International Journal of
HEMATOLOGY

The Serum Cytokine Profiles of Lymphoma-Associated Hemophagocytic Syndrome: A Comparative Analysis of B-Cell and T-Cell/Natural Killer Cell Lymphomas

Tatsuharu Ohno,[a] Yasunori Ueda,[b] Ken-ichi Nagai,[c] Takayuki Takahashi,[c] Yoshiteru Konaka,[d] Teruyuki Takamatsu,[e] Takayo Suzuki,[f] Masataka Sasada,[g] Takashi Uchiyama,[h] members of the Kyoto University Hematology/Oncology Study Group*

[a]Division of Hematology and Immunology, Department of Internal Medicine, Ohtsu Red Cross Hospital, Ohtsu; [b]Department of Hematology and Oncology, Kurashiki Central Hospital, Kurashiki; [c]Department of Hematology and Immunology, Kobe Central Municipal Hospital, Kobe; [d]Division of Hematology and Rheumatology, Department of Medicine, Kitano Hospital, Osaka; [e]Department of Hematology and Immunology, Rakuwakai Otowa Hospital, Kyoto; [f]Department of Hematology, Shiga Prefectural Center for Adult Disease, Moriyama; [g]College of Medical Technology, Kyoto University, Kyoto; [h]Department of Hematology/Oncology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Received October 1, 2002; received in revised form December 4, 2002; accepted December 9, 2002

Abstract

To elucidate the differences in pathogenesis between lymphoma-associated hemophagocytic syndromes (LAHS) of the T-cell/natural killer cell (T/NK) and B-cell (B) types, we comparatively analyzed the clinical features and serum cytokine profiles of 33 patients with LAHS registered in the Kyoto University Hematology/Oncology Study Group. The serum cytokine levels of each patient group (B-LAHS versus T/NK-LAHS) were expressed as the ratio of the median to the upper normal values of the respective cytokines and were as follows: 19.05 versus 13.99 for soluble interleukin 2 (IL-2) receptor, 0.67 versus 0.67 for granulocyte-macrophage colony-stimulating factor (GM-CSF), 0.64 versus 1.26 for G-CSF, 5.70 versus 3.61 for M-CSF, 1.54 versus 3.39 for interferon γ (IFN-γ), 13.17 versus 1.17 for IL-6, 6.88 versus 1.58 for tumor necrosis factor α (TNF-α), 0.71 versus 0.41 for IL-1β, 1.99 versus 0.21 for IL-12, and 105.32 versus 29.65 for IL-10. The serum levels of IL-6, TNF-α, and IL-10 were significantly higher in the B-LAHS group, whereas those of IFN-γ were significantly lower. These differences between the 2 groups may reflect a difference in the pathogenesis. Higher serum levels of IL-6, TNF-α, and IL-10 may be derived at least partly from neoplastic B-cells themselves. In addition, the extremely high serum levels of IL-10 suggest that a compensatory anti-inflammatory process may operate in both groups and give rise to a profound immunosuppressive state and a poor outcome. *Int J Hematol.* 2003;77:286-294.
©2003 The Japanese Society of Hematology

Key words: Malignant lymphoma; Hemophagocytic syndrome; Cytokine; Intravascular lymphomatosis; Systemic inflammatory response syndrome

1. Introduction

Hemophagocytic syndrome (HPS) is a clinicopathologic entity characterized by persistent high-grade fever, hepatosplenomegaly, pancytopenia, coagulation abnormality, liver dysfunction, and generalized proliferation of macrophages that exhibit hemophagocytosis [1,2]. This syndrome has been ascribed to hyperactivation of T-cells and macrophages and is characterized by marked elevations in levels of serum cytokines [2-12]. Reactive HPS is induced by a variety of causes, such as infections, autoimmune diseases, disseminated carcinomas, and hematologic malignancies, mainly non-Hodgkin's lymphomas (lymphoma-associated hemophagocytic syndrome, LAHS) [1,2]. LAHS is largely diagnosed at initial presentation; however, it occasionally occurs at remission, relapse, or transformation and may even precede the lymphoma discovery [13,14].

*Participants and their affiliations are listed in the Acknowledgments.

Correspondence and reprint requests: Tatsuharu Ohno, MD, Division of Hematology and Immunology, Department of Internal Medicine, Ohtsu Red Cross Hospital, 1-35, 1 Chome, Nagara, Ohtsu 520, Japan; 81-77-522-4131; fax: 81-77-525-8018 (e-mail: tatsohno@ja2.so-net.ne.jp).

Most LAHS cases reported in Western countries have been T-cell/natural killer cell lymphomas (T/NK-LAHS) with only a small number of HPS cases being derived from B-cell lymphomas (B-LAHS), and the data on their clinicopathologic features are very limited [1,15-21]. Recently, the number of reported B-LAHS cases, including those in our previous report, has been increasing, both in Western and in Asian countries, mainly in Japan [22-30]. In a large survey of 142 Japanese LAHS cases conducted by Takahashi et al, approximately half the cases were demonstrated to be B-LAHS, and their clinicopathologic features are becoming increasingly clear [28]. However, these patients, whether with the B-cell or T/NK-cell phenotypes, usually shared common clinical features at presentation of HPS and could not be classified into the 2 phenotypes until completion of the full pathologic examination.

Neoplastic T/NK cells in T/NK-LAHS, as well as the reactive T/NK cells in Epstein-Barr virus (EBV)–associated HPS (VAHS), have been confirmed to be infected with clonal EBV, suggesting that the clinical symptoms are ascribed to elevated serum cytokines produced by the uncontrolled proliferation of EBV-infected T/NK cells [14,31-38]. On the other hand, the mechanisms operating in B-LAHS remain ill defined. These observations have prompted us to comparatively analyze both B-LAHS and T/NK-LAHS for the similarities and differences in their cytokine patterns, in addition to the clinical pictures. We then discuss the mechanisms operating in LAHS.

2. Materials and Methods

2.1. Patients

Thirty-five adult patients with malignant lymphoma for whom the possibility of concomitant infection had been excluded were registered in the Kyoto University Hematology/Oncology Study Group after their informed consent was obtained, and on examination 33 patients fulfilled the diagnostic criteria proposed by Imashuku [2]. Of these patients, 18 had B-LAHS, and 13 had T/NK-LAHS. The cell lineages of the remaining 2 patients were not determined. The profiles and routine laboratory characteristics of these 33 patients are listed in Table 1. Lymphomas were, in principle, classified according to the revised European-American classification of lymphoid neoplasms. The serum concentrations of granulocyte-macrophage colony-stimulating factor (GM-CSF), G-CSF, M-CSF, interleukin 1β (IL-1β), IL-6, IL-10, IL-12, tumor necrosis factor α (TNF-α), interferon γ (IFN-γ), and soluble IL-2 receptor (sIL-2R) were measured in 33 patients with LAHS.

