT). Therapy of graft failure includes a second marrow transplantation and, more recently, the administration of human recombinant growth factors (colony-stimulating factors [CSF]). However, in a considerable number of patients sustained graft function cannot
be established by these measures. As the addition of CSF-mobilized peripheral blood progenitor cells (PBPC) to autologous bone marrow (BM) grafts markedly improves hematopoietic recovery, infusion of donor-derived PBPC may also help to overcome insufficient hematopoiesis after allogeneic BMT: here we report the first case of successful transplantation of granulocyte CSF (G-CSF) – mobilized allogeneic PBPC after the repeated failure of CSF-promoted BM grafts.

A 47-year-old cytomegalovirus (CMV)-positive female with AML in first CR received a BM graft (cell dose $1.56 \times 10^8$/kg) from her HLA-matched blood group mismatched sister (donor A, recipient B) after standard conditioning with 12 Gy TBI and 120 mg/kg cyclophosphamide. Prophylaxis for graft-versus-host disease (GVHD) consisted of cyclosporine A and short course methotrexate. Although restriction fragment length polymorphism (RFLP) typing of marrow aspirates showed DNA of donor origin, there was no clinical evidence of engraftment (white blood cell [WBC] count < 0.1/nL, BM cellularity < 5%). On day +32, a second BM graft from the same donor (cell dose $2.46 \times 10^9$/kg) was given after preparation with anti-thymocyte globulin ($4 \times 0.5$ mL/kg Lymphoglobuline; Pasteur Merieux, Lyon, France). To promote marrow recovery, granulocyte-macrophage CSF (GM-CSF) ($250 \mu$g/m$^2$; Sandoz, Nuremberg, Germany) was administered from day 0 to +18 and interleukin-3 (IL-3, $10 \mathrm{pg/kg}$; Sandoz) from day +19 to +35 after the second BMT. Peripheral blood leukocytes appeared on day +6, and the WBC count increased rapidly to 15/nL, although the BM cellularity remained poor (<5%). The patient developed mild acute GVHD, which was limited to the skin (grade I). When IL-3 was stopped, a rapid decrease of the WBC count to <0.5/nL occurred, which proved irreversible despite a further course of IL-3. At this time, CMV cultures from throat and urine became positive, and serology demonstrated development of anti-CMV-IgM. Analysis of blood leukocytes for CMV DNA by polymerase chain reaction (PCR) confirmed active CMV infection, indicating a possible role of CMV in BM failure. Thus, ganciclovir was administered for 4 consecutive days before infusion of PBPC.

PBPC were mobilized from the marrow donor by daily subcutaneous injections of G-CSF ($6 \mu$g/kg; Amgen, Munich, Germany), which resulted in a leukocytosis of 38 to 50 cells/nL. On days 7, 8, and 9 after initiation of G-CSF, three leukapheresis procedures yielded a total number of $13.52 \times 10^8$/kg mononuclear cells. The leukapheresis product contained $4.04 \times 10^8$/kg CD3+ cells and $8.16 \times 10^6$/kg CD34+ cells and was infused without further processing. The donor tolerated the mobilization and cell separation procedures well.

