

Elevation of the Serum Fas Ligand in Patients With Hemophagocytic Syndrome and Diamond-Blackfan Anemia

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Fas ligand (FasL) is a membrane protein that is expressed in activated T cells and natural killer cells. FasL binds to Fas on target cells and induces apoptosis. There exists a soluble form of FasL (sFasL), and sFasL also induces apoptosis of Fas-bearing cells. The serum sFasL concentrations were reported to be elevated in patients with large granular lymphocytic leukemia and natural killer cell lymphoma. In this study, we have measured serum sFasL concentrations in other hematological disorders, including severe aplastic anemia (SAA), hemophagocytic lymphohistiocytosis (HLH), and Diamond-Blackfan anemia (DBA). The serum sFasL con-

Fas (ALSO KNOWN AS APO-1 or CD95) is a member of the tumor necrosis factor (TNF) receptor/nerve growth factor receptor family and transduces an apoptotic signal by activating a cascade of interleukin-1 β -converting enzyme (ICE)-like cysteine proteases (caspases).¹ Fas is constitutively expressed in a variety of tissues, including thymus, liver, heart, and kidney. Fas is barely detectable in bone marrow CD34⁺ cells isolated from healthy volunteers. Treatment of these cells with interferon- γ (IFN- γ) or TNF- α increases the expression of Fas and these cells become susceptible to anti-Fas antibody-induced apoptosis.^{2,3} The ligand for Fas (FasL) is a type II membrane protein that is expressed in the cells having cytotoxic activity, including activated T cells and natural killer (NK) cells, and in the cells in immune-privileged sites such as the testes, the brain, and the anterior chamber of the eye.^{4,5} Mice carrying an abnormality in either the Fas (*lpr*) or FasL (*gld*) gene show marked lymphoadenopathy and autoimmune disease.^{6,7} Moreover, an abnormality of the Fas gene was shown in human children suffering from autoimmune lymphoproliferative syndrome.^{8,9} These results indicate that the Fas/FasL system plays an important role in the clonal selection of T cells. The role of the Fas/FasL system has also been implicated in cell-mediated cytotoxicity during virus infections, autoimmune diseases, and tumor immunity.¹ It has been recently reported that the Fas/FasL system is involved in the pathogenesis of Hashimoto's thyroiditis and hepatitis.^{10,11}

Cleavage of membrane-bound FasL by a metalloproteinase-like enzyme results in the formation of soluble FasL (sFasL).¹² sFasL binds to Fas antigen and transduces an apoptotic signal in Fas-bearing cells.^{13,14} Therefore, cytotoxic cells can kill the target cells either via direct contact or by secreting sFasL. Tanaka et al¹² reported that serum sFasL concentrations are elevated in the patients with large granular lymphocytic (LGL) leukemia and NK lymphoma. They suggested that neutropenia and liver dysfunction, both of which are common complications of these diseases, can be attributed to the elevated levels of sFasL.

In this study, we have measured serum sFasL concentrations in the patients with hematological disorders in which immunological mechanisms are suspected to play a role in the pathophysiology, including severe aplastic anemia (SAA), hemophagocytic lymphohistiocytosis (HLH), and Diamond-Blackfan

anemia (DBA), to clarify the role of the Fas/FasL system in these disorders.

concentration of age-matched healthy controls was 0.16 ± 0.11 ng/mL (mean \pm SD, $n = 22$). The serum sFasL levels in the patients with HLH and DBA were 3.75 ± 3.82 ($n = 19$; $P < .0001$, HLH v control) and 2.76 ± 2.43 ng/mL ($n = 6$; $P = .012$, DBA v control), respectively. Serum interferon- γ concentration was elevated in the patients with HLH (1.61 ± 2.62 ng/mL) but not in those with DBA (below the detectable level). These results suggest that the Fas-FasL system plays a role, at least in part, in the pathophysiology of HLH and DBA.

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anemia (DBA), to clarify the role of the Fas/FasL system in these disorders.

MATERIALS AND METHODS

Patients. Eleven patients with SAA, 6 patients with DBA, 19 patients with HLH, and 5 patients with infectious mononucleosis (IM) were studied. The diagnosis of HLH was based on clinical findings (fever and hepatosplenomegaly), laboratory findings (cytopenia affecting at least 2 lineages in the peripheral blood, hypertriglyceridemia, and/or hypofibrinogenemia), and histopathologic findings (hemophagocytosis in the bone marrow, spleen, or lymph nodes without evidence of malignancy).¹⁵ The diagnosis of familial hemophagocytic lymphoproliferative syndrome (FHL) was made with a positive family history. SAA was defined as pancytopenia with at least two of the following abnormalities: an absolute neutrophil count of less than 500/ μ L, a platelet count of less than 20,000/ μ L, and a reticulocyte count of less than 60,000/ μ L, in association with a bone marrow cellularity of less than 30%. Patients were diagnosed as having posthepatitis aplastic anemia when they developed the disease within 3 months after documented hepatitis.¹⁶ The diagnosis of DBA was based on the following criteria: (1) normochromic anemia in early infancy, (2) reticulocytopenia, (3) normocellular bone marrow with selective deficiency of red blood cell precursors, (4) normal or slightly decreased leukocyte counts, and (5) normal or often increased platelet counts.¹⁷