2.2. Cytokine Assay

The sera collected at diagnosis were frozen at –20°C until use. Serum cytokines were measured by sandwich enzyme-linked immunosorbent assay (ELISA). The ELISA kits used were as follows: GM-CSF, human GM-CSF ELISA system (Amersham Biosciences, Tokyo, Japan; detection limit, 2.0 pg/mL); M-CSF, human M-CSF ELISA system (Amersham Biosciences; detection limit, 9 pg/mL); G-CSF, G-CSF CLEIA kit (Chugai, Tokyo, Japan; detection limit, 1.0 pg/mL); IL-1β, Quantikine HS human IL-1β immunoassay kit (R&D Systems, Minneapolis, MN, USA; detection limit, 0.10 pg/mL); IL-6, Quantikine HS human IL-6 immunoassay kit (R&D Systems; detection limit, 0.1 pg/mL); IFN-γ, IFN-γ ELISA (Bender MedSystems, Vienna, Austria; detection limit, 1.5 pg/mL); TNF-α, Quantikine HS human TNF-α immunoassay kit (R&D Systems; detection limit, 0.18 pg/mL); sIL-2R, Cellfree sIL-2R bead assay kit (Yamanouchi, Tokyo, Japan; detection limit, 85 U/mL). Assays for IL-10 were conducted with the Cytoscreen human IL-10 immunoassay kit (BioSource, Camarillo, CA, USA; detection limit, 0.2 pg/mL), which is specific for human IL-10 and is not affected by viral IL-10 produced by EBV. Assays for IL-12 were conducted with the Quantikine HS human IL-12 immunoassay kit (R&D Systems; detection limit, 0.78 pg/mL), which recognizes only the IL-12 heterodimer and not the individual subunits of the dimer.

2.3. Detection of EBV

EBV DNA was detected by a polymerase chain reaction assay using the EBV *Bam*HI–W region amplification primers as described previously [25]. In situ hybridization with the probe complementary to EBV-encoded small nuclear RNA 1 (EBER1) was performed as described previously to visualize EBV-infected neoplastic cells [25]. Southern blot analysis to assess the monoclonality of EBV was performed as previously described with the probe against the terminal repetitive sequence [39,40].

2.4. Statistics

All data obtained in the ELISA assays were examined to determine if they were normally distributed. When the data in both B-LAHS and T/NK-LAHS groups conformed to normal distributions, the means were analyzed with the Student or Welch *t* test. Otherwise, the medians were analyzed with the Wilcoxon rank sum test. Survival analysis was performed with the Kaplan-Meier method, and the difference was examined with the log-rank test. Correlations between the 2 parameters were calculated with the Spearman rank test.

3. Results

3.1. Patient Profiles

Of the 35 patients registered, 33 patients were eligible for the cytokine analysis according to Imashuku's criteria [2]. As summarized in Table 1, 18 and 13 patients were classified into B-LAHS and T/NK-LAHS groups, respectively. HPS was diagnosed on the initial presentation in 13 patients of the former group and in 11 patients of the latter group. Malignant lymphoma was diagnosed in 2 patients, with the clinical features mimicking malignant histiocytosis, by bone marrow puncture. Considering these 2 patients' deteriorated conditions, chemotherapy preceded the determination of the cell lineage. The patients with B-LAHS were significantly older

Table 1.
Characteristics of the Patients with Lymphoma-Associated Hemophagocytic Syndrome*

Patient	Age, y	Sex	Stage	Diagnosis (Site or Method)	B or T/NK Cell Type	Survival, wk	Onset of HPS	VCA IgG	VCA IgM	EA IgG	EBNA	PCR	EBER
S.H.	70	M	VI	IVL (autopsy)	B	10.6	IP	ND	<10	<10	10	ND	ND
K.K.	56	F	VI	IVL (autopsy)	B	55.7	IP	160	<10	<10	10	(–)	(–)
T.U.	68	M	VI	IVL (autopsy)	B	3.1	IP	80	<10	<10	20	(–)	(–)
Y.A.	83	M	VI	IVL (autopsy)	B	41.6	IP	160	<10	<10	40	(–)	(–)
A.S.	75	F	VI	IVL (kidney)	B	18.7	IP	160	<10	<10	40	ND	ND
C.N.	68	F	VI	IVL (uterus)	B	12.8+	IP	80	<10	<10	20	ND	ND
T.S.	67	M	VI	IVL (autopsy)	B	7.1	IP	160	<10	<10	40	ND	ND
O.M.	65	M	VI	DL (bone marrow)	B	6.0	IP	160	<10	<10	80	ND	(–)
H.H.	83	M	VI	DL (lymph node)	B	NS	IP	160	<10	<10	40	ND	(–)
A.N.	83	F	VI	DL (lymph node)	B	NS	IP	80	<10	<10	40	ND	(–)
M.K.	65	F	VI	DL (lymph node)	B	NS	IP	160	<10	<10	20	ND	ND
K.S.	86	F	VI	DL (lymph node)	B	NS	IP	80	<10	<10	40	ND	ND
Y.I.	76	M	VI	DL (bone marrow)	B	6.0	IP	160	<10	<10	10	ND	ND
M.H.	65	F	VI	DL (lymph node)	B	6.7	TC	40	<10	<10	10	(–)	(–)
F.S.	64	F	III	DL (lymph node)	B	3.0	TC	160	<10	<10	40	(–)	(–)
S.O.	77	M	III	DL (lymph node)	B	6.6	TC	80	<10	<10	40	ND	(–)
M.T.	65	M	VI	DL (lymph node)	B	6.0	TC	80	<10	<10	20	ND	ND
R.K.	68	M	III	DL (lymph node)	B	3.1	TC	80	<10	<10	40	ND	ND
H.O.	49	M	VI	LGL leukemia (bone marrow)	NK	24.9+	IP	ND	ND	80	ND	(MS)	ND
M.O.	61	M	VI	AC (maxillary tumor)	NK	5.0+	IP	640	<10	40	40	ND	(+)
M.I.	17	F	VI	AC (skin)	NK	34.1+	IP	160	<10	10	<10	(MS)	(+)
T.H.	60	M	VI	AC (nasal tumor)	NK	15.7	IP	80	<10	<10	40	ND	ND
I.K.	68	M	III	ALCL (adrenal gland)	T	7.3	IP	160	<10	<10	20	ND	(+)
S.K.	78	M	VI	ALCL (lymph node)	T	21.5+	IP	1280	<10	40	20	ND	ND
Y.O.	67	M	VI	AILD (lymph node)	T	6.9	IP	1280	<10	20	10	ND	(+)
M.Y.	86	F	VI	AILD (lymph node)	T	NS	IP	640	<10	ND	ND	ND	ND
T.I.	56	M	VI	PTCL (autopsy)	T	1.9	IP	1280	<10	<10	40	ND	(+)
S.M.	67	M	VI	PTCL (autopsy)	T	0.4	IP	2560	<10	<10	40	ND	(+)
Y.K.	39	M	VI	PTCL (autopsy)	T	3.3	IP	640	<10	<10	20	ND	(+)
R.K.	43	M	III	AC (nasal tumor)	NK	0.6	TC	10	<10	<10	<10	ND	ND
T.I.	63	M	VI	PTCL (autopsy)	T	7.1	TC	160	<10	<10	20	ND	ND
A.N.	37	F	VI	MH-like (bone marrow)	ND	33.0+	IP	80	ND	ND	10	(–)	ND
Y.H.	69	M	VI	MH-like (bone marrow)	ND	52.1+	IP	160	<10	<10	40	ND	ND

