

Letter to the Editor

Autologous Hematopoietic Recovery with Aberrant Antigen Expression after Allogeneic Bone Marrow Transplantation

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TO THE EDITOR

A 50-year-old woman was admitted in December 2006 with progressive petechiae. Bone marrow (BM) aspiration showed massive proliferation of leukemic myeloblasts with myeloperoxidase staining. Three-color flow cytometry (FCM) with a CD45 gate for the BM cells was performed in our laboratory¹; the blasts were positive for CD7, CD11c, CD13, CD15, CD33, myeloperoxidase and HLA-DR and negative for CD34 and CD117. Karyotypes of the BM cells were normal. A diagnosis of acute myeloblastic leukemia (AML)-M2 was made on the basis of French-American-British classification. The patient needed standard induction chemotherapy twice to achieve complete remission. Refractoriness to platelet transfusions due to anti-HLA antibody developed. She received an allogeneic BM transplant from an ABO-matched and HLA-DR-mismatched unrelated female donor in September 2007. The conditioning regimen consisted of total body irradiation (2 Gy twice daily for 3 days) followed by cyclophosphamide (60 mg/kg/day for 2 days). On day 0, 4.0×10^8 BM cells per recipient body weight were infused. Tacrolimus and short-course methotrexate were used as prophylaxis for graft-versus-host disease. After BM transplantation, severe pancytopenia persisted. BM aspiration on day 22 revealed marked hypocellularity, suggesting graft rejection. Peripheral blood neutrophils gradually increased from day 50. BM aspiration smears on day 75 showed recovery of the BM

cells, especially myeloid cells, without significant morphologic abnormalities and proliferation of myeloblasts, indicating that complete remission was maintained. Chromosomal analysis of the BM cells showed various cytogenetic abnormalities (Table 1). A chimerism-based analysis of the BM cells using short tandem repeat-polymerase chain reaction showed that 100% of the cells originated from the recipient. A diagnosis of autologous (recipient) hematopoietic recovery after graft rejection was made. Multiparametric FCM based on a four-color method (ReproCELL, Yokohama, Japan), which had been approved by the Jichi Medical University Institutional Review Board (no. 06-70), did not show the abnormal expression of antigens in the blasts in the BM on days 75 and 183. However, it showed small populations of CD34⁺CD7⁺ cells and CD34⁺CD15⁺ cells in the blasts on day 253 (Fig. 1). The latter cells were characterized by a high intensity of CD34, which indicates abnormal expression of the antigen. BM aspiration was performed every three to six months; various chromosomal abnormalities of BM cells were found in each sample (Table 1). After day 253, routine three-color FCM instead of the multiparametric FCM was conducted to analyze phenotypes of the blasts in the BM. This FCM can detect CD34⁺CD7⁺ cells but not CD34⁺CD15⁺ cells because of antibody combinations. The proportions of CD34⁺CD7⁺ cells among the blasts of the BM were as follows: on day 323, 37.2%; on day 421, 30.9%; on day 603, 35.5%; on day 785, 21.5%; on day 975, 49.2%; on day 1,149, 36.0%; and on day 1,232, 24.5%. Although chromosomal abnormalities were detected on and after day 75, obvious dysplastic features associated with myelodysplastic syndrome (MDS) were not found. The patient is clinically well with normal peripheral blood cell counts.

There are several reports on autologous (recipient) hematopoietic recovery after allogeneic hematopoietic stem cell transplantation.²⁻⁶ All patients received total body irradiation