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Serological diagnosis of primary Epstein-Barr virus (EBV) infection was made by the detection of either an increase in EBV viral capsid antigen (VCA)-specific IgG antibody in the absence of antibody against EBV nuclear antigen or an increase in VCA-specific IgM antibody titers. Detection of the EBV genome in the patients' peripheral blood was performed with the polymerase chain reaction as described by Saito et al.¹⁸

Enzyme-linked immunosorbent assay (ELISA) for sFasL and IFN- γ . The serum sFasL levels of age-matched healthy controls and patients with hematological disorders were measured with ELISA essentially as described by Tanaka et al¹² using monoclonal anti-FasL antibodies and recombinant human FasL. The limit of detection was 5 pg/mL. The serum IFN- γ level was determined with a commercially available kit obtained from Diaclone Research (Besançon, France). The limit of detection was 5 pg/mL.

Statistical analysis. The differences in the sFasL levels among controls and SAA, HLH, DBA, and IM patients were analyzed with Mann-Whitney's U-test using the computer program DASTat (developed by O. Nagata), whereas that in the sFasL levels between the active phase and remission phase of HLH patients were analyzed with Student's *t*-test. The values below detectable level in the sFasL level were treated as 5 pg/mL in the statistical analysis. The differences in the IFN- γ level among controls and HLH, DBA, IM, and AA patients were also analyzed with Mann-Whitney's U-test. The values below detectable level in the IFN- γ level were treated as 5 pg/mL in the statistical analysis. The difference was considered to be significant when the *P* value was less than .05.

Flow cytometric analysis. Quantitative analysis of fluorescence was performed using a Coulter Epics Flow Cytometry System (Coulter, Hialeah, FL). Phycoerythrin (PE)-conjugated mouse anti-Fas monoclonal antibody (clone UB2; MBL, Nagoya, Japan) and fluorescein isothiocyanate (FITC)-conjugated mouse anti-glycophorin A (GPA) monoclonal antibody (clone D2.10; MBL) were used to determine the Fas expression on bone marrow erythroid lineage cells. Appropriate isotypic controls were used in all of the experiments.

RESULTS

We have measured serum sFasL concentrations in a total of 41 patients with hematological disorders, including 11 SAA, 19 HLH, 6 DBA, and 5 IM patients and 22 age-matched healthy controls. The values in each group were plotted and shown in Fig 1. The serum samples from SAA, HLH, DBA, and IM patients were taken upon diagnosis and kept at -80°C until analysis. The serum sFasL concentration in healthy controls was 0.16 ± 0.11 ng/mL (mean \pm SD; range, <0.005 to 0.37 ng/mL). We tentatively set the upper normal limit of serum sFasL at 0.5 ng/mL. IM patients showed a moderate increase in their serum sFasL levels (1.39 ± 0.56 ng/mL; mean \pm SD). These IM patients showed mild liver dysfunction with a moderate increase in serum aminotransferases and leukocytosis with atypical lymphocytes. None of the IM patients analyzed showed anemia or thrombocytopenia.

The serum sFasL concentration exceeded 0.5 ng/mL in 15 of 19 HLH patients. The mean \pm SD value was 3.75 ± 3.82 ng/mL (range, <0.005 to 12.25 ng/mL), and this was significantly higher than the controls ($P < .0001$). The clinical profiles of the HLH patients are shown in Table 1. Three patients underwent bone marrow transplantation and 2 patients died. The other patients were treated with steroids with or without other immunosuppressive and cytotoxic agents, including cyclo-

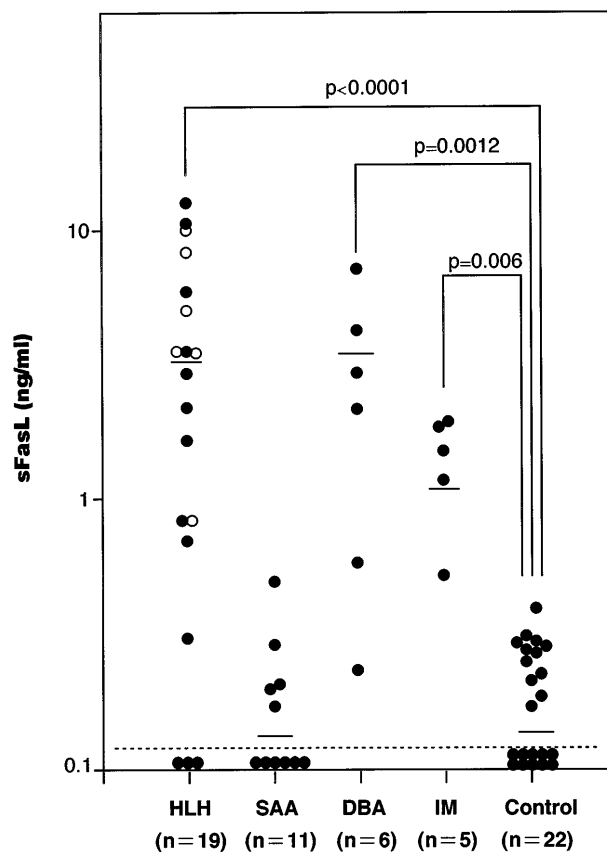


Fig 1. The serum sFasL concentrations in patients with hematologic disorders. Sera from patients and age-matched healthy controls were assayed by ELISA for sFasL as described before.¹² The mean values are shown by horizontal bars. (○) Familial cases of HLH (FHL). Statistical analysis was performed as described in the Materials and Methods and the *P* value is presented.

sporin A, FK506, OKT3, and etoposide; 4 of these patients died from the disease.

