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Case Report Open access

Bone Marrow Necrosis and Elevated Plasma Levels of Fas Ligand in a Patient with Aggressive Natural Killer Cell Leukemia

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Abstract

Fas is type I membrane protein, expressed on various tissue including hematopoietic cell. Its ligand (FasL), a member of the tumor necrosis factor (TNF) family, which includes two forms of a membrane-bound form and a soluble one, induces apoptosis of Fas-bearing cells through cytotoxic activity. FasL is undetectable in the serum of healthy person, whereas it is high in that from patients with T-large granular lymphocytic leukemia and natural killer (NK) cell lymphoma. We experienced a case of aggressive NK cell leukemia presenting severe bone marrow necrosis (BMN) after administrating granulocyte colony stimulating factor (G-CSF). In concern with a relation to cytokine, his serum FasL was examined and found to be extremely elevated. Administration of G-CSF under a large quantity of soluble FasL might play an important role in the pathogenesis of BMN through apoptosis of hematopoietic cells in the bone marrow.

Keywords: Aggressive natural killer cell leukemia (ANKL); Fas ligand; Bone marrow necrosis

Introduction

Aggressive natural killer cell leukemia (ANKL) has aggressive features such as systemic tissue injury including bone marrow, resulting in pancytopenia. Although leukemic cells are thought to cause these damages, the mechanism is unclear.

Fas, type I membrane protein, is expressed on various tissue including hematopoietic cells and its ligand FasL, a member of the tumor necrosis factor (TNF) family, is limitedly expressed on activated T-cells or activated NK-cells. Although membrane-bound FasL can be converted to a soluble form (sFasL) [1], sFasL is undetectable in the serum of healthy person and that of patients with most subtypes of lymphoma or leukemia. It is contrarily elevated in patients with T-large granular lymphocytic (LGL) leukemia and natural killer (NK) cell lymphoma [2-6], suggesting that Fas-FasL system plays a crucial role in characteristic systemic tissue damage observed in these types of hematological tumor [7-9]. We report a case of ANKL accompanied by bone marrow necrosis (BMN) with severe bone pain after granulocyte colony-stimulating factor (G-CSF) administration. Serum level of sFasL was increased and G-CSF might trigger FasL-induced apoptosis of bone marrow.

Case Report

A 25-year-old man presented to local doctor because of high fever (39.3°C) and thrombocytopenia (platelet count 57 × 10E9/l) in September 2007. He was diagnosed as Epstein-Barr virus (EBV) associated hemophagocytic lymphohistiocytosis (HLH). Even though he was treated with methylprednisolone pulse therapy, his symptom did not improve, so he was introduced to our hospital in October 2007. Physical examination exhibited severe hepatosplenomegaly. Laboratory findings on admission revealed moderate anemia, thrombocytopenia (hemoglobin 11.3 g/dl, platelet count 12 × 10E9/l) and elevation of lactate dehydrogenase (LDH) (995 IU/l), ferritin (9915 ng/ml), and soluble interleukin-2 receptor (sIL-2R) (6450 U/ml) (Table 1). Serological analyses of EBV show the pattern of infection in the past, although EBV-DNA was elevated (4.7 × 10E4 copy/μgDNA) (Table 1).

WBC	4.8 × 10	9/L	LDH	995	IU/1	PrItINR	0.98	
Neut	93	%	AST	50	IU/1	APTT	36.4	sec
Lym	3	%	ALT	81	IU/1	FIG	202	mg/d1
Eos	0	%	ALP	235	IU/1	FDP	16.3	pg/m1
Baso	0	%	y-GTP	82	IU/1	sIL-2R	6540	U/ml

Mono	2	%	TP	5.8	g/dl	VCA-IgG		80 folds
					g/dl	VCA-IgM		<10
Hb	11.3	g/dl	Alb	3.1	mg/di	EADR-IgG		<10
Ht	32.4	%	T-Bil	1.36	mg/di	EADR-IgA		<10
PLT	12 × 10	9/L	CRP	1.77		EBNA		40 folds
					EBV-DNA		4.7×10^4	copy/nDNA

Table 1: Laboratory data on admission.

Bone marrow aspiration showed marked hemophagocytosis (3.2%) but no atypical cells were detected. Flow cytometry of bone marrow disclosed 5.4% of CD3-negative and CD56-positive cells (Figure 1).

