Ann Hematol (1996) 72:337–339

CASE REPORT

S. Hashino · M. Imamura · J. Tanaka · S. Kobayashi
M. Musashi · M. Kasai · M. Asaka

Transformation of severe aplastic anemia into acute myeloblastic leukemia with monosomy 7

Received: 27 September 1995 / Accepted: 19 December 1995

Abstract A cytogenetically normal man with severe aplastic anemia was treated with granulocyte colony-stimulating factor (G-CSF), erythropoietin (EPO), cyclosporin A, anti-thymocyte globulin, and interleukin-6 (IL-6), which resulted in a gradual improvement in his neutrophil count and hemoglobin level. After 2 years of the therapy, monosomy 7 was detected during cytogenetic analysis of his bone marrow, which evolved during a period of 5 months into acute myeloblastic leukemia. An in vitro proliferation assay of cytokine responses showed that leukemic blasts were sensitive only to G-CSF, and not to EPO or IL-6. Although allogeneic bone marrow transplantation from an HLA-matched unrelated donor was carried out in the non-remission stage, the patient died of systemic fungal infection on day 25, without any evidence of hematological engraftment. As long-term use of cytokines and immunomosuppressants in patients with severe aplastic anemia may induce or hasten the onset of a malignant transformation, careful attention must be paid to clonal evolution. Due to the poor prognosis of secondary myelodysplasia and leukemia, allogeneic bone marrow transplantation for such patients must be carried out early in the course of the disease.

Key words Bone marrow transplantation · Cyclosporin A · Granulocyte colony-stimulating factor · Monosomy 7 · Severe aplastic anemia

S. Hashino (✉) · M. Imamura · J. Tanaka · S. Kobayashi
M. Musashi · M. Asaka
Third Department of Internal Medicine,
Hokkaido University School of Medicine, Kita-15, Nishi-7,
Kita-ku, Sapporo 060, Japan

M. Kasai
Division of Internal Medicine, Sapporo Hokuyu Hospital,
Higashi-Sapporo 6-6, Shiroishi-ku, Sapporo 003, Japan

Introduction

Recently, cytokines and immunosuppressants have been widely used for patients with severe aplastic anemia (SAA), and the effects of these drugs on the improvement of pancytopenia have been evaluated. However, little is known about the long-term side effects and toxicities of these agents. SAA has the potential to transform into myelodysplastic syndrome (MDS), leukemia, and paroxysmal nocturnal hemoglobinuria (PNH), and this transformation has been thought to be a clonal evolution of hematopoietic cells [1, 4, 11, 12]. In this paper we report on a patient with SAA who developed acute myeloblastic leukemia after long-term treatment with various cytokines and immunosuppressants and show the necessity for early bone marrow transplantation for these patients.

Case report

A 20-year-old man was examined for evaluation of general fatigue and palpitation. Pancytopenia was observed, and the patient was admitted to the hospital for differential diagnosis and treatment of pancytopenia. The patient showed a hemoglobin concentration of 5.9 g/dl, reticulocytes of 2.3%, platelet count of 20000/μl, and leukocyte count of 1400/μl with 53% granulocytes and 47% lymphocytes. Bone marrow aspiration and biopsy revealed markedly hypocellular fatty marrow (less than 5% of normal cellularity) without leukemic blasts or myelodysplasia. A bone marrow scintigram using a radioisotope of $^{111}InCl_3$ showed decreased expression in the spleen and central marrow and increased expression in the kidneys and peripheral marrow, especially in the joints of the extremities. Cytogenetic analysis of the bone marrow showed normal karyotype. Nonsevere aplastic anemia was diagnosed according to the criteria of Camitta and co-workers [3], and the patient was treated with two courses of methylprednisolone pulse therapy (1000 mg × 3 days) which resulted in no hematological effect. Two months after the initial treatment, his pancytopenia progressed and fulfilled the criteria of SAA; he had a hemoglobin concentration of 5.2 g/dl with reticulocytes less than 0.9%, a platelet count of 6000/μl, and granulocytes of 156/μl. Therefore, the patient was treated with cyclosporin A (CsA) at a dose of 300–580 mg/day, granulocyte colony-stimulating factor (G-CSF) at a dose of 250 mg/day, and erythropoietin (EPO) at a

Table 1 Cytokine responses of leukemic blasts

Cytokine (concentration)		^{3}H-TdR incorporation (DPM) (mean ± SD)	*t*-Test (vs none)
None		2362 ± 322	
G-CSF	(10 ng/ml)	2377 ± 120	NS
	(100 ng/ml)	3388 ± 558	$p < 0.05$
	(1000 ng/ml)	4795 ± 896	$p < 0.01$
IL-6	(10 ng/ml)	2474 ± 227	NS
	(100 ng/ml)	2765 ± 749	NS
	(1000 mg/ml)	2334 ± 441	NS
EPO	(10 units/ml)	2695 ± 115	NS
	(100 units/ml)	2927 ± 532	NS
	(1000 units/ml)	2548 ± 484	NS

dose of 12000 units/2 days, which raised the granulocyte count to within the normal range (3000–5000/μl) within 2 months and the hemoglobin to a level (7.0–8.0 g/dl) that did not necessitate transfusion therapy. However, the platelet count remained low (6000–15000/μl) despite the above treatment for 22 months.