The serum sFasL levels exceeded 0.5 ng/mL in 5 of the 6 DBA patients. Three patients were steroid-dependent and the others were transfusion-dependent. The serum sFasL concentration was 2.75 ± 2.42 ng/mL (mean \pm SD; range, 0.23 to 6.78 ng/mL). There was a significant difference between the controls and DBA patients ($P = .0012$).

We have measured the serum sFasL concentrations in 11 SAA patients, including 3 with posthepatitis SAA. Although the involvement of CTL has been implicated in SAA,¹⁹ none of the patients analyzed exceeded 0.5 ng/mL. The mean \pm SD was 0.15 ± 0.14 ng/mL. There was no significant difference between the controls and SAA patients ($P = .41$).

Administration of agonistic anti-Fas antibodies to mice causes liver damages *in vivo*.²⁰ The same antibodies decrease hematopoietic colony formation^{2,3} and induce apoptosis in a variety of cell types, including hepatocytes and leukemic cells *in vitro*. Therefore, it is conceivable that a high level of sFasL produced by circulating and/or tissue-infiltrating cytotoxic cells causes cell damage in patients with HLH or DBA. However, DBA patients do not show liver dysfunction and pancytopenia,

Table 1. Clinical Characteristics of the Patients With HLH

Case No.	Age/Sex	Family History	Etiology	Fever	Hepato-megaly	Spleno-megaly	DIC	Pancytopenia	Liver Dysfunction	Hyperferritinemia	Hypertriglycemia	BMT	Outcome
1	1/F	-	Unknown	+	+	-	+	+	+	+	ND		Alive
2	3/F	-	Unknown	+	-	-	-	+	+	+	-		Alive
3	3/F	-	EBV	+	+	+	+	-	+	+	ND		Alive
4	13/F	-	Ki-lymphoma	+	-	-	+	+	+	+	ND		Alive
5	4/F	+	FHL	+	+	+	+	+	+	+	+	Allogeneic	Dead
6	1/M	+	FHL	+	+	-	+	+	+	+	ND	Allogeneic	Dead
7	0/M	+	FHL	+	+	+	+	+	+	+	+		Alive
8	1/F	+	FHL	+	+	+	+	+	+	+	+		Alive
9	1/M	+	FHL/EBV	+	+	+	+	+	+	+	+		Dead
10	1/F	+	FHL/EBV	+	+	+	+	+	+	+	+		Dead
11	2/F	-	EBV	+	+	+	+	+	+	+	+		Alive
12	0/M	-	EBV	+	+	+	+	+	+	+	+		Dead
13	1/F	-	Influenza AH3	+	+	-	+	+	+	+	-		Alive
14	1/F	-	EBV	+	+	+	+	+	+	-	-		Alive
15	5/M	-	Unknown	+	+	+	+	+	+	+	+		Alive
16	10/F	-	Unknown	+	+	+	+	+	+	+	+		Alive
17	2/M	-	Unknown	+	+	+	-	+	+	+	-		Alive
18	7/F	-	EBV	+	+	-	+	+	+	+	-	Syngeneic	Alive
19	4/F	-	EBV	+	+	-	+	+	+	+	-		Dead

Abbreviations: BMT, bone marrow transplantation; ND, not determined.

both of which are commonly observed in HLH. Therefore, the elevation of serum sFasL per se may not be enough to cause cell damage in the liver and bone marrow. It is known that IFN- γ upregulates the Fas expression and makes target cells susceptible to Fas-mediated apoptosis in a variety of cell types.^{21,22} High serum levels of IFN- γ are reported to be a common feature in HLH patients.²³ We then measured the serum IFN- γ concentrations in our HLH and DBA patients. As shown in Fig 2, most of the HLH patients showed high serum IFN- γ levels (mean \pm SD; 1.61 ± 2.62 ng/mL), whereas those in all of the DBA patients were below the detectable level. IM patients showed a moderate increase in their serum IFN- γ concentrations. These results raise the possibility that IFN- γ upregulates Fas in the target cells in HLH and makes them susceptible to FasL-mediated cell death.

A typical example of the changes in the serum sFasL and IFN- γ levels and the clinical course of one HLH patient is shown in Fig 3. The peak of serum sFasL and IFN- γ levels paralleled with the degree of leukocytopenia and preceded the peak of the serum lactate dehydrogenase (LDH) level in this patient. These values decreased after chemotherapy with steroids and etoposide. This patient underwent syngeneic bone marrow transplantation using an identical twin sister as the donor. She is alive and has been well for 9 months after the bone marrow transplantation. We have compared the serum sFasL levels during the acute phase and remission state in 9 HLH patients who showed elevated serum sFasL levels during the acute phase. As shown Fig 4, the serum sFasL level decreased with remission in all of the patients analyzed.