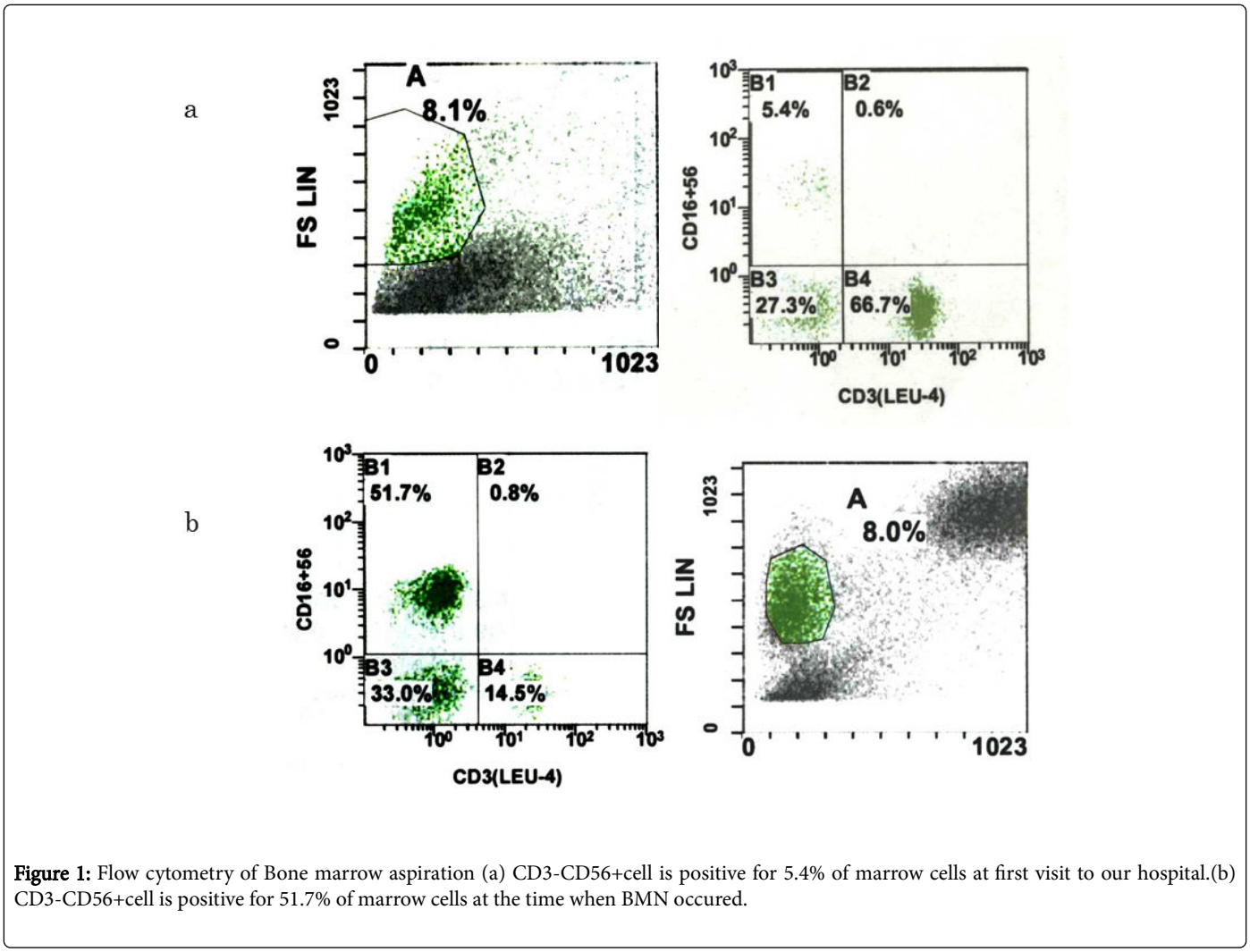

Figure 1: Flow cytometry of Bone marrow aspiration (a) CD3-CD56+cell is positive for 5.4% of marrow cells at first visit to our hospital.(b) CD3-CD56+cell is positive for 51.7% of marrow cells at the time when BMN occured.

Monoclonality of EBV terminal repeat sequence was confirmed in mononuclear cells obtained from peripheral blood.Combined immunochemotherapy consisting of cyclosporine A, dexamethasone, and etoposide was begun on 2 hospital day. His fever fell to a low grade level, but pancytopenia was progressive. Concerning of myelosuppression by etoposide, 75 μg of G-CSF was daily administered. On the third day of G-CSF, sudden onset of bone pain and fever occurred. Laboratory investigation showed pancytopenia (WBC 0.3 × 10E9/l, platelet count 9 × 10E9/l) and elevation of LDH level (1515 IU/l). Peripheral blood smear showed leukoerythroblastosis. Bone marrow aspiration smear exhibited increase of small to medium-sized naked lymphoid cellsand nucleated cells containing basophilic cytoplasm. Most of granulocytic cells were destroyed (Figure 2).