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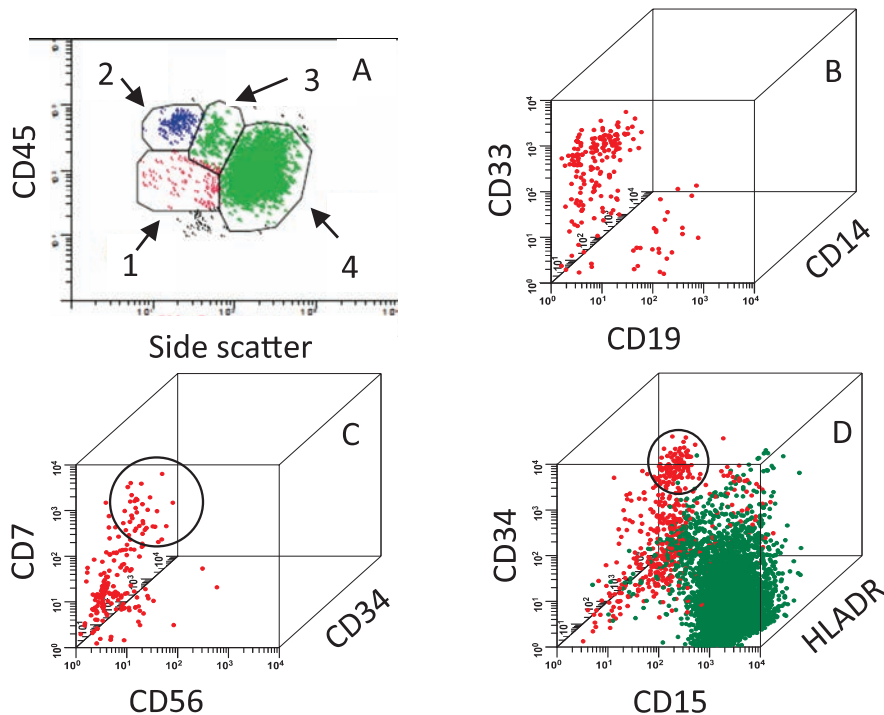


Fig. 1. Flow cytometric analysis of bone marrow cells on day 253 after bone marrow transplantation. 1, blasts ; 2, lymphocytes ; 3, monocytes ; 4, granulocytes. Antigen levels in the blasts are plotted in *IB* (right upper), *IC* (left lower) and *ID* (right lower), while those in granulocytes are plotted in *ID*. The cycle indicates the blasts expressing abnormal antigens.

with a total of 12 Gy or more as conditioning. In these patients, various chromosomal abnormalities in the BM cells were found to be associated with autologous hematopoietic recovery. Because chromosomal abnormalities in these patients were random and not related to the patients' underlying diseases, such aberrations indicate that normal hematopoietic progenitors may have been injured by the irradiation used for conditioning. In our case, various chromosomal abnormalities were found concomitantly with small populations of CD34⁺CD7⁺ cells and CD34⁺CD15⁺ cells in the BM, which have been used as markers for aberrant antigen expression in AML.⁷⁻¹⁰ Taking these findings together, the CD34⁺CD7⁺ cells and CD34⁺CD15⁺ cells in the blasts of our patient were derived from injured normal hematopoietic progenitors that have self-renewal activity. Although the reason why neither CD34⁺CD7⁺ cells nor CD34⁺CD15⁺ cells were detected on day 75 or 183 is not known, it may be due to clonal changes to the injured hematopoietic stem cells. To the best of our knowledge, there is no report on progression to MDS or AML in patients who showed autologous hematopoietic recovery.²⁻⁶ It is necessary to follow up the patient carefully for the long term to clarify whether the disappearance of CD34⁺CD7⁺ cells in the BM leads to the normalization of BM karyotypes or an increase in these cells causes hematopoietic diseases includ-

ing MDS and AML.