The serum sFasL level was high in most of the DBA patients (Fig 1). Given that cytotoxic cells are involved in the DBA, these cells might recognize some foreign or self antigen on the cell surface of erythroid progenitors and cause apoptosis via the Fas/FasL and/or perforin/granzyme pathways. We have exam-

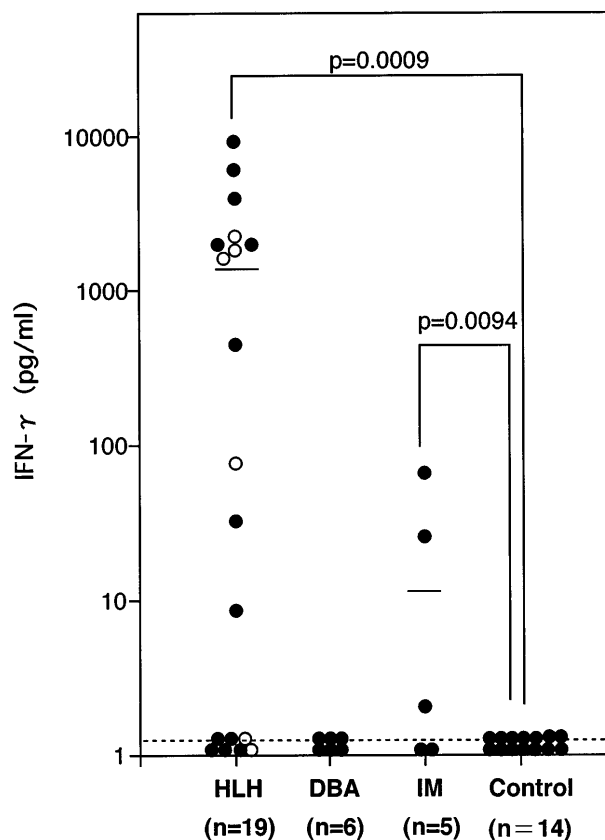


Fig 2. The serum IFN- γ concentrations in patients with hematologic disorders. The mean values are shown by horizontal bars. (○) FHL cases. Statistical analysis was performed as described in the Materials and Methods and the P value is presented.

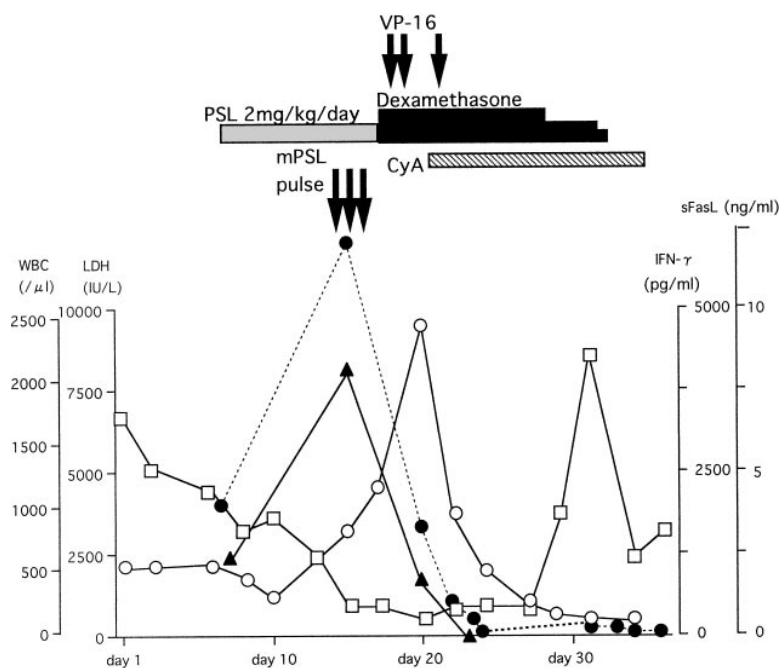


Fig 3. The clinical course and serum sFasL and IFN- γ concentrations in an HLH patient. γ -glob, γ -globulin; PSL, prednisolone; mPSL, methylprednisolone; CyA, cyclosporine A. (□) WBC; (○) LDH; (●) sFasL; (▲) IFN- γ .

ined the bone marrow cells obtained from 2 DBA patients and 4 healthy volunteers for the expression of the Fas antigen on the erythroid lineage (GPA⁺) cells. Representative results of the flow cytometry analysis are shown in Fig 5. In the bone marrow cells obtained from the DBA patients, 63% and 79% of GPA⁺

cells were Fas⁺ (Fig 5A). The expression of Fas antigen on erythroid lineage cells is not restricted to DBA patients. In bone marrow cells obtained from healthy volunteers, 87%, 46%, 54%, and 65% of GPA⁺ cells were Fas⁺ (Fig 5B).

