Acute Promyelocytic Leukemia Presenting Initially with Pancytopenia and Severe Hypocellular Marrow Which was Successfully Treated with All-Trans Retinoic Acid and Chemotherapy

Munehiro Suzukawa, Akiko Sugiyama, Tatsuki Nakazora, Yasufumi Kawasaki, Takayuki Tominaga and Kenji Shinohara

Division of Hematology, Department of Medicine, Yamaguchi Prefectural Medical Center, 77 Ohsaki, Hofu, Yamaguchi 747-8511, Japan
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Abstract   A 62-year-old male developed pancytopenia due to severe hypocellular marrow. He was initially diagnosed with aplastic anemia (AA), and was treated with antilymphocyte globulin (ATG), cyclosporine (CyA), and granulocyte colony-stimulating factor (G-CSF). However, 3 days after starting the therapy, the leukocyte count increased abruptly and the bone marrow became markedly hypercellular with hypergranulated promyelocytes. Cytogenetic analysis demonstrated t(15;17)(q22;q12) and promyleocytic leukemia/retinoic acid receptor-α (PML/RAR-α) fusion genes on employing interphase fluorescence in situ-hybridization (FISH) and nested PCR. He was diagnosed with acute promyelocytic leukemia (APL). He was initially treated with all-trans retinoic acid (ATRA) for 60 days. Hypergranulated promyelocytes decreased, and anemia and thrombocytopenia improved; however, neutropenia and dysplastic cells markedly remained, while the bone marrow retained its hypocellularity. Finally complete hematological and cytogenetic remissions were achieved. He was subsequently treated with 3 courses of reduced doses of chemotherapeutic agents consisting of cytosine arabinoside (Ara-C) and alternating regimens of mitoxantrone (MIT), daunorubicin (DNR), and idarubicin (IDR) as consolidation chemotherapy. However, bone marrow cellularity remained hypocellular, although peripheral blood counts returned to normal.

Key words: acute promyelocytic leukemia, hypoplastic marrow

Introduction

The diagnosis of aplastic anemia (AA) is sometimes difficult solely based on characteristics of pancytopenia and hypocellular bone marrow. The pathogenetic characteristics of AA, myelodysplastic syndrome (MDS), and acute myelogeneous leukemia (AML) partly overlap, and clonal hematopoiesis has been observed in these disorders.[1-4] There are several reports of the development of MDS and leukemia during the clinical course of AA, especially in cases treated with immunosup-pressants or G-CSF.[1-8] The concepts of hypoplastic leukemia or hypoplastic MDS are also recognized.[9,10] These share morphological characteristics of pancytopenia, bone marrow hypocellularity, and dysplasia or a low percentage of leukemic hematopoietic cells. Cytogenetic abnormalities have been reported in patients with otherwise typical AA, involving chromosome 7 such as monosomy 7 or trisomy 8.[11] Leukopenia is present more commonly in patients with acute promyelocytic leukemia (APL); however the bone marrow is usually hypercellular.[12] Hypoplastic

leukemia usually occurs in AML, and is rare in APL.[9)10)] We encountered a case of APL who initially presented with pancytopenia and hypocellular marrow and was initially diagnosed with AA, which was successfully treated with all-trans retinoic acid (ATRA) and subsequent chemotherapy.

Case report

A 62-year-old male who complained of purpura of the lower extremities was pointed out to have pancytopenia in another hospital, and was referred to us. Laboratory data on admission are shown in Table 1. CBC demonstrated severe pancytopenia, and a bone marrow aspiration smear demonstrated markedly hypocellular marrow (Fig. 1). Cytogenetic analysis of bone marrow cells was not performed at this time. Magnetic resonance imaging (MRI) of the bone demonstrated hypocellularity of the bone marrow. Hemostatic findings were normal. Based on these data, diagnosis of aplastic anemia (AA) was made. Paroxysmal nocturnal he-
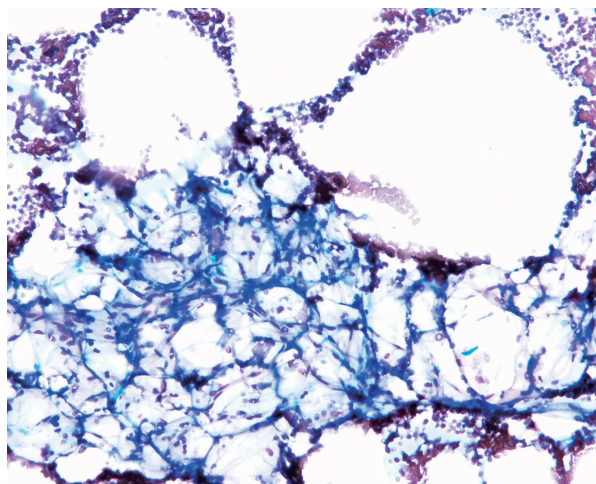