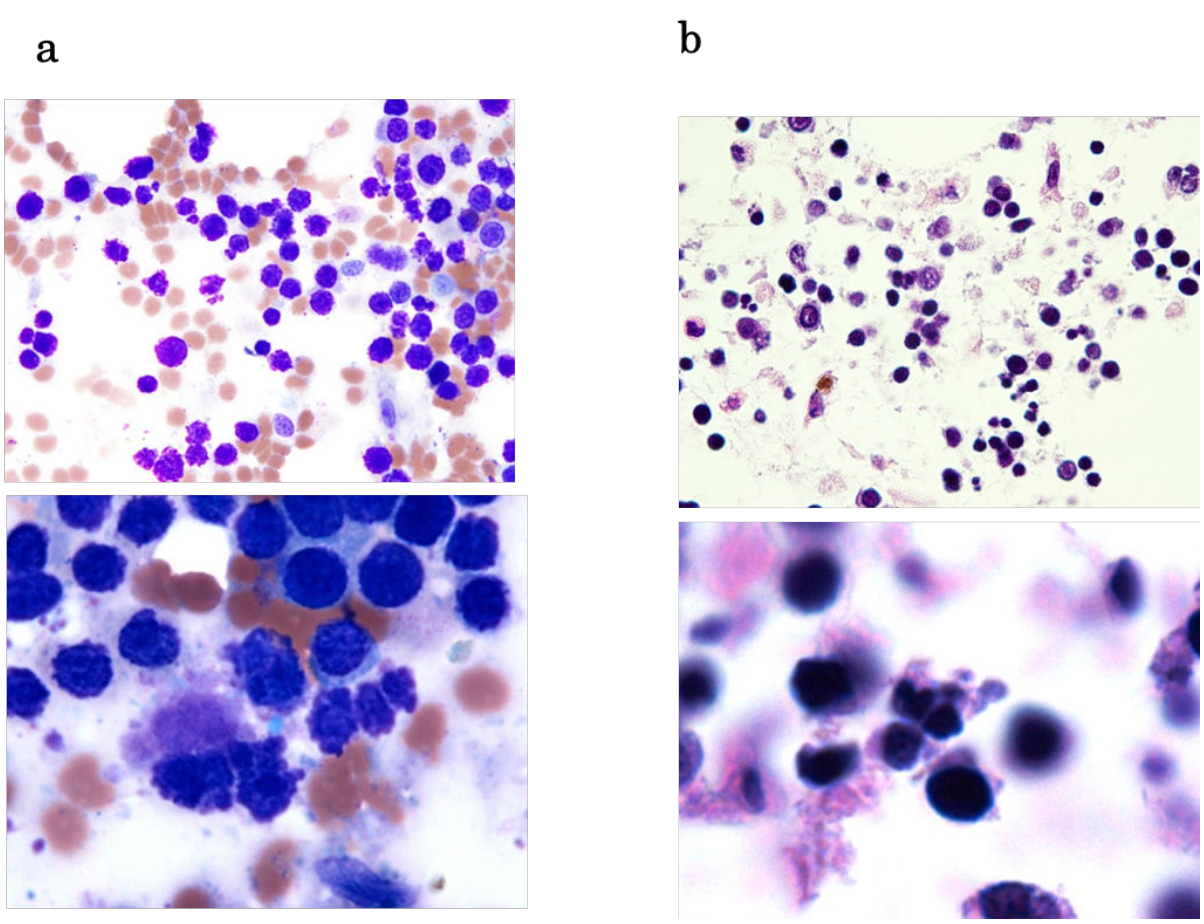

Figure 2: Bone marrow aspirates at the time when BMN occurred a) May-Giemsa stain ×100. b) Haematoxylin and eosin ×40). Most of nucleated cells contain picnotic nucleus. Outline of their cytoplasm is often illegible. Elevation of small to medium-sized naked lymphoid cellsand nucleated cells containing basophilic cytoplasm. Most of granulocytic cells were destroyed and hemophagocytosis. These indicate massive necrosis of hematopoietic cells.