DISCUSSION

FasL is a 40-kD glycoprotein that is expressed on the cell membrane of cytotoxic cells, including CD4⁺ and CD8⁺ T cells and NK cells.⁴ FasL is cleaved by a metalloproteinase-like enzyme to release a 26-kD glycoprotein, sFasL.¹² Therefore, serum sFasL levels can be a marker for activation of cytotoxic cells in a variety of human diseases. A moderate increase in serum sFasL was observed in IM patients (Fig 1). These results might reflect the facts that CD8⁺ T cells are activated in IM and that these cells play a major role in host defense against EBV.²⁴

HLH is a term applied to a life-threatening disease characterized by a generalized histiocytic proliferation with marked

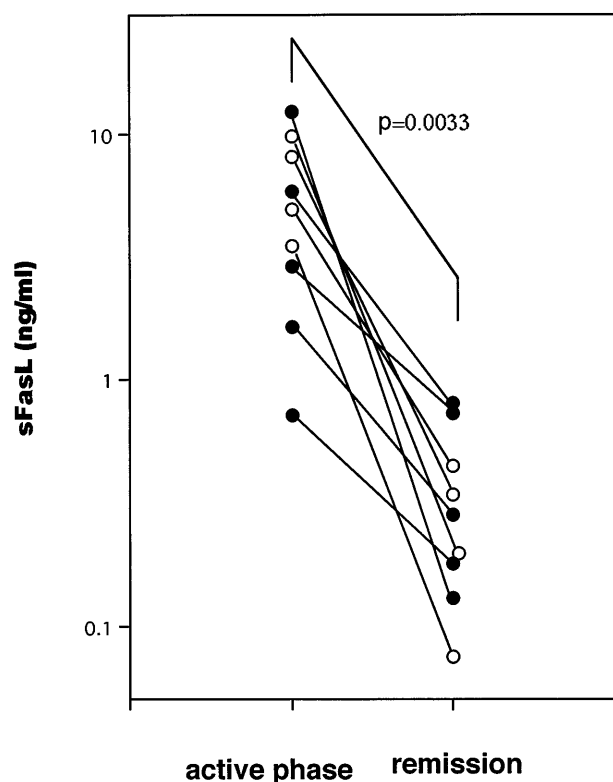
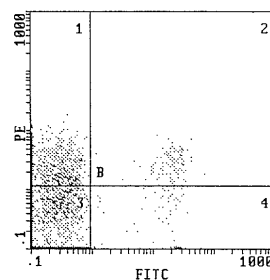


Fig 4. The serum sFasL concentrations during active phase and remission state in 9 HLH patients. (○) FHL cases.

A. DBA



B. control

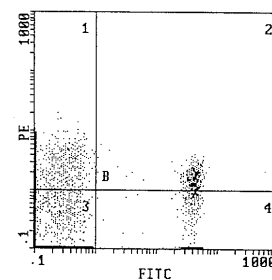


Fig 5. Expression of the Fas antigen in erythroid lineage cells in bone marrow. Bone marrow cells obtained from a DBA patient (A) and a healthy volunteer (B) were stained with the FITC-conjugated anti-GPA and PE-conjugated anti-Fas antibodies. The panel shows the fluorescence intensity of GPA (x-axis) and Fas (y-axis).

hemophagocytosis.²⁵ HLH occurs in several disorders, including an autosomal recessive inherited disease called FHL and virus- or infection-associated hemophagocytic syndrome (VAHS or IAHS). The distinction between FHL and VAHS/IAHS is not always easy without a positive family history, because some FHL cases seem to be triggered by an infection of viral nature. Therefore, the Histiocyte Society has proposed to call these disorders HLH.¹⁵ The patients have intermittent fever, lymph node enlargement, and hepatosplenomegaly.²⁶ The most common virus implicated in this syndrome is EBV. IM is a benign, self-limiting disease associated with a primary EBV infection. Most of the IM patients show lymphocytosis with atypical lymphocytes, liver enzyme abnormalities, neutropenia, and mild thrombocytopenia. In rare cases, patients develop severe or fatal IM with clinical characteristics that resemble those of HLH. Laboratory studies of HLH patients demonstrate severe cytopenia and abnormal liver function tests with elevated levels of serum transaminases and LDH. Most of the patients show elevated serum levels of soluble interleukin-2 (IL-2) receptor and cytokines, including IFN- γ and TNF- α , suggesting that the pathogenesis of this syndrome is attributable to cytotoxic damage mediated by abnormally activated T and/or NK cells.²³ However, the precise mechanism causing this syndrome is not known. The serum sFasL concentration is elevated in most of the HLH patients and its level is closely related to the disease status (Figs 1, 3, and 4). These results indicate that cytotoxic cells are activated in HLH. It is well established that sFasL or agonistic anti-Fas antibody causes apoptosis of Fas-bearing cells, including hepatocytes, both in vivo and in vitro.^{11,14,27} As little as 1 ng/mL of sFasL kills 90% of a mouse T-cell line expressing Fas in 15 hours and 3.2 ng/mL of sFasL kills 40% of a mouse fibroblast cell line expressing Fas in 7 hours.¹³ The mean serum sFasL concentration in HLH patients was 3.75 ng/mL (Fig 1), and 2 patients exceeded 10 ng/mL. These values were high enough to cause cell damage to these cells in vitro. Hepatocytes naturally express Fas and can be a target for FasL-triggered cell death. However, Fas surface expression does not necessarily render cells susceptible to Fas ligand-induced cell death.¹ Tanaka et al¹⁴ showed that injection of recombinant sFasL alone does not kill the mice. Simultaneous administration of killed bacteria, *Propionibacterium acnes*, renders them susceptible to sFasL, and the treated mice die of hepatic failure within 24 hours after intravenous injection of 30 μ g of sFasL. Because the administration of killed *P acnes* stimulates the production of a variety of cytokines, including IFN- γ , TNF, and IL-1, it is conceivable that these cytokines make the animals sensitive to sFasL. Tamura et al²⁸ reported that IFN- γ upregulates the ICE gene in the U937 monocytic cell line. Because both IFN- γ and sFasL are elevated in HLH patients (Figs 1 and 2), these cytokines may act together to cause hepatic damage in these patients.