*HPS indicates hemophagocytic syndrome; VCA IgG, viral capsid antigen immunoglobulin G; EA, Epstein-Barr virus early antigen; EBNA, Epstein-Barr virus nuclear antigen; PCR, polymerase chain reaction; EBER, Epstein-Barr virus–encoded small nuclear RNA; M, male; IVL, intravascular lymphomatosis; IP, initial presentation; ND, not done; F, female; DL, diffuse large cell lymphoma; NS, not specified; TC, terminal complication; LGL, large granular lymphocyte; NK, natural killer; MS, monoclonal by Southern blotting; AC, angiocentric lymphoma; ALCL, anaplastic large cell lymphoma; AILD, angioimmunoblastic T-cell lymphoma; PTCL, peripheral T-cell lymphoma, unspecified; MH-like, malignant histiocytosis-like lymphoma.

than those with T/NK-LAHS, who showed a wide age distribution. Pathologically, all B-LAHS cases are classified as diffuse, large B-cell lymphoma. Of note is that 7 cases were included in the specific subtype, intravascular lymphomatosis. Of the 13 T/NK-LAHS cases, 5 and 8 cases were derived from NK cells and T-cells, respectively. Patterns of antibodies against EBV suggested remote infection in essentially all patients with B-LAHS, and EBV could not be detected in the tumor samples of 9 patients with B-LAHS examined with the polymerase chain reaction and/or EBER in situ hybridization. On the other hand, aberrant antibody patterns (viral capsid antigen immunoglobulin G $\geq \times 640$; EBV early antigen immunoglobulin G $\geq \times 20$; EBV nuclear antigen $< \times 10$) were observed in 10 patients with T/NK-LAHS, and EBV was detected in the tumor samples of 8 patients with T/NK-LAHS examined with Southern blot analysis and/or EBER in situ hybridization. Fifty percent survival rates for B-LAHS and T/NK-LAHS were 6.64 weeks and 3.29 weeks, respectively (median survival rates, 6.71 weeks and 5.08 weeks) and were not significantly different. Anemia and thrombocytopenia tended to be severe; C-reactive protein levels were higher in B-LAHS cases, and neutropenia and liver damage were more prominent in T/NK-LAHS cases (Table 2).

3.2. Cytokines

As shown in Figure 1, the serum levels of GM-CSF were less than the minimum detectable concentration in all LAHS patients except the 2 NK-LAHS cases (2.7 pg/mL and 2.9 pg/mL), which were still considered to be within the normal range. The serum levels of G-CSF in the B-LAHS patients ranged from 7.3 pg/mL to 127.0 pg/mL (median, 24.8 pg/mL), whereas those in T/NK-LAHS patients ranged from 13.4 pg/mL to 112.0 pg/mL (median, 49.0 pg/mL). Although the difference between the B-LAHS and T/NK-LAHS groups was significant according to the Wilcoxon

Table 2.
Representative Clinical Parameters in the Patients with Lymphoma-Associated Hemophagocytic Syndrome*

	Age, y	WBC, $\times 10^9$/L	Neutrophil, $\times 10^9$/L	Hb, g/dL	Plt, $\times 10^9$/L	CRP, mg/dL	T. Bil, mg/dL	GOT, /UNL	GPT, /UNL	LDH, /UNL	Ferritin pg/mL	NCC, /μL	Macrophage, %
B-LAHS													
Mean	71.30	3.15	2.42	8.44	31.2	13.30	1.72	2.25	0.92	4.47	3810	75,023	5.73
SD	8.38	1.87	1.64	1.42	17.7	5.51	1.58	1.98	0.67	1.68	2593	43,032	2.98
T/NK-LAHS													
Mean	58.00	2.12	1.40	9.66	52.3	9.58	3.12	7.18	3.98	5.68	6097	70,260	10.66
SD	17.90	1.36	1.04	2.15	32.0	5.77	2.60	4.26	2.54	3.66	2078	50,564	13.40
Student *t* test													
P	.01	.10	.05	.02	.16	.02	.08	.01	.00	.19	.10	.56	.31

*WBC indicates white blood cell; Hb, hemoglobin; Plt, platelets; CRP, C-reactive protein; T.Bil, total bilirubin; GOT, glutamic-oxaloacetic transaminase; UNL, upper normal limit; GPT, glutamic-pyruvic transaminase; LDH, lactate dehydrogenase; NCC, nucleated cell count; B-LAHS, B-cell lymphoma-associated hemophagocytic syndrome; T/NK, T-cell/natural killer cell.

rank sum test ($P = .00341$), this result may simply reflect the difference in neutrophil counts (Table 2) [41].

The serum levels of M-CSF in the B-LAHS group ranged from 617 pg/mL to 8470 pg/mL with a median of 3760 pg/mL, and the levels in T/NK-LAHS patients ranged from 412 pg/mL to 8810 pg/mL with a median of 2380 pg/mL. The difference in the medians was significant ($P = .00341$). These high serum levels in both LAHS groups are presumably due to the effect of activated macrophages. The serum levels of sIL-2R were very high without exception. The levels in the B-LAHS patients ranged from 3890 U/mL to 43,800 U/mL with a median of 13,600 U/mL, and the levels in T/NK-LAHS patients ranged from 2590 U/mL to 27,700 U/mL with a median of 12,500 U/mL. The median value in the B-LAHS group was significantly higher than that of the T/NK-LAHS group ($P = .00002$).

