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Case report

Acute leukemia of donor origin arising after stem cell transplantation for acute promyelocytic leukemia

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Abstract

We report a patient with PML/RARα-positive acute promyelocytic leukemia (APL) who developed PML/RARα-negative acute myeloid leukemia 37 months after allogeneic bone marrow (BMT) transplant for molecular relapse. Features of myelodysplasia were noted 11 months earlier, chimerism testing by analysis of short tandem repeats was consistent with development of myelodysplasia and acute leukemia within cells of donor origin. To our knowledge, this is the first report of donor cell leukemia following BMT for APL. We hypothesize that replicative stress may lead to the development of some cases of donor cell acute leukemia.

Keywords: Donor cell leukemia; Acute promyelocytic leukemia; Allogeneic bone marrow transplantation

1. Introduction

Recurrence of acute leukemia following allogeneic bone marrow transplantation (BMT) most frequently results from outgrowth of residual host tumor cells that evade eradication during or following the transplant procedure. In a minority of cases a secondary acute leukemia may arise in cells of donor origin, although the exact incidence of this complication is unclear. Donor cell acute leukemia is uncommonly diagnosed, as methods to determine the donor or host origin of the cells involved in the malignant process reliably have been routinely applied only recently [1].

The events leading to the development of secondary malignancies after bone marrow transplantation have been reviewed [2]. Exposure to Epstein–Barr virus in the setting of reduced immune surveillance is a well-established cause of post-transplant lymphoproliferative disease, while exposure to high-dose chemotherapy and radiotherapy are believed to increase the risk of epidermal malignancies substantially. The events leading to the development of donor cell acute leukemia after allogeneic BMT have not been well described. Reduced immune surveillance and the transfer of leukemogenic viruses or DNA from host cells to those of the donor have been proposed as possible mechanisms of leukemogenesis following transplantation, although to date no such viruses have been identified in cases of donor cell acute leukemia. Immune surveillance, difficult to evaluate even outside of the post-transplant setting, has not been examined in patients with donor cell acute leukemia. In this report we describe a patient who underwent BMT for a molecular relapse of acute promyelocytic leukemia and who subsequently developed myelodysplastic syndrome with transformation to acute leukemia after one year of clinical observation. The secondary acute leukemia was shown to have arisen in cells of donor origin. The long period of myelodysplasia suggests that the acquisition of multiple genetic "hits" may be necessary for the development of some cases of donor-derived acute leukemia following allogeneic BMT.

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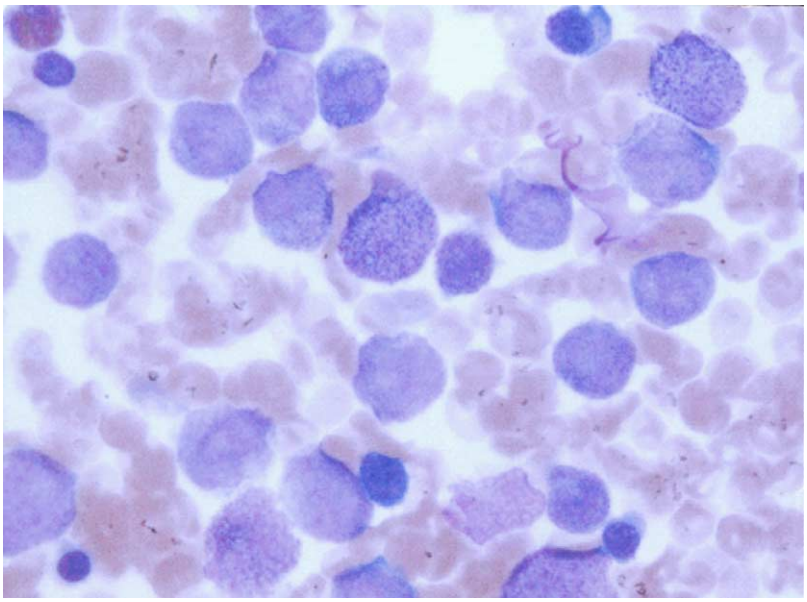

Fig. 1. Morphology of a Wright–Giemsa stained bone marrow sample obtained at the time of diagnosis of APL demonstrating heavily granulated abnormal promyelocytes.