IFN- γ is a potent inhibitor of myeloid colony formation in vitro.²⁹ On the other hand, IFN- γ has been used to treat chronic granulomatous disease patients without serious side effects.³⁰ Transgenic mice expressing high levels of IFN- γ in their bone marrow did not show decreased hematopoiesis despite the decreased number of hematopoietic progenitor cells.³¹ Therefore, IFN- γ alone may not cause myelosuppression in animals. It has been reported that IFN- γ induces Fas receptor expression

on CD34⁺ human bone marrow cells and agonistic anti-Fas antibody enhances IFN- γ -mediated suppression of hematopoietic colony formation.^{2,3} Neutrophils are highly susceptible to agonistic anti-Fas antibody-mediated apoptosis.³² Therefore, it is possible that sFasL is involved in the cytopenia observed at least in some of the HLH patients. However, whether sFasL and IFN- γ suppress hematopoiesis in vivo should be studied further.

Immunosuppressive therapy consisting of antilymphocyte globulin and cyclosporin A is effective in the majority of patients with SAA,³³ suggesting that T-cell-mediated cytotoxicity is involved in the pathogenesis of SAA. The bone marrow cells obtained from the patients with SAA show markedly increased percentages of Fas-expressing CD34⁺ cells, which correlated with their increased sensitivity to anti-Fas antibody-mediated inhibition of colony formation in culture.¹⁹ Aberrant IFN- γ gene expression and elevated numbers of activated suppressor T cells are present in the bone marrow of the SAA patients.^{34,35} These results suggest that Fas-mediated cell death plays a role in the pathogenesis of SAA. However, our results showed that serum sFasL is not elevated in SAA patients, including primary and posthepatitis diseases (Fig 1). These results do not exclude the possibility that FasL-expressing cytotoxic cells are present in the patients' bone marrow and play a role in the pathogenesis of SAA. However, if FasL-expressing cytotoxic cells actively cause the apoptosis of CD34⁺ cells, Fas⁺-CD34⁺ cells might not be able to survive in the bone marrow. The presence of Fas⁺-CD34⁺ cells might reflect the high IFN- γ levels in the patients' bone marrow and does not necessarily indicate that Fas-mediated cell death occurs in SAA. Nakao et al³⁶ established a CD4⁺-CTL clone from an SAA patient. The cytolytic activity of this clone is EGTA-sensitive, indicating that this clone uses perforin/granzyme pathway to kill the target cells.³⁷ The involvement of the Fas/FasL system in SAA should be re-evaluated in future studies.

DBA is a severe congenital abnormality of erythropoiesis developing early in childhood. The bone marrows of affected individuals have markedly reduced or absent red blood cell precursors with normal marrow cellularity and preservation of other hematopoietic cell lineages. Although congenital genetic abnormalities have been suspected in this disorder, the analysis of erythropoietin,¹⁷ erythropoietin receptor,³⁸ c-kit, and c-kit ligand^{39,40} genes has failed to detect any abnormalities in DBA patients. Some of DBA patients respond to corticosteroids, suggesting that an immunological abnormality underlies this disease. However, no clear explanation has been given for its defective erythropoiesis. We showed here that the serum sFasL concentration is high in most of the DBA patients (Fig 1), indicating that activation of cytotoxic cells occurs in DBA. The origin of increased serum sFasL is not clear at present. Lymphocyte-mediated inhibition of erythroid colony formation was implicated in some DBA cases.^{41,42} Therefore, it might be possible that certain subsets of lymphocytes overproduce sFasL in DBA patients. This should be clarified in future study. We have clearly shown that erythroid lineage (GPA⁺) cells in BM express Fas antigen (Fig 5). Bouscary et al⁴³ examined Fas expression on BM cells obtained from normal subjects and myelodysplastic syndrome patients. They reported that GPA⁺ cells did not express Fas antigen to a detectable extent by their FACS analysis using FITC-labeled UB2 anti-Fas MoAb. The

exact reason for the discrepancy between our results (Fig 5) and theirs is not certain. However, it could be due to a different labeling of the UB2 MoAb. We used PE-labeled UB2, which might give brighter fluorescence than the FITC-labeled one. Based on our findings shown in Fig 5, it is possible that erythroid lineage cells are killed by cytotoxic cells via the Fas-mediated pathway in certain conditions. However, an analysis of large samples is required to establish the role of the Fas/FasL system in DBA.