As shown in Figure 2, the serum levels of IL-1β in B-LAHS patients ranged from 0.28 pg/mL to 5.13 pg/mL with a median of 1.41 pg/mL, and the levels in the T/NK-LAHS group ranged from 0.13 pg/mL to 3.43 pg/mL with a median of 0.82 pg/mL. Although the difference in the values of the 2 groups was significant ($P = .00005$), the elevation of IL-1β in the sera of LAHS patients was not striking, because 12 of 18 B-LAHS cases and 11 of 13 T/NK-LAHS cases fell within the normal range. The serum levels of IFN-γ in B-LAHS patients ranged from 1.6 pg/mL to 436.0 pg/mL with a median of 14.4 pg/mL, and those in T/NK-LAHS patients ranged from 12.4 pg/mL to 1910 pg/mL with a median of 31.9 pg/mL. The median value was significantly higher in the T/NK-LAHS group ($P = .00008$).

The serum levels of TNF-α in B-LAHS patients ranged from 4.34 pg/mL to 83.5 pg/mL with a mean of 32.8 pg/mL

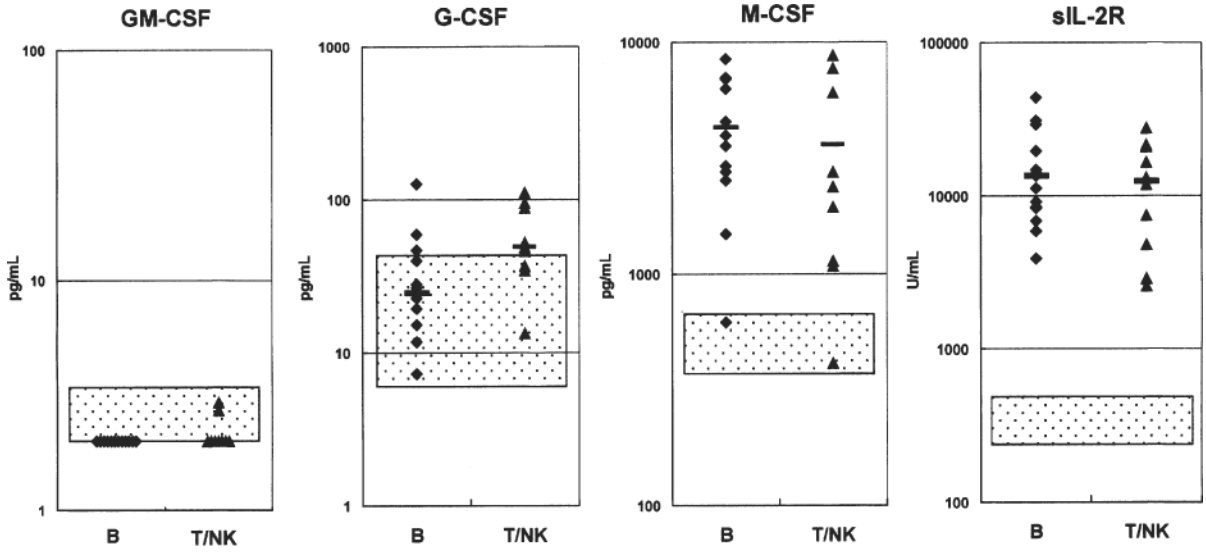

Figure 1. The serum levels of granulocyte-macrophage colony-stimulating factor (GM-CSF), G-CSF, M-CSF, and soluble interleukin 2 receptor (sIL-2R) in healthy control subjects (n = 20) ranged from an undetectable level (75%) to 3 pg/mL, from 7.7 pg/mL to 38.9 pg/mL, from 436 pg/mL to 660 pg/mL, and from 254 to 534 U/mL, respectively. These normal ranges are depicted as dotted areas in the figure. Serum levels of these cytokines were determined in 18 cases of B-cell (B) and 13 cases of T-cell/natural killer cell (T/NK) lymphoma-associated hemophagocytic syndrome. Of these cytokines, only the M-CSF data followed a normal distribution and were analyzed with the Student *t* test; the other data were analyzed with the Wilcoxon rank sum test. The short horizontal lines represent the mean of M-CSF levels and the median for the other cytokines.