Fig. 1 Hypocellular bone marrow at admission (x10).

Table 1 Laboratory data on admission
PNH-type cells, G: granulocyte, E: erythrocyte Cytokine (   ): normal value

CBC			Bone Marrow	Markedly Hypocellular
RBC (x10^4/μl)	321		Myeloblasts (%)	4.0
Hb (g/dl)	10.2		Promyelocytes (%)	7.0
Ht (%)	29.7		N Myelocytes (%)	2.0
Ret (%)	1.2		N Metamyelocytes (%)	1.0
Platelets (x104/μl)	6.7		N Bands (%)	1.0
WBC (/μl)	1200		N Segmented (%)	25.0
Promyelocytes (%)	8.0		Eo (%)	0.0
N Myelocytes (%)	2.0		Ba (%)	0.0
N Metamyelocytes (%)	0.0		Lymphocytes (%)	52.0
N Band (%)	2.0		Monocytes (%)	2.0
N Segmented (%)	30.0		Histiocytes (%)	2.0
Eo (%)	1.0		Erythroblasts	
Ba (%)	0.0		Polyochromatic (%)	2.0
Lymphocytes (%)	54.0		Orthochromatic (%)	0.0
Monocytes (%)	1.0			
Others (%)	2.0		Cytokines	
			TNF-α (pg/ml)(<5)	1.3
PNH-type cells			IFN-γ (IU/ml)(<0.1)	0.1
G	-		Epo (mU/ml)(8-36)	24.8
E	-		G-CSF (pg/mL)(<18.1)	41.7
Hemostatic Study				
PT (sec)	12.7			
APTT (sec)	30.1			
Fibrinogen (mg/dl)	249			

moglobunuria (PNH)-type cells measured by flow cytometry were not detected in the red cells and granulocytes.[13)14)] Plasma levels of tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and erythropoietin (Epo) were normal, and that of granulocyte colony-stimulating factor (G-CSF) was elevated. The clinical course is shown in Fig. 2. The patient started treatment in early December 2008, consisting of anti-thymocyte globulin (ATG, Lymphoglobuline, Genzyme), cyclosporine (CyA), and granulocyte colony-stimulating factor (G-CSF).[15)] Surprisingly, an increase in the leukocyte count and dysplastic granulocytes was observed 3 days after starting treatment. The laboratory data at that time are shown in Table 2. A bone marrow aspiration smear demonstrated markedly hypercellular marrow (Fig. 3A) with hypergranulated promyelocytes showing Auer rods and Fagott and dysplastic cells (Fig. 3B). Flow

cytometric analysis demonstrated increased expressions of CD2, CD13, CD33, and CD56. Cytogenetic analysis of bone marrow cells
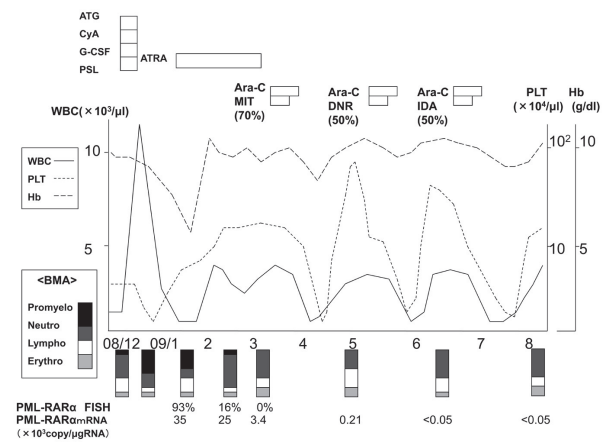