Flow cytometry of bone marrow showed 51.7% of CD3-negative and CD56-positive cells (Figure 1), whereas that were only 5.6% at the initial presentation (Figure 1). Furthermore, Magnetic Antigen Cell Sorting System (MACS) revealed a higher proportion of EBV infection among CD56 positive-cells than other types of lymphocytes (Figure 3). Based on these findings, the patient was diagnosed as ANKL. After G-CSF was discontinued, the patient was free from pain within 3 days, however, the elevation of LDH continued for about a month. Salvage regimen consisting of ifosfamide, dexamethasone, and L-asparaginase brought only a transient improvement in clinical symptoms. No further conventional chemotherapy was undertaken since treatment toxicities without benefit were anticipated. Finally, the patient received allogeneic peripheral blood stem cell transplantation (PBSCT) from HLA-one locus mismatched EBV-seropositive father. The conditioning regimen consisted of total body irradiation (TBI) of 10Gy and high-dose intravenous cyclophosphamide (60 mg/kg once daily for 2 days), followed by the infusion of PBSC containing 3.7 × 10E6 CD34 positive cells/kg.

Mononuclear cell	EBV copy number
CD3+ lymphocyte	41,736 copy/μgDNA
CD8+ lymphocyte	34,529 copy/μgDNA
CD19+ lymphocyte	28,391 copy/μgDNA
CD56+ lymphocyte	262,069 copy/μgDNA

Figure 3: EB viral copy number in peripheral blood mononuclear cells. Magnetic Antigen Cell Scoring System (MACS) showed EBV infected CD56 positive cells dominantly.

Standard-dose FK506 and short-term methotrexate were used for prophylaxis of graft-versus-host disease. Although engraftment was

completed at day 10, ANKL was relapsed presenting fever up, elevation of LDH, and exacerbation of hepatosplenomegaly within a few weeks. The disease was progressive despite administration of dexamethasone and etoposide. Although the second transplantation was intended, he died of fungal infection.

Since we hypothesized that the interaction between Fas and FasL caused of BMN, we examined his serum sFasL concentration sequentially at the timings of BMN and after chemotherapy. His serum was sent to the Department of HSCT Data Management, Nagoya University School of Medicine. Enzyme immunoassay was employed and sFasL was found to be elevated to 1050 pg/ml on the day of BMN diagnosis (lower than 0.1 ng/ml in normal serum). Maximum titer was 1350 pg/ml 10 days after (Figure 4).

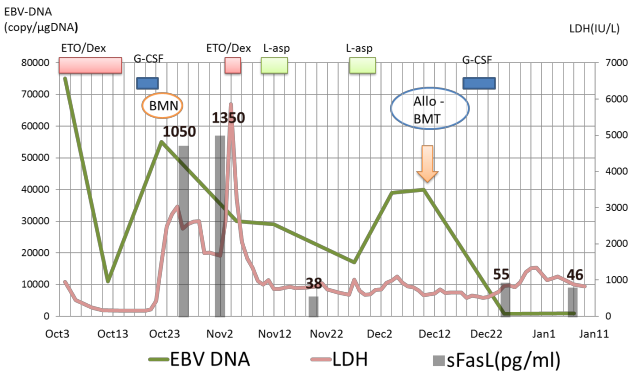

Figure 4: Clinical course and serum level of sFas, LDH, and EBV DNA before and after chemotherapy. At the onset of BMN, the levels of LDH, sFasL and EBV-DNA were elevated, and decreased after chemotherapy.
ETO; Etoposide, Dex; Dexamethasone, L-asp; L-asparaginase, sFasL; soluble Fas ligand, LDH; lactate dehydrogenase, BMN; Bone marrow necrosis, BMT; Bone marrow transplantation.

Discussion

Standard therapy for ANKL has not yet been established. P-glycoprotein expressed by NK cells is reported to be responsible for its refractoriness to chemotherapy [8]. On the other hand, Kawa et al. [9] introduced immunosuppressive and cytoreductive therapy with prednisolone, cyclosporine A and etoposide to a case of systemic active EBV infection. However, it did not benefit our case and pancytopenia was progressive even though symptoms fell down. Several researchers reported high serum levels of sFasL in patients with NK- or T-cell LGL leukemia as well as NK cell lymphoma [2-6]. However, despite over production of FasL in NK cell leukemia, tissue damage such as BMN does not always occur. It is presumed that the sensitivity of the target cells to the sFasL is different among the patients [7]. In this case, serum level of sFasL was increased and subsequently to G-CSF administration, BMN happened [10-13]. Takenaka et al. [12] reported that hematopoietic cells cultured in the presence of G-CSF showed induction of Fas expression. These results suggest that G-CSF triggered FasL-induced apoptosis of bone marrow under the high concentration of sFasL. However, controversial reported that G-CSF inhibits Fas-triggered apoptosis in bone marrow cells [14]. The participation of G-CSF to BMN is thus unclear. Further accumulation of similar cases is required.

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