Because RFLP typing of peripheral blood and bone marrow had demonstrated only donor bands (see Fig 2), PBPC transplantation was performed without further immunosuppressive therapy on days 91 to 93 after first BMT. G-CSF ($10 \mu$g/kg) was administered for 26 days after PBPC infusion. The WBC count exceeded 1.0/nL on day +11, and BM cellularity was 10% on day +14. Moderate acute GVHD (grade II, skin) started on day +18. Intensification of immunosuppressive treatment with high-dose steroids (16 mg/kg) and anti-IL-2 receptor monoclonal antibody (Biotest, Dreieich, Germany) was followed by a decrease of the WBC count to 1.5/nL; CMV-PCR became positive again, suggesting a reactivation of CMV infection (Fig 1). After cessation of the monoclonal antibody, reduction of the prednisolone dose (1.5 mg/kg with slow tapering), antiviral treatment (ganciclovir day +37 to +49), and renewed G-CSF administration (day +39 to +58), a stable WBC count (3 to 4/nL) was achieved. On day +66, BM histology showed a cellularity of 15% with trilineage engraftment. RFLP typing demonstrated DNA of donor origin only (Fig 2). Subsequently, the BM function improved slowly; the patient
Identification of posttransplant cell populations by RFLP analysis after PBPC transplantation. DNA was isolated from blood or BM specimen, digested with Hind, electrophoresed in a 0.8% agarose gel, and directly hybridized in the dried gel with probe O-AY-29. (A) Analysis before PBPC infusion (from left to right): 1. BM donor; 2. peripheral blood day + 91; 3. BM day + 60; 4. peripheral blood day + 60; 5. peripheral blood day + 50 after first BMT; 6. BM recipient pre-BMT; 7. peripheral blood recipient pre-BMT. (B) Analysis after PBPC infusion (from left to right): 1. BM donor; 2. peripheral blood day + 56; 3. BM day + 56; 4. peripheral blood day + 28; 5. BM day + 14 after PBPC infusion; 6. BM recipient pre-BMT. Only donor DNA is detectable after PBPC transplantation.

now (day +260) has a WBC count of 7.5/nL without growth factor support and is independent of platelet and red blood cell (RBC) support. BM histology shows trilineage engraftment (cellularity 100%) without evidence of leukemia. As blood group serology and RFLP typing revealed, hematopoiesis is exclusively donor derived. CMV DNA is absent from peripheral blood leukocytes. A generalized mild maculo-squamous exanthema is consistent with chronic GVHD, as confirmed by skin biopsy.

Peripheral blood progenitor cells, mobilized by CSF and/or cytotoxic chemotherapy, can be harvested for the purpose of stem cell rescue after myeloablative high-dose chemotherapy. The resulting preparations usually contain high numbers of progenitors when compared with conventional BM grafts.\textsuperscript{2} Infusion of growth factor-mobilized PBPC markedly accelerates blood cell recovery after autologous BMT,\textsuperscript{24} indicating that PBPC can add substantial hematopoietic potential to marrow grafts. The course of events in this patient (see Fig 1) demonstrates that otherwise refractory graft failure after allogeneic BMT can be overcome by infusion of G-CSF-mobilized PBPC, even in a situation where the chance of successful engraftment of BM cells would appear very low. Severe GVHD did not develop although the extremely high number of T cells infused with the PBPC caused considerable concern in that direction. In particular, the intensity of acute GVHD was not worse than the GVHD episode after the second marrow graft, suggesting that the quality (ie, specificity) rather than the quantity of donor T cells seems to be the more important determinant influencing GVHD intensity. On the other hand, the T cells contained in the PBPC inoculum may have facilitated engraftment.\textsuperscript{3} Since recurrent CMV viremia was demonstrated in our patient, CMV infection may have contributed to the poor function of the BM graft.\textsuperscript{6} Thus, it cannot be ruled out that hematopoietic recovery after PBPC infusion was influenced by control of CMV infection. However, the striking increase of the leukocyte count at a time typically expected after PBPC infusion, and the fact that graft function was not grossly affected during subsequent renewed CMV reactivation argue against this interpretation.

Taken together, this case indicates that allogeneic PBPC transplantation is feasible without causing detrimental GVHD and may be useful, at least in situations in which a BM graft fails or is not available.

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PETER DREGER
MEINOLF SUTTORP
TORSSEN HAFLERLACH
HELMUT LöffLER
NORBERT SCHMITZ
Bone Marrow Transplantation Unit
and Second Department of Medicine
University of Kiel
Kiel, Germany
WILFRIED SCHROYENS
Department of Hematology
University of Gießen
Gießen, Germany
REFERENCES


Allogeneic granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells for treatment of engraftment failure after bone marrow transplantation [letter]

P Dreger, M Suttrop, T Haferlach, H Loffler, N Schmitz and W Schroyens