Fig. 2 Clinical course.
Ara-C: cytosine arabinoside, MIT: mitoxantrone, DNR: daunorubicin, IDA: idarubicin

Table 2 Laboratory data at diagnosis of APL
  Hemostatic study ( ): normal value

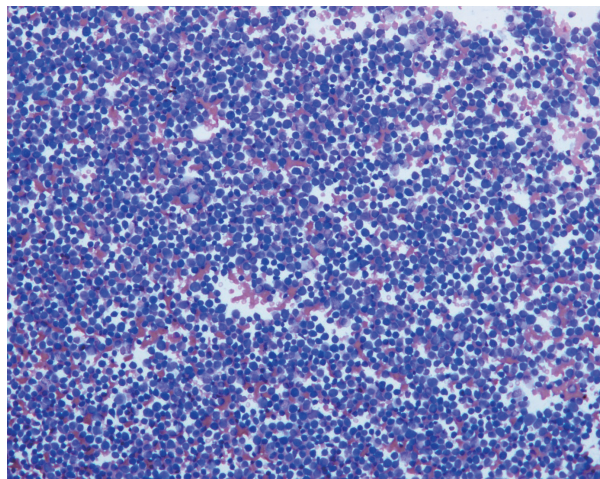| CBC			Bone Marrow	Markedly Hypercellular
RBC (x10⁴/μl)		320	Myeloblasts (%)	13.4
Hb (g/dl)		10.0	Promyelocytes (%)	56.0
Ht (%)		30.6	N Myelocytes (%)	9.0
Ret (%)			N Metamyelocytes (%)	2.0
Platelets (x10⁴/μl)		3.2	N Band (%)	1.8
WBC (/μl)		17000	N Segmented (%)	2.6
Promyelocytes (%)		11.0	Eo (%)	0.2
N Myelocytes (%)		9.0	Ba (%)	0.0
N Metamyelocytes (%)		2.0	Lymphocytes (%)	5.0
N Band (%)		0.5	Monocyts (%)	1.6
N Segmented (%)		15.0	Histiocytes (%)	0.0
Eo (%)		0.0	Erythroblasts	
Ba (%)		0.0	Polyochromatic (%)	6.8
Lymphocytes (%)		1.5	Orthochromatic (%)	1.4
Monocytes (%)		6.0		
Others (%)		54.5	Chromosome	
			46,XY,t(15;17)(q22;q12)	20/20
Hemostatic Study			FISH,PML-RARα(%)	93
PT (sec)		12.7	PML-RARα m-RNA	+
APTT (sec)		24.1		
Fibrinogen (mg/dl)		106.0	Flow Cytometry	
FDP (μg/ml)		22.2	CD2 (%)	86.3
D-dimer (μg/ml)		11.1	CD13 (%)	56.9
SFMC (-)		1+	CD33 (%)	93.5
TAT (ng/mL)(<3.0)		11.1	CD56 (%)	70.4
PIC (μg/mL)(<0.8)		4.5		

Fig. 3A Hypercellular marrow at diagnosis
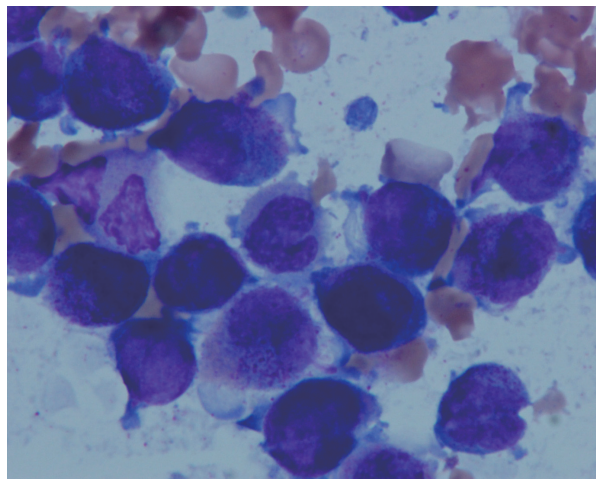   of APL (x10).