2. Patient and methods

A 44-year-old woman with PML/RARα-positive acute myelocytic leukemia (APL) (Fig. 1) received all-trans retinoic acid (ATRA) 40 mg/m^2 daily, Daunorubicin 60 mg/m^2 daily for 3 days and Cytarabine 200 mg/m^2 daily by continuous infusion for 7 days. Treatment with ATRA continued uneventfully for 30 days, and a repeat bone marrow aspirate demonstrated a morphological and molecular complete remission. Two cycles of consolidation with the above regimen were given. Treatment was completed in December 1998 and the patient did not receive maintenance therapy.

In February 1999, a follow-up sample of bone marrow was once again positive for PML/RARα fusion transcripts by RT-PCR, although the patient remained in morphological complete remission. Treatment with ATRA 40 mg/m^2 daily was given and after a molecular complete remission was achieved the patient underwent BMT from her

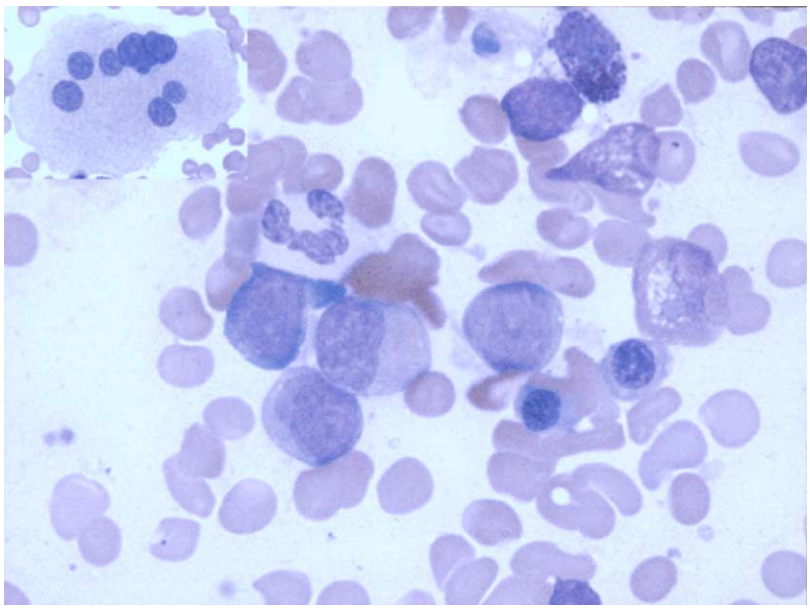

Fig. 2. Morphology of a bone marrow sample obtained at the time of diagnosis of donor cell leukemia demonstrating blasts, a hypogranular neutrophil and a dysplastic megakaryocyte (insert).

genotypically HLA-matched half-sister. Transplantation was carried out in May 1999, following a conditioning regimen consisting of Cyclophosphamide 60 mg/kg IV daily for three days and 1200 cGy total body irradiation in six fractions over three days. Infusion of 1.76×10^8 kg/L unmanipulated bone marrow mononuclear cells took place after the last dose of radiation. Graft-versus-host disease (GVHD) prophylaxis consisted of standard Cyclosporine A and Methotrexate. The neutrophil count exceeded 0.5×10^9/L on day +21 and grade 3 acute GVHD occurred on day +26. GVHD responded to treatment with Prednisone 1.25 mg/kg daily. On day +60 a repeat bone marrow aspirate demonstrated continued morphological remission and was once again negative for PML/RARα fusion transcripts by RT-PCR.

New onset of anemia (hemoglobin 83 g/l) and thrombocytopenia (platelets 111×10^9/L) was first noted in July 2001, approximately 26 months after transplantation. No blast cells were identified on microscopic examination of the peripheral blood or bone marrow, and RT-PCR testing for PML/RARα fusion transcripts was negative. Features of trilineage dysplasia were identified on examination of bone marrow. Serology for Parvovirus B19 was negative and the cytopenias did not resolve with intravenous immunoglobulin infusions. The patient required transfusions of packed red blood cells on a bi- or tri-weekly basis. After 4 months of cytopenias, repeat bone marrow examination demonstrated continued trilineage dysplasia, now with the presence of 5% blast cells. Cytogenetic testing of bone marrow revealed a normal female karyotype. The patient's blood counts remained unchanged and she continued to receive regular red blood cell transfusions until June 2002 when blast cells were noted on examination of the peripheral blood. Bone marrow examination at this time (Fig. 2) demonstrated the presence of 20% blast cells in addition to marked trilineage dysplasia. The sample failed to produce dividing cells for cytogenetic analysis, and fluorescence in-situ hybridization (FISH) for rearrangement of the *MLL* gene was negative. Flow cytometry carried out on a sample of the patient's bone