Because of the continuously low platelet count (9000–13000/μl) and a gradual decrease of hemoglobin (4.7– 5.6 g/dl) in spite of the continuous administration of these three agents, anti-thymocyte globulin and interleukin-6 were added 22 and 24 months, respectively, after the start of the treatment, which resulted in a rise in the hemoglobin level (10–11 g/dl), and an allogeneic bone marrow transplantation (BMT) was suspended in spite of the discovery of an HLA-matched unrelated donor from the Japan Marrow Donor Program. After 2 years of treatment with CsA, G-CSF, and EPO, cytogenetic analysis of the bone marrow revealed monosomy 7, which had not been detected previously but was repeatedly found thereafter. However, to guarantee a continuously good hematological response (10–11 g/dl), long-term administration of these three agents was required. Thereafter, myeloblasts appeared in the peripheral blood and bone marrow, and his condition gradually evolved into acute leukemia. The use of these agents, which had been administered for a 32-month period, was then stopped. We examined the responsiveness of the leukemic blast clone to several cytokines used in his clinical course. Briefly, the leukemic blasts were obtained from a heparinized fresh bone marrow sample by centrifugation on a Ficoll-Hypaque gradient. The cells were suspended in RPMI1640 medium supplemented with 10% heat-inactivated FCS and 5×10^{-5} *M* 2-mercaptoethanol; 1×10^5/100 μl cells were co-cultured with several concentrations of cytokines in flat-bottomed 96-well microtiter plates. All the cultures were set up in triplicate. Two days later, 37 kBq of ^{3}H-TdR was added to each well and after a 6-h incubation period, each sample was harvested onto paper filters with a multi-sample harvester. After the samples had been dried, they were put into a liquid scintillation counter. The in vitro proliferation assay of leukemic blasts showed sensitivity to G-CSF, but not to EPO or IL-6 (Table 1). One month later, allogeneic BMT from an HLA-matched unrelated donor was carried out, even though the patient was not in remission. The conditioning regimen consisted of busulfan (4 mg/kg × 4 days) and cyclophosphamide (60 mg/kg × 2 days), and prophylaxis of acute graft-versus-host disease consisted of FK-506 (0.1 mg/kg × 2/day) and short-term methotrexate. The patient died of systemic candidiasis on day 25 after BMT, before engraftment. The autopsy revealed no clear evidence of marrow engraftment and we were unable to evaluate the residual leukemia because of his early death after BMT.

Discussion

Although Marsh et al. suggest that patients with SAA should not be treated with hematopoietic growth factors alone [9], in Japan G-CSF has been widely used in patients with SAA who have no suitable BMT donor in order to avoid severe infections [7]. CsA has also been used in patients with SAA due to its strong immunosuppressive activity. Patients receiving long-term treatment of G-CSF or CsA have generally tolerated the treatment well. Recently, a combination therapy of CsA and G-CSF in a patient with SAA was reported [6].

A serious concern regarding patients with SAA has been the evolution into clonal diseases, particularly MDS, PNH, and acute leukemia [1, 4, 11, 12, 14]. There have been previous reports of SAA and monosomy 7, some evolving into leukemia [1, 2, 4, 8], and one report has suggested that monosomy 7 was directly related to the administration of CsA [2]. The incidence of malignancy in patients with SAA has been difficult to evaluate because most patients receive cytokines or immunosuppressants, which are thought to have the potential to transform into clonal diseases.

A theoretical concern regarding the use of G-CSF in patients with SAA has been the possibility of stimulation of the malignant clone [8, 13]. In this case, in vitro proliferation assay of leukemic blasts showed sensitivity of the blasts to G-CSF but not to IL-6 or EPO. The fact that the blasts responded only to G-CSF suggests the possibility that the administration of G-CSF, in his later stage at least, enhanced the rapid proliferation of transformed leukemic blasts. Although not all of the in vitro proliferation assays of leukemic blasts to cytokines may be parallel to the in vivo clinical manifestations, the transformation into acute myeloblastic leukemia in our patient may have been due to the use of G-CSF. On the other hand, immunosuppressants containing CsA have the potential to transform into PNH, MDS, and leukemia in patients with SAA [10]. The possible side effects of CsA treatment of SAA have not yet been clarified. However, the possibility of clonal evolution, even in an indirect manner, cannot be disregarded. Although various cytokines and immunosuppressants were administered to our patient, the long-term administration of G-CSF and CsA was thought to be mainly responsible for the transformation to leukemic acceleration from the preleukemic state. The detection of monosomy 7 during the evolutionary phase of SAA into acute leukemia was compatible with the findings in the previous report.

As secondary myelodysplasia and leukemia from SAA have a poor prognosis, the possibility of allogeneic BMT might be considered before the transformation occurs, particularly if an HLA-matched sibling or unrelated donor is available [1, 4, 5]. In our patient, hematological engraftment was not observed and he died of infectious complications. Therefore, allogeneic BMT should be carried out as soon as possible, in order to avoid graft failure due to transfusion-induced sensitization to minor histocompatibility antigens. The incidence of SAA evolving into MDS and leukemia may increase as survival rates improve, and there is a high

risk of malignant conditions developing after immunosuppressive therapy compared with BMT [12]. Therefore, long-term administration of these agents should be restricted to patients who have no appropriately matched donors.

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