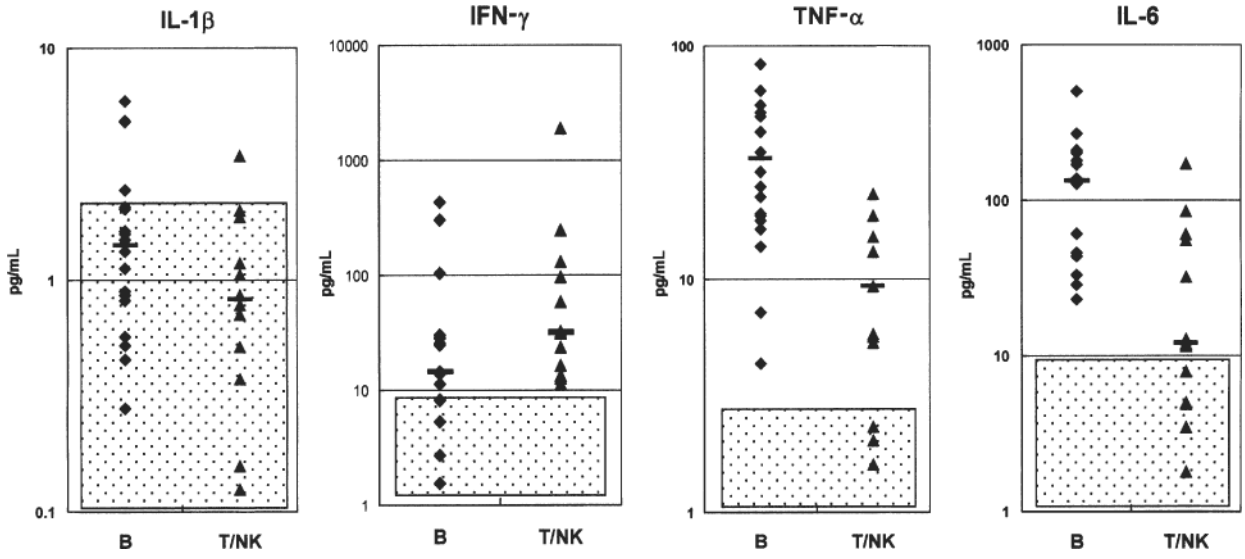

Figure 2. The serum levels of interleukin 1β (IL-1β), interferon γ (IFN-γ), tumor necrosis factor α (TNF-α), and IL-6 in healthy control subjects (n = 20) ranged from an undetectable level (30%) to 2.00 pg/mL, from an undetectable level (65%) to 9.2 pg/mL, from an undetectable level (5%) to 3.62 pg/mL, and from 0.4 pg/mL to 10.1 pg/mL, respectively. These normal ranges are depicted as dotted areas in the figure. Serum levels of these cytokines were determined in 18 cases of B-cell (B) and 13 cases of T-cell/natural killer cell (T/NK) lymphoma-associated hemophagocytic syndrome. Of these cytokines, only the TNF-α data followed a normal distribution and were analyzed with the Student *t* test; the other data were analyzed with the Wilcoxon rank sum test. The short horizontal lines represent the mean of TNF-α levels and the median for the other cytokines.

(median, 24.9 pg/mL), and the levels in the T/NK-LAHS group ranged from 1.62 pg/mL to 23.2 pg/mL with a mean of 9.29 pg/mL (median, 5.8 pg/mL). The data in both groups followed a normal distribution, and serum TNF-α levels in the B-LAHS group were significantly higher than those of the T/NK-LAHS group (P = .00056). The serum levels of IL-6 in the B-LAHS patients ranged from 23.0 pg/mL to 500 pg/mL with a median of 133 pg/mL, and the levels in the T/NK-LAHS patients ranged from 1.8 pg/mL to 173 pg/mL with a median of 12.0 pg/mL. The median serum IL-6 level of the B-LAHS group was significantly higher than that of the T/NK-LAHS group (P = .00002).

As shown in Figure 3, the serum levels of IL-10 were markedly high in both B-LAHS and T/NK-LAHS groups. Serum IL-10 levels in the B-LAHS patients ranged from 7.2 pg/mL to 6340 pg/mL with a median of 743 pg/mL, and levels in the T/NK-LAHS patients ranged from 28.7 pg/mL to 8900 pg/mL with a median of 209 pg/mL. The median serum IL-10 level of the B-LAHS group was significantly higher than that of the T/NK-LAHS group (P = .00341). The serum levels of IL-12 in B-LAHS patients ranged from an undetectable level to 43.9 pg/mL with a median of 7.45 pg/mL, and the levels in the T/NK-LAHS patients ranged from an undetectable level to 6.21 pg/mL. In 7 of 12 B-LAHS cases and in 8 of 9 T/NK-LAHS cases, serum IL-12 levels were within the normal range. The difference in the median values between the 2 groups was not significant (P = .10988).

As shown in the radar chart (Figure 4), the serum cytokine levels of each patient group were expressed as the ratio of the median to the upper normal value of the respective cytokines, and the results (B-LAHS versus T/NK-LAHS) were 19.05 versus 13.99 for sIL-2R, 0.67 versus 0.67 for GM-CSF, 0.64 versus 1.26 for G-CSF, 5.70 versus 3.61 for M-CSF, 1.54 versus 3.39 for IFN-γ, 13.17 versus 1.17 for IL-6, 6.88 versus 1.58 for TNF-α, 0.71 versus 0.41 for IL-1β, 1.99 versus 0.21 for IL-12, and 105.32 versus 29.65 for IL-10. The LAHS patients as a whole did not exhibit marked elevations in the serum levels of GM-CSF, G-CSF, IL-1β, and IL-12, but both groups showed striking elevations in the levels of M-CSF, sIL-2R, and IL-10. Discrepancies between the B-LAHS and the T/NK-LAHS groups were evident in the serum levels of IL-6, TNF-α, IFN-γ, and IL-10. The results were substantially the same among the patients who had developed HPS at initial presentation. No correlation between cytokine levels and patient prognosis was found.

4. Discussion

In this study, we analyzed 33 patients with LAHS. Approximately half (54.5%, 18 of 33 patients) of the LAHS cases were B-cell lymphomas of the diffuse, large cell type, and the predominance of the specific subtype (intravascular lymphomatosis) was noted (53.8%, 7 of 13 B-LAHS cases with HPS at initial presentation). These cases may fall within the Asian variant of intravascular lymphomatosis proposed by Murase et al [24]. As far as we were able to determine, an association with EBV was suggested in all patients with T/NK-LAHS, in contrast to the patients with B-LAHS, in whom no sign of involvement with EBV could be detected. This finding is consistent with our previous observations and recently reported data from Japan [24,25,28] and suggests that EBV infection of lymphoma cells is not a factor contributing to the onset of B-LAHS. In addition to hemophagocytic macrophages, many reactive $CD3^+$ T-cells were

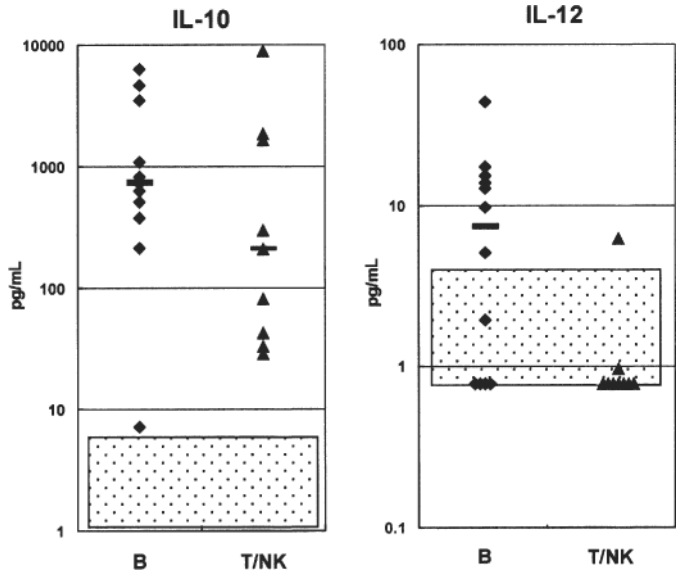

Figure 3. The serum levels of interleukin 10 (IL-10) and IL-12 in healthy control subjects (n = 20) ranged from an undetectable level (65%) to 7.1 pg/mL and from an undetectable level (65%) to 3.74 pg/mL, respectively. These normal ranges are depicted as dotted areas in the figure. Serum levels of these cytokines were determined in 12 cases of B-cell (B) and 9 cases of T-cell/natural killer cell (T/NK) lymphoma-associated hemophagocytic syndrome. Neither cytokine followed a normal distribution, and the data were analyzed with the Wilcoxon rank sum test. The short horizontal lines represent the median.

present in the bone marrow of all B-LAHS patients who have been reported [29,30]. The morphological characteristics of these reactive T-cells suggest that they are functionally activated, but these reactive T-cell populations were not assessable in T/NK-LAHS cases [30]. Thus, as was suggested previously [25], reactive T-cells may be candidates for cytokine production and play a causative role in B-LAHS.