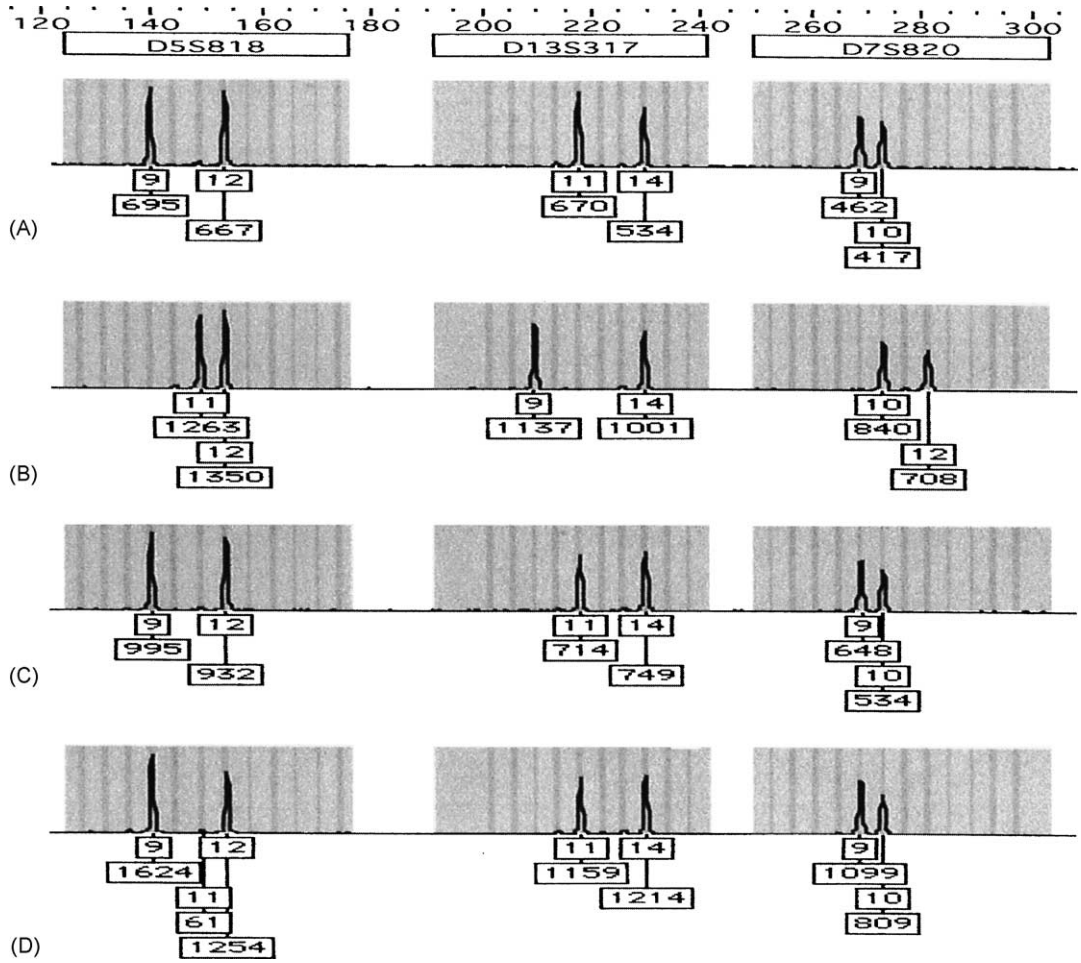

Fig. 3. Electrophoretic profiles of PCR products from three representative short tandem repeat sequences: row A represents the profile of the donor and row B that of the recipient prior to undergoing BMT. Row C shows the profile after myelodysplasia was diagnosed, and row D shows it from July 2002, after acute secondary leukemia was diagnosed. The profiles in rows C and D are predominantly those of the donor. Calculations based on the relative heights of donor and recipient peaks at these times indicate that chimerism was 99.7% donor in November 2001 and 99% donor in July 2002. This test has a reported sensitivity of 1 in 1000 cells.

marrow demonstrated that blast cells were immunoreactive for myeloid cell-associated markers (myeloperoxidase, CD13, CD33, CD11b, CD15 and CD65) and were negative for T-, B- and NK-cell associated markers. A diagnosis of donor cell acute myeloid leukemia was suggested by the results of molecular testing, which demonstrated undetectable levels of recipient-derived bone marrow mononuclear cells by donor-recipient chimerism studies on short tandem repeat (STR) sequences (Fig. 3). Bone marrow was negative for PML/RARα fusion transcripts by PCR (data not shown). The patient was given chemotherapy to induce complete remission, and the patient received a second BMT from the same donor 15 months ago, and has remained in remission. Workup of the donor prior to retransplantation documented normal blood counts and bone marrow morphology, and cytogenetic testing on a sample of donor bone marrow demonstrated a normal female karyotype.