Fig. 3B Increased hypergranulated promy-
        elocytes and dyslobulated granulo-
        cytes (x100).

demonstrated t(15;17)(q22;q12) by G-banding, fusion signals of promyeocytic leukemia/ retinoic acid receptor-α (PML/RAR-α) genes by interphase fluorescence in situ-hybridization (FISH), and chimeric PML/RAR-α fusion m-RNA by nested PCR. Laboratory data for disseminated intravascular coagulation (DIC) were observed. Acute promyelocytic leukemia (APL), M3 based on the French-American-British (FAB) classification, was diagnosed. The treatment for AA was interrupted 3 days after commencing therapy. Leukopenia and bone marrow hypocellularity returned to the same levels as before the start of AA treatment in early January 2009. Subsequently, treatment for APL was performed according to the Japan Adult Leukemia Study Group (JALSG), APL 204 protocol. Initially, all-trans retinoic acid (ATRA), at 45 mg/m^2/ day, was administered. Anemia and thrombocytopenia improved but recovery from leukopenia was insufficient. Leukocytosis or ATRA differentiation syndrome were not observed. A bone marrow aspiration smear revealed the disappearance of promyelocytes from the peripheral blood and bone marrow; however, it demonstrated hypocellular and dysplastic granulocytes with bi-nucleated or reniform nuclei, and erythroblasts with intercellular bridges were observed (Fig. 4). FISH analysis revealed a decrease in the fusion signal of PML/RAR-α genes in 16% after 40 days of ATRA treatment, and finally completely disappeared 60 days after the start of
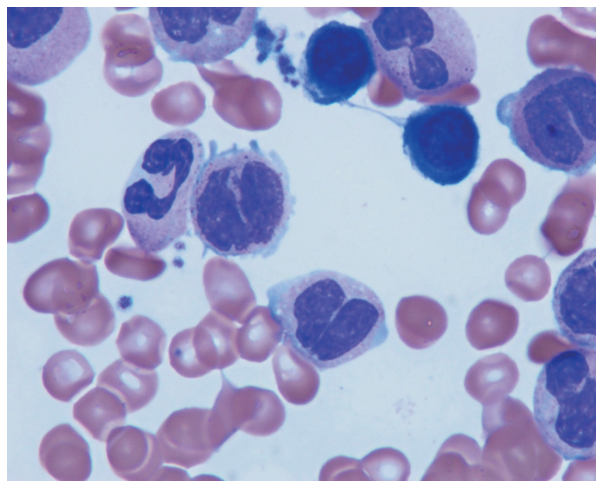

Fig. 4 Dysplastic cells after treatment of
       ATRA. Bi-nucleated granulocytes
       and erythroblasts with intercellular
       bridges (x100).

treatment. A bone marrow aspiration smear demonstrated a decrease of dysplastic cells; however, hypocellularity remained. Subsequently, 3 courses of consolidation chemotherapy consisting of cytosine arabinoside (Ara-C) and alternating regimens of mitoxantrone (MIT), daunorubicin (DNR), and idarubicin (IDR) as consolidation chemotherapy were performed with reduced doses of chemotherapeutic agents. During and after these treatments, severe pancytopenia recovered; Hb recovered and the platelet count markedly rose above normal. The recovery of neutrophils was delayed, although it finally recovered; however, the bone marrow remained

hypocellular. Quantitative measurement of chimeric PML/RAR-α fusion m-RNA showed a decrease to undetectable levels. The patient was discharged, and will subsequently be treated with ATRA.

Discussion

Typical AA is usually distinct from typical MDS and leukemia with respect to pancytopenia and BM hypocellularity. However, clonal hematopoiesis was demonstrated in AA.[1-4] The development of MDS/AML occurs in 5 to 15% of long-term survivors with AA.[1-7] There are also the concepts of hypoplastic MDS and leukemia,[9,10] and these disorders frequently evolve into AML.[1-10] Cytogenetic abnormalities such as monosomy 7 or trisomy 8 are not informative for the differential diagnosis of these disorders, and the results are not informative in AA since a sufficient number of metaphase cells is difficult to obtain in this disorder.[11] Thus, there are overlapping aspects among AA, MDS, and AML regarding the morphology and pathogenesis.[1-10] Thus, it may be sometimes difficult to differentiate MDS from AA, and some patients with MDS or hypoplastic leukemia may be inadvertently included among those with a diagnosis of AA, especially in patients with pancytopenia and hypocellular bone marrow. The present patient was initially diagnosed with AA based on pnacytopenia, an extremely hypocellular marrow, and MRI findings, although PNH-type cells suggestive of immunologically mediated AA were negative in granulocytes and red cells. Repeated examinations involving bone marrow aspiration or bone marrow biopsy were not performed.