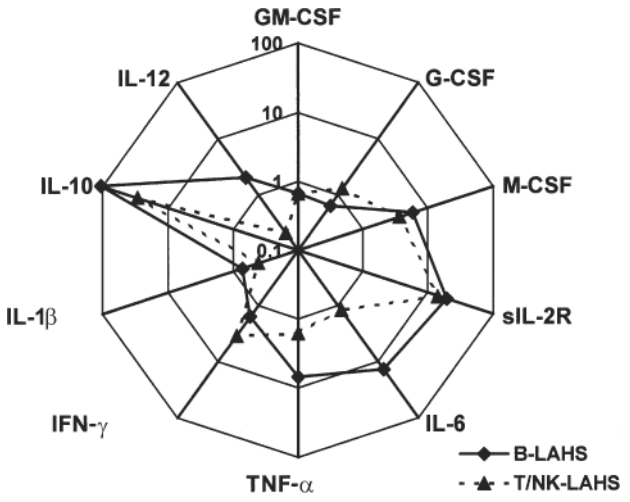

Figure 4. The radar chart showing similarities and differences between the serum cytokine profiles of the B-cell lymphoma-associated hemophagocytic syndrome (B-LAHS) group and the T-cell/natural killer cell (T/NK)-LAHS group. Each point expresses the ratio of the median of the respective serum cytokine levels of the LAHS cases to the upper normal limit of control subjects. GM-CSF indicates granulocyte-macrophage colony-stimulating factor; sIL-2R, soluble interleukin 2 receptor; TNF-α, tumor necrosis factor α; IFN-γ, interferon γ.

In the great majority of T/NK-LAHS cases, including those of the present study, the neoplastic cells have been shown to be infected with clonal EBV similar to the reactive T-cells in VAHS [14,31-38]. Lay et al showed that the infection of T-cells by EBV selectively up-regulates TNF-α expression, which can activate macrophages in combination with IFN-γ and probably other cytokines [37]. These findings suggest that EBV functions in T/NK-LAHS patients as an inducer of a complex activation program of the infected neoplastic T-cells, and the clinical symptoms are ascribed to elevated levels of serum cytokines produced by these EBV-infected neoplastic T-cells.

By analyzing the serum cytokine levels of these patients, we have shown the similarities and differences between cases of B-LAHS and T/NK-LAHS. Although GM-CSF and G-CSF are produced by T-cells or macrophages, neither B-LAHS nor T/NK-LAHS patients showed marked changes in serum levels, suggesting that the commitment of these cytokines to the pathogenesis of HPS may be negligible. On the other hand, M-CSF exhibited a marked increase in both B-LAHS and T/NK-LAHS cases, reflecting a systemic hyperactivation of macrophages. In this context, the beneficial use of G-CSF, but not of M-CSF, during the neutropenic phase after chemotherapy against LAHS may be substantiated.

Markedly elevated serum levels of sIL-2R have been reported in LAHS as well as in HPS induced by other pathologic processes and have been interpreted as an index of T-cell activation in HPS [3,5,6,42]. Given that serum sIL-2R has been assumed to be a product shed out of proliferating neoplastic T/NK cells in T/NK-LAHS cases, how should the

present findings that serum levels of sIL-2R in B-LAHS cases are significantly higher than in T/NK-LAHS cases be interpreted? In our previous study, we showed that the neoplastic cells of our B-LAHS cases expressed CD25 uniformly [25], and normal and neoplastic B-cells in some instances do express CD25 and release sIL-2R from their cell surfaces [43,44]. It is not likely, however, that serum sIL-2R is solely derived from neoplastic B-cells in B-LAHS cases. As mentioned above, tumor-infiltrating T-cells reacting with disseminated lymphoma cells may play an important additive role in elevating levels of sIL-2R, as well as the other cytokines in B-LAHS cases, as is discussed below [29,30].

The serum levels of IL-1β were not a good indicator in the present findings for either B-LAHS or T/NK-LAHS. This result is consistent with previous data including VAHS cases, which showed that serum IL-1 levels did not correlate with other clinical parameters or with survival [4]. However, it is possible that IL-1β takes part in the pathogenesis of HPS in the microenvironment surrounding neoplastic cells, where the exquisitely controlled cytokine network is perturbed. Comparable to the findings of previous studies, the serum levels of IFN-γ in this study were significantly higher in the T/NK-LAHS group [5-8,45]. The difference between the 2 groups may suggest the involvement of EBV, although the magnitude of the difference is small compared with that found in children [45]. IFN-γ has been speculated to be one of the key cytokines that induce HPS, because IFN-γ is a potent activator of macrophages and is a representative cytokine produced by subtype 1 helper T-cells (Th1). HPS has been regarded as a pathologic state created by excessive Th1 polarization [2,11,12]. Possibly, the situation is similar to that of IL-1β. These 2 cytokines should be estimated locally via detection of the expression of messenger RNA or its product.

TNF-α is an autocrine growth factor for normal B-cells [46,47]. Stimulation of B-cells by several triggers induces rapid TNF-α gene transcription and the proliferation of B-cells, which is augmented by TNF-α. Furthermore, TNF-α up-regulates Bcl-2 expression and decreases the apoptosis of B-cells [48]. Thus, the higher serum levels of TNF-α in the B-LAHS group compared with the T/NK-LAHS group do not appear to be strange. Presumably, serum TNF-α is mainly derived from actively proliferating neoplastic B-cells. If T/NK-LAHS is triggered by TNF-α, which is excessively produced by EBV-infected neoplastic T/NK cells, as has been shown by Lay et al [37], one may assume that neoplastic B-cells themselves trigger B-LAHS via TNF-α.