3. Discussion

In this report we describe a patient with APL who developed myelodysplastic syndrome of donor origin 26 months after allogeneic BMT. Progression to acute leukemia of donor origin was noted over 11 months of clinical observation. We have previously described a case of donor origin myelodysplastic syndrome with a monosomy 7 abnormality in which the donor had previously been treated with carmustine and bacillus Calmette–Guerin for limited-stage malignant melanoma [3]. Bone marrow morphology and karyotype from the donor in that case were normal. In the present case we have been unable to identify any prior genotoxic exposures in the donor. In addition, the donor's bone marrow morphology and karyotype, repeated in preparation for retransplantation, remain normal. To date these are the only cases in which a period of myelodysplasia has been identified prior to the onset of donor cell acute leukemia. It is possible that brief periods of myelodysplasia occurred in other cases of donor cell acute myeloid leukemia but were not noticed clinically because of less frequent follow-up.

Donor cell acute leukemia has occurred following transplantation for a wide range of hematological disorders [4]. Transplants for malignant diseases such as chronic myeloid leukemia [3], acute myelogenous leukemia [5] and acute lymphoblastic leukemia [6] have been complicated by the later development of donor cell acute leukemia. It has also been diagnosed following BMT for non-malignant hematological disorders such as aplastic anemia and thalassemia [7,8]. The literature describes a wide range of pretransplant therapies and divergent indications for transplantation, and it seems likely that the development of leukemia in this setting relates more to events occurring after transplantation than to pretransplant events in the recipient. It is possible that the donor's state of health and prior exposures to genotoxic agents may increase the risk of donor cell acute leukemia occurring in the recipient, although to date these issues have not been examined in detail.

The diagnosis of donor cell myelodysplastic syndrome and acute leukemia were facilitated in this case by analysis of nine STRs. Other features, such as the appearance of new trilineage dysplasia and the reappearance of acute leukemia without the *t*(15; 17) translocation or PML/RARα fusion transcripts, suggested that this might be the case but could not be taken as proof in the absence of the molecular chimerism data. Other techniques, such as G-banded and quinacrine-banded cytogenetics, Southern blotting of restriction fragment length polymorphisms and interphase fluorescence in situ hybridization have been applied to this problem [9–11]. Conventional cytogenetics may misclassify leukemia cells as being of donor origin if donor cells outpace a slowly dividing or static populations of malignant recipient cells in culture [11] and has largely been replaced by molecular biology techniques for determination of donor–recipient chimerism [1]. In one case $CD34^+$ leukemic cells were isolated from whole bone marrow and subjected to molecular chimerism testing for more formal demonstration that these cells were in fact donor-derived [12]. In the present case such an isolation step was not possible since the leukemia cells did not express CD34. It was also felt that such a step was unnecessary given the degree by which the proportion of leukemia blast cells (20%) exceeded the detection limit of our STR analysis (0.1%), and the chimerism was 99% donor at the time of secondary leukemia.

In this report we have described the development of myelodysplasia in cells of donor origin in a patient one year following allogeneic stem cell transplant. Transformation to acute leukemia was observed over the course of the next year. To our knowledge, this is the first report of donor origin acute leukemia occurring following APL and the second report of a preceding myelodysplastic syndrome. We suspect that some of the events leading to the development of donor cell leukemia may take place in the early post-engraftment phase, when rapidly dividing early progenitor cells may be most susceptible to genotoxic events. Clonal dominance has been shown on occasion in the first few weeks after BMT [13]. While this situation may be transient in the majority of cases, it is possible that if it is sustained, replicative stress may lead to the emergence of clonal disorders through critical telomere shortening or other mechanisms [14]. We hypothesize that the events reported in this case support a "multi-hit" mechanism of leukemogensis in at least some cases of donor cell acute leukemia.

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