References

- 1 Oka S, Muroi K, Matsuyama T, Sato K, Ueda M, *et al.* : Correlation between flow cytometric identification of CD33-positive cells and morphological evaluation of myeloblasts in bone marrow of patients with acute myeloblastic leukemia. *Hematology* 14:133-138, 2009
- 2 Lin YW, Hamahata K, Watanabe K, Adachi S, Akiyama Y, *et al.* : Repetitious appearance and disappearance of different kinds of clonal cytogenetic abnormalities after allogeneic bone marrow transplantation. *Int J Hematol* 74:86-89, 2001
- 3 Kebraie P, Winter JN, Laport GG, Le Beau MM, Dewald G, *et al.* : Multiple unrelated clonal abnormalities in host bone marrow cells after allogeneic stem cell transplantation. *Leuk Res* 28:537-540, 2004
- 4 Yoshihara T, Hibi S, Yamane Y, Morimoto A, Hashida T, *et al.* : Numerous nonclonal chromosomal aberrations arising in residual recipient hematopoietic cells following allogeneic bone marrow transplantation. *Bone Marrow Transplant* 35:587-589, 2005
- 5 Masuko M, Furukawa T, Yersser O, Narita M, Toba K, *et al.* : Persistence of various chromosomal aberrations in recipient cells during complete remission after bone marrow transplantation fol-

Table 1. Karyotypes of the bone marrow cells before and after bone marrow transplantation

| Day | Karyotype |
|--------------|---|
| At diagnosis | 46, XX (20/20) |
| Day-14 | 46, XX (20/20) |
| Day 22 | No metaphases because of insufficient specimen |
| Day 75 | 46, X, -X, add(1)(p32), -5, add(6)(q21), add(11)(p15), +mar1 (4/16) 46, XX, -8, del(15)(q22), +mar2 (2/16) 46, XX (10/16) |
| Day 183 | 45-46, X, X, -2, -6, -10, -16, -22, +1-3mar (8/20) 46, XX (12/20) |
| Day 253 | 46, XX, add(7)(p11) (7/17) 46, XX, add(7)(q32) (5/17) 46, XX, t(3;15)(q29;q15) (1/17) 46, XX(4/17) |
| Day 323 | No metaphases because of insufficient specimen |
| Day 421 | 46, XX, -3, add(7)(q32), +1 (3/5) 46, XX (2/5) |
| Day 603 | 46, XX, -3, add(7)(q32), +1-5mar (3/5) 46, XX (2/5) |
| Day 785 | 45, XX, add(11)(p15), -22, +mar (4/9) 46, XX, del(10)(q24) (3/9) 44-45, XX, add(7)(q36) (2/9) |
| Day 975 | 46, XX, add(3)(q23), add(7)(q32) (2/6) 46, XX (4/6) |
| Day 1,149 | 45-46, XX, -7, -9, +1-8mar (4/7) 46, XX (3/7) |
| Day 1,232 | 46, XX (3/4) AK (1/4) |

AK, unidentified abnormal karyotypes.

The numerators and denominators in parentheses indicate identified karyotype numbers and total metaphase numbers.

- lowed by graft rejection. *Leuk Res* 29:1083-1087, 2005
- 6 Kim IS, Kim HJ, Yoo KH, Sung KW, Kim SH : A boy with acute lymphoblastic leukemia acquired clonal and nonclonal cytogenetic abnormalities including del (7q) and del (20q) without clinical evidence of disease after sex-mismatched cord blood transplantation. *J Pediatr Hematol Oncol* 28:540-543, 2006
 - 7 Macedo A, Orfao A, Ciudad J, Gonzalez M, Vidriales B, *et al.* : Phenotypic analysis of CD34 subpopulations in normal human bone marrow and its application for the detection of minimal residual disease. *Leukemia* 9:1896-1901, 1995
 - 8 Fritsch G, Stimpfl M, Kurz M, Printz D, Buchinger P, *et al.* : The composition of CD34 subpopulations differs between bone marrow, blood and cord blood. *Bone Marrow Transplant* 17:169-178, 1996
 - 9 San Miguel JF, Martínez A, Macedo A, Vidriales MB, López-Berges C, *et al.* : Immunophenotyping investigation of minimal residual disease is a useful approach for predicting relapse in acute myeloid leukemia patients. *Blood* 90:2465-2470, 1997
 - 10 Ossenkoppele GJ, van de Loosdrecht AA, Schuurhuis GJ : Review of the relevance of aberrant antigen expression by flow cytometry in myeloid neoplasms. *Br J Haematol* 153:421-436, 2011