Although IL-6 is produced by various kinds of cells, elevated serum levels of IL-6 have been shown to be derived from neoplastic B-cells in patients with B-cell lymphoma [49,50]. In B-LAHS, higher serum levels of IL-6 may also be caused at least partly by neoplastic B-cells. In addition to TNF-α and IL-6, B-cells produce IL-10 [51,52], which has a potent stimulating effect on B-cells. Increased serum IL-10 levels have been found in vivo in patients with B-cell lymphoma [51]. Markedly higher levels of serum IL-10 in B-LAHS may also at least partly be due to their production by lymphoma cells themselves. Because IL-6 and TNF-α, as well as IL-10, are produced by neoplastic B-cells, the distinctive cytokine feature that serum levels of TNF-α, IL-6, and IL-10 were higher in B-LAHS than in T/NK-LAHS may confirm the previous findings that these cytokines act as cooperative growth factors for neoplastic B-cells [46-53]. Thus, the higher profiles of these 3 cytokines may be unique characteristics for B-LAHS. Of note here is that the serum levels of both TNF-α and IL-10 remained high in both B-LAHS and T/NK-LAHS groups and were so even when these unique B-cell–derived factors are subtracted. If stable Th1 polarization is characteristic of HPS, the main source of serum IL-10 and TNF-α may be derived from activated macrophages in LAHS. From the viewpoint of critical care medicine, clinical signs and symptoms of HPS may be regarded as a kind of systemic inflammatory response syndrome (SIRS) [54,55]. Actually, almost all HPS cases fulfill the criteria for SIRS [56]. The elevation of the serum IL-10 levels may be derived from the Th1 cells and macrophages themselves and may be regarded as a compensatory anti-inflammatory response.

IL-12 is produced mainly by antigen-presenting cells such as B-cells and macrophages, which are essential effectors during the early phase of inflammation, and is the dominant factor for the induction of Th1 development [57,58]. The serum levels of IL-12 in the B-LAHS group were somewhat higher than in the T/NK-LAHS group, and some cases of the former group and most cases of the latter group fell within the normal range. The difference in serum levels may reflect the ability of B-cells to produce IL-12 [57]. The serum levels of IL-12 did not show a huge impact on the serum cytokine profiles in LAHS, especially in the T/NK-LAHS group, and although these findings were contrary to our expectations, they were comparable with those of previous studies [11,12,58].

On the basis of these findings and those of previous studies, we can summarize the difference in the pathophysiology between B-LAHS and T/NK-LAHS as follows: LAHS is caused by the hyperactivation of T-cells and macrophages, and the clinical characteristics associated with this disease result from the overproduction of Th1 cytokines, including IFN-γ, IL-2, and TNF-α, by activated Th1 cells and macrophages. Stable Th1 polarization is caused directly or indirectly by EBV-infected neoplastic cells in T/NK-LAHS, whereas this polarization is developed by reactive T-cells in B-LAHS. Serum TNF-α, IL-6, IL-10, and possibly IL-12 are derived at least partly from the neoplastic cells themselves in B-LAHS.

Acknowledgments

Participants in this study: Takashi Uchiyama (study chairman), Hitoshi Ohno, Toshiyuki Hori, Hirofumi Takayama, Toshirou Okazaki, Takayuki Ishikawa, Takahashi Atushi, Kazunori Imada, Tatsuo Ichinohe, Akifumi Takaori, Norimitsu Kadowaki, Masataka Sasada, Kaoru Tohyama, Masao Matsuoka (Kyoto University, Kyoto); Toshihiro Mochizuki (Shizuoka Municipal Hospital, Shizuoka); Tadahiko Shiomura (Shizuoka Prefectural General Hospital, Shizuoka); Daizaburou Kagawa (Shimada Municipal Hospital, Shimada); Tetsuya Okuda (Nagahama Municipal Hospital, Nagahama); Takayo Suzuki (Shiga Prefectural Hospital for Adult Disease, Moriyama); Tatsuharu Ohno (Ohtsu Red Cross Hospital, Ohtsu); Kohkichi Yamamoto (Shiga University, Ohtsu); Kenji Kanaji (Maizuru

Municipal Hospital); Norimasa Yasuda (Kyoto Municipal Hospital, Kyoto); Shoichi Doi (Katsura Hospital, Kyoto); Yasushi Takeda (Kyoto Keisatsu Hospital, Kyoto); Teruyuki Takamatsu (Otowa Hospital, Kyoto); Yataro Yoshida (Takeda General Hospital, Kyoto); Mitsuhiko Koya (Unitica Central Hospital, Kyoto); Yoichiro Ohno (Tenri General Hospital, Tenri); Hitoshi Sawada (Takanohara Central Hospital, Takanohara); Masao Tajima (Takatsuki Red Cross Hospital, Takatsuki); Mitsuru Tsudo (Osaka Red Cross Hospital, Osaka); Naochika Domae (Osaka Dental College, Osaka); Naofumi Nezumi (Kansai Denryoku Hospital, Osaka); Yoshiteru Konaka (Kitano Hospital, Osaka); Hiroto Sakota (Sumitomo Hospital, Osaka); Satoshi Tanabe (Torigata Hospital, Osaka); Tateo Sugiyama (Wakayama Red Cross Medical Center, Wakayama); Michihiko Uchida (Hyogo Prefectural Amagasaki Hospital, Amagasaki); Takayuki Takahashi, Ken-ichi Nagai (Kobe Municipal Central Hospital, Kobe); Yasuichi Kotaka (Shinko Hospital, Kobe), Asako Nagai (Hyogo Prefectural Tukaguchi Hospital, Kobe); Akemi Shimasaki (National Himeji Hospital, Himeji); Yasunori Ueda (Kurashiki Central Hospital, Kurashiki); Yoichiro Izumi (Kokura Memorial Hospital, Kokura).

The authors are grateful to Mr. Ryuichi Mitsuhashi and Ms. Hiroko Imamura (Department of Laboratory Medicine, Ohtsu Red Cross Hospital) for expert technical assistance.