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REFERENCES

- Nagata S: Apoptosis by death factor. *Cell* 88:355, 1997
- Maciejewski J, Selleri C, Anderson S, Young NS: Fas antigen expression on CD34⁺ human marrow cells is induced by interferon- γ and tumor necrosis factor α and potentiates cytokine-mediated hematopoietic suppression in vitro. *Blood* 85:3183, 1995
- Nagafuji K, Shibuya T, Harada M, Mizuno S, Takenaka K, Miyamoto T, Okamura T, Gondo H, Niho Y: Functional expression of Fas antigen (CD95) on hematopoietic progenitor cells. *Blood* 86:883, 1995
- Suda T, Takahashi T, Golstein P, Nagata S: Molecular cloning and expression of the Fas ligand: A novel member of the tumor necrosis factor family. *Cell* 75:1169, 1993
- Griffith TS, Brunner T, Fletcher SM, Green DR, Ferguson TA: Fas ligand-induced apoptosis as a mechanism of immune privilege. *Science* 270:1189, 1995
- Watanabe-Fukunaga R, Brannan CI, Copeland NG, Jenkins NA, Nagata S: Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature* 356:314, 1992
- Takahashi T, Tanaka M, Brannan CI, Jenkins NA, Copeland NG, Suda T, Nagata S: Generalized lymphoproliferative disease in mice, caused by a point mutation in the Fas ligand. *Cell* 76:969, 1994
- Fisher GH, Roseberg FJ, Straus SE, Dale JK, Middleton LA, Lin AJ, Strober W, Lenardo MJ, Puck JM: Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. *Cell* 81:935, 1995
- Rieux-Laucat F, Le-Deist F, Hivroz C, Roberts IA, Debatin KM, Fischer A, de-Villartay JP: Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. *Science* 268:1347, 1995
- Giordano C, Stassi G, De-Maria R, Todaro M, Richiusa P, Papoff G, Ruberti G, Bagnasco M, Testi R, Galluzzo A: Potential involvement of Fas and its ligand in the pathogenesis of Hashimoto's thyroiditis. *Science* 275:960, 1997
- Kondo T, Suda T, Fukuyama H, Adachi M, Nagata S: Essential roles of the Fas ligand in the development of hepatitis. *Nat Med* 3:409, 1997
- Tanaka M, Suda T, Haze K, Nakamura N, Sato K, Kimura F, Motoyoshi K, Mizuki M, Tagawa S, Ohga S, Hatake K, Drummond AH, Nagata S: Fas ligand in human serum. *Nat Med* 2:317, 1996
- Tanaka M, Suda T, Takahashi T, Nagata S: Expression of the functional soluble form of a human Fas ligand in activated lymphocytes. *EMBO J* 14:1129, 1995
- Tanaka M, Suda T, Yatomi T, Nakamura N, Nagata S: Lethal effect of recombinant human Fas ligand in mice pretreated with *Propionibacterium acnes*. *J Immunol* 158:2303, 1997
- Henter J-I, Elinder G, Ost A, the FHL Study Group of the Histiocytosis Society: Diagnostic guidelines for hemophagocytic lymphohistiocytosis. *Semin Oncol* 18:29, 1991
- Brown KE, Tisdale J, Barret J, Dunbar CE, Young NS: Hepatitis-associated aplastic anemia. *N Engl J Med* 336:1059, 1997
- Halperin DS, Freedman MH: Diamond-Blackfan anaemia: Etiology, pathophysiology, and treatment. *Am J Pediatr Hematol Oncol* 11:380, 1989
- Saito I, Serenius B, Compton T, Fox RI: Detection of Epstein-Barr virus DNA by polymerase chain reaction in blood and tissue biopsies from patients with Sjögren's syndrome. *J Exp Med* 169:2191, 1989
- Maciejewski JP, Selleri C, Sato T, Anderson S, Young NS: Increased expression of Fas antigen on bone marrow CD34⁺ cells of patients with aplastic anaemia. *Br J Haematol* 91:245, 1995
- Ogasawara J, Watanabe-Fukunaga R, Adachi M, Matsuzawa A, Kasugai T, Kitamura Y, Itoh N, Suda T, Nagata S: Lethal effect of the anti-Fas antibody in mice. *Nature* 364:806, 1993
- Itoh N, Yonehara S, Ishii A, Yonehara M, Mizushima S, Sameshima M, Hase A, Seto Y, Nagata S: The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell* 66:233, 1991
- Yano H, Fukuda K, Haramaki M, Momosaki S, Ogasawara S, Higaki K, Kojiro M: Expression of Fas and anti-Fas-mediated apoptosis in human hepatocellular carcinoma cell lines. *J Hepatol* 25:454, 1996
- Imashuku S, Hibi S, Fujiwara F, Ikushima S, Todo S: Hemophagocytic lymphohistiocytosis, interferon-gamma-anemia and Epstein-Barr virus involvement. *Br J Haematol* 88:656, 1994
- Rickinson AB: Cellular immunological responses to the virus infection, in Epstein MA, Achong BG (eds): *The Epstein-Barr Virus: Recent Advances*. New York, NY, Wiley, 1990, p 75
- Risdall RJ, McKenna RW, Nesbit ME, Krivit W, Balfour HH, Simmons RL, Bruning RD: Virus-associated hemophagocytic syndrome: A benign histiocytic proliferation distinct from malignant histiocytosis. *Cancer* 44:993, 1979
- Arico M, Janka G, Fisher A, Henter JI, Blanche S, Elinder G, Marinetti M, Ruska MP: Hemophagocytic lymphohistiocytosis. Report of 122 children from the international registry. *Leukemia* 10:197, 1996
- Galle PR, Hofmann WJ, Walczak H, Schaller H, Otto G, Stremmel W, Krammer PH, Runkel L: Involvement of the CD95 (APO-1/Fas) receptor and ligand in liver damage. *J Exp Med* 182:1223, 1995
- Tamura T, Ueda S, Yoshida M, Matsuzaki M, Mohri H, Okubo T: Interferon- γ induces ICE gene expression and enhances cellular susceptibility to apoptosis in the U937 leukemia cell line. *Biochem Biophys Res Commun* 229:21, 1996
- Broxmeyer HE, Lu L, Platzer E, Feit C, Juliano L, Rubin BY: Comparative analysis of the influences of human gamma, alpha and beta interferons on human multipotential (CFU-GEMM), erythroid (BFU-E) and granulocyte-macrophage (CFU-GM) progenitor cells. *J Immunol* 131:1300, 1983
- Sechler JM, Malech HL, White CJ, Gallin JI: Recombinant human interferon-gamma reconstitutes defective phagocyte function in patients with chronic granulomatous disease of childhood. *Proc Natl Acad Sci USA* 85:4874, 1988
- Young HA, Klinman DM, Reynolds DA, Grzegorzewski KJ, Nii A, Ward JM, Winkler-Pickett RT, Ortaldo JR, Kenny JJ, Komschilies KL: Bone marrow and thymus expression of interferon- γ results in severe B-cell lineage reduction, T-cell lineage alterations, and hematopoietic progenitor deficiencies. *Blood* 89:583, 1997
- Liles WC, Kiener PA, Ledbetter JA, Aruffo A, Klebanoff SJ: Differential expression of Fas (CD95) and Fas ligand on normal human phagocytes: Implications for the regulation of apoptosis in neutrophils. *J Exp Med* 184:429, 1996