However, the leukocyte count increased and bone marrow became extremely hypercellular shortly after the start of treatment for AA, demonstrating an increase in promyelocytes and dysplastic granulocytes, responding to the administration of G-CSF. Documeneted infection was not observed after the treatment for AA. Retrospectively considered, low percentages of myeloblasts and hypergranulated promyelocytes were observed in the peripheral blood and bone marrow aspiration smear at the diagnosis of AA,

although lymphocytes were predominant and hypocellularity of the bone marrow was marked. The increase in the leukocyte count and hypercellular bone marrow returned to the pretreatment hypocellular state seen before treatment shortly after the cessation of AA treatment. The cytogenetic analysis of an increase in promyelocytes confirmed the diagnosis of APL. G-CSF administered for the treatment of AA might have stimulated the proliferation of APL cells.

Treatment by ATRA only successfully brought about hematological and cytogenetic responses. Any beneficial effect of G-CSF on ATRA treatment, if present, is unknown. It might be potentially effective for the treatment of APL, tageting the leukemic cells entering the cell cycle and making them susceptible to differentiation into mature neutrophils by ATRA. However, the recovery of neutrophils in peripheral blood was delayed, although they finally recovered, dysplastic granulocytes in the bone marrow remained for a period, and the bone marrow remained hypocellular. The dysplatic granulocytes with bi-nuceated or reniform nuclei may represent unique morphological characteristics, as observed in the microgranular variant of APL (M3v, based on FAB classification)[12], differentiation of blood cells by ATRA, and therapy-related APL treated with ATRA with or without chemotherapeutic agents.[16-18] ATRA used solely could bring about a complete hematological and cytogenetic remission. However, ATRA alone usually cannot achieve durable remission.[12,19] So, the present patient was subsequently treated with consolidation chemotherapy. Peripheral blood Hb recovered, and the platelet count markedly recovered above normal, which is observed in the treatment of APL after treatments with ATRA and chemotherapy[20]; however, the recovery of neutrophils was delayed although they finally recovered and bone marrow cellularity remained hypocellular throughout the clinical course, even after complete hematological and cytogenetic responses were obtained.

The reason for the hypocellularity of bone marrow in APL remains unknown. Impaired hematopoiesis may have a clonal nature involving multipotent hematopoietic stem cells

including leukemic stem and granulocyte-committed stem cells.[1-4)21)] APL cells themselves, or the cytokines such as leukemia inhibitory factor (LIF), TNF-α, or IFN-γ secreted by microenvironmental cells might inhibit hematopoiesis. TNF-α or IFN-γ was not increased and G-CSF was slightly elevated, while Epo was normal. These results are inconsistent with a diagnosis of AA in which these cytokines are elevated, but are compatible with APL in which G-CSF stimulates the proliferation and differentiation of promyelocytes.[22)] The cytogenetically abnormal clone might affect impaired hematopoiesis. A rare case of hypocellular APL with a tetraploid clone characterized by two t(15;17) was reported;[23)] however, this karyotypic abnormality, or an additional abnormality such as -7 or +8 which is observed in AA, hypoplastic MDS, or leukemia[11)] was not observed in the present patient. The dysplastic cells observed might represent the clonal nature of impaired hematopoiesis. The expression of CD2 and CD56 in addition to CD13 and CD33 is more frequently observed in M3v with microgranules with a poorer prognosis than in M3 with hypergranules in APL; however, marrow hypocellularity was mentioned,[24)] and the present patient exhibited hypergranulated promyelocytes of M3.

These results require further analysis regarding the significance of bone marrow hypocellularity in APL in respect of pathophysiology and treatment.

Acknowledgements

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