33. Rosenfeld SJ, Kimball J, Vining D, Young NS: Intensive immunosuppression with antithymocyte globulin and cyclosporine as treatment for severe acquired aplastic anemia. *Blood* 85:3058, 1995
34. Nakao S, Yamaguchi M, Shiobara S, Yokoi T, Miyawaki T, Taniguchi T, Matsuda T: Interferon-gamma gene expression in unstimulated bone marrow mononuclear cells predicts a good response to cyclosporine therapy in aplastic anemia. *Blood* 79:2532, 1992
35. Maciejewski JP, Hibbs JR, Anderson S, Katevas P, Young NS: Bone marrow and peripheral blood lymphocyte phenotype in patients with bone marrow failure. *Exp Hematol* 22:1102, 1994
36. Nakao S, Takami A, Takamatsu H, Zeng W, Sugimori N, Yamazaki H, Miura Y, Ueda M, Shiobara S, Yoshioka T, Kaneshige T, Yasukawa M, Matsuda T: Isolation of a T-cell clone showing HLA-DRB1*0405-restricted cytotoxicity for hematopoietic cells in a patient with aplastic anemia. *Blood* 89:3691, 1997
37. Nakao S: (1997) Expression of Fas gene in aplastic anemia, in Takaku F, Miyazaki S, Saito H, Mizoguchi H, Sakata Y (eds): *Annual Review Ketsueki*. Tokyo, Japan, Chugai Igakusha, 1997, p 43 (in Japanese)
38. Dianzani I, Garelli E, Dompe C, Crescenzo N, Locatelli F, Schillo G, Castaman G, Bagnara GP, Olivieri NF, Gabutti V, Ramenghi U: Mutations in the erythropoietin receptor gene are not a common cause of Diamond-Blackfan anemia. *Blood* 87:2568, 1996
39. Abkowitz JL, Broudy VC, Benet LG, Zsebo KM, Martin FH: Absence of abnormalities of c-kit or its ligand in two patients with Diamond Blackfan anemia. *Blood* 79:25, 1992
40. Drachtman RA, Geissler EN, Alter BP: The SCF and c-kit genes in Diamond Blackfan anemia. *Blood* 79:2177, 1992
41. Hoffman R, Zanjani ED, Vila J, Zalusky R, Lutton JD, Wasserman LR: Diamond-Blackfan syndrome: Lymphocyte-mediated suppression of erythropoiesis. *Science* 193:899, 1976
42. Steinberg MH, Coleman MF, Pennebaker JB: Diamond-Blackfan syndrome: Evidence for T-cell mediated suppression of erythroid development and a serum blocking factor associated with remission. *Br J Haematol* 41:57, 1979
43. Bouscary D, De Vos J, Guesnu M, Jondeau K, Viguier F, Melle J, Picard F, Dreyfus F, Fontenay-Roupie M: Fas/Apo-1 (CD95) expression and apoptosis in patients with myelodysplastic syndromes. *Leukemia* 11:839, 1997