References

1. Wong KF, Chan JK. Reactive hemophagocytic syndrome: a clinicopathologic study of 40 patients in an Oriental population. *Am J Med*. 1992;93:177-180.
2. Imashuku S. Differential diagnosis of hemophagocytic syndrome: underlying disorders and selection of the most effective treatment. *Int J Hematol*. 1997;66:135-151.
3. Komp DM, McNamara J, Buckley P. Elevated soluble interleukin-2 receptor in childhood hemophagocytic histiocytic syndromes. *Blood*. 1989;73:2128-2132.
4. Ishii E, Ohga S, Aoki T, et al. Prognosis of children with virus-associated hemophagocytic syndrome and malignant histiocytosis: correlation with levels of serum interleukin-1 and tumor necrosis factor. *Acta Haematol*. 1991;85:93-99.
5. Imashuku S, Ikushima S, Esumi N, Todo S, Saito M. Serum levels of interferon-gamma, cytotoxic factor and soluble interleukin-2 receptor in childhood hemophagocytic syndromes. *Leuk Lymphoma*. 1991;3:287-292.
6. Fujiwara F, Hibi S, Imashuku S. Hypercytokinemia in hemophagocytic syndrome. *Am J Pediatr Hematol Oncol*. 1993;15:92-98.
7. Akashi K, Hayashi S, Gondo H, et al. Involvement of interferon-γ and macrophage colony-stimulating factor in pathogenesis of haemophagocytic lymphohistiocytosis in adults. *Br J Haematol*. 1994;87:243-250.
8. Imashuku S, Hibi S, Fujiwara F, Ikushima S, Todo S. Haemophagocytic lymphohistiocytosis, interferon-gamma-naemia and Epstein-Barr virus involvement. *Br J Haematol*. 1994;88:656-658.
9. Seymour JF, Talpaz M, Cabanillas F, Wetzler M, Kurzrock R. Serum interleukin-6 levels correlate with prognosis in diffuse large-cell lymphoma. *J Clin Oncol*. 1995;13:575-582.
10. Imashuku S, Hibi S, Fujiwara F, Toda S. Hyper-interleukin (IL)-6-naemia in haemophagocytic lymphohistiocytosis. *Br J Haematol*. 1996;93:803-807.
11. Osugi Y, Hara J, Tagawa S, et al. Cytokine production regulating Th1 and Th2 cytokines in hemophagocytic lymphohistiocytosis. *Blood*. 1997;89:4100-4103.
12. Takada H, Ohga S, Mizuno Y, et al. Oversecretion of IL-18 in haemophagocytic lymphohistiocytosis: a novel marker of disease activity. *Br J Haematol*. 1999;106:182-189.
13. Takeshita M, Kikuchi M, Ohshima K, et al. Bone marrow findings in malignant histiocytosis and/or malignant lymphoma with concurrent hemophagocytic syndrome. *Leuk Lymphoma*. 1993;12: 79-89.
14. Yao M, Cheng AL, Su IJ, et al. Clinicopathological spectrum of hemophagocytic syndrome in Epstein-Barr virus-associated peripheral T cell lymphoma. *Br J Haematol*. 1994;87:535-543.
15. Wilson MS, Weiss LM, Gatter KC, Mason DY, Dorfman RF, Warnke RA. Malignant histiocytosis: a reassessment of cases previously reported in 1975 based on paraffin section immunophenotyping studies. *Cancer*. 1990;66:530-536.
16. Reiner AP, Spivak JL. Hematophagic histiocytosis: a report of 23 new patients and a review of the literature. *Medicine*. 1988;67: 369-388.
17. Falini B, Pileri S, De Solas I, et al. Peripheral T-cell lymphoma associated with hemophagocytic syndrome. *Blood*. 1990;75:434-444.
18. Cheng AL, Su IJ, Chen YC, Uen WC, Wang CH. Characteristic clinicopathological features of Epstein-Barr virus-associated peripheral T-cell lymphoma. *Cancer*. 1993;72:909-916.
19. Chang CS, Wang CH, Su IJ, Chen YC, Shen MC. Hematophagic histiocytosis: a clinicopathologic analysis of 23 cases with special reference to the association with peripheral T-cell lymphoma. *J Formos Med Assoc*. 1994;93:421-428.
20. Su IJ, Wang CH, Cheng AL, Chen RL. Hemophagocytic syndrome in Epstein-Barr virus-associated T-lymphoproliferative disorders: disease spectrum, pathogenesis, and management. *Leuk Lymphoma*. 1995;19:401-406.
21. Wong KF, Chan JKC, Lo ESF, Wong CSC. A study of the possible etiologic association of Epstein-Barr virus with reactive hemophagocytic syndrome in Hong Kong Chinese. *Hum Pathol*. 1996;27: 1239-1242.
22. Kuratsune H, Machii T, Aozaka K, et al. B cell lymphoma showing clinicopathological features of malignant histiocytosis. *Acta Haematol*. 1988;79:94-98.
23. Nakamoto T, Ogawa S, Mano H, Hirai H, Yazaki Y. Hemophagocytic syndrome associated with non-Hodgkin's lymphoma of B-cell type. *Am J Hematol*. 1994;47:335-336.
24. Murase T, Nakamura S, Tashiro K, et al. Malignant histiocytosis-like B-cell lymphoma, a distinct variant of intravascular lymphomatosis: a report of five cases and review of the literature. *Br J Haematol*. 1997;99:656-664.
25. Ohno T, Miyake N, Hada S, et al. Hemophagocytic syndrome in five patients with Epstein-Barr virus-negative B-cell lymphoma. *Cancer*. 1998;82:1963-1972.
26. Takahashi T, Kanda Y, Mori M, et al. B-cell lymphoma-associated hemophagocytic syndrome after PBSCT. *Bone Marrow Transplant*. 1998;21:623-625.
27. Shimazaki C, Inaba T, Shimura K, et al. B-cell lymphoma associated with haemophagocytic syndrome: a clinical, immunological and cytogenetic study. *Br J Haematol*. 1999;104:672-679.
28. Takahashi N, Chubati A, Miura I, Nakamura S, Miura T. Lymphoma-associated hemophagocytic syndrome in Japan [in Japanese, abstract in English]. *Jpn J Clin Hematol*. 1999;40:542-549.
29. Miyahara M, Sano M, Shibata K, et al. B-cell lymphoma-associated hemophagocytic syndrome: clinicopathological characteristics. *Ann Hematol*. 2000;79:378-388.
30. Allory Y, Challine D, Haioun C, et al. Bone marrow involvement in lymphomas with hemophagocytic syndrome at presentation: a clinicopathologic study of 11 patients in a Western institution. *Am J Surg Pathol*. 2001;25:865-874.
31. Su IJ, Hsieh HC, Lin KH, et al. Aggressive peripheral T-cell lymphomas containing Epstein-Barr viral DNA: a clinicopathologic and molecular analysis. *Blood*. 1991;77:799-808.
32. Craig FE, Clare CN, Sklar JL, Banks PM. T-cell lymphoma and the virus-associated hemophagocytic syndrome. *Am J Clin Pathol*. 1992;97:189-194.
33. Kikuta H, Sakiyama Y, Matsumoto S, et al. Fatal Epstein-Barr

virus-associated hemophagocytic syndrome. *Blood*. 1993;82: 3259-3264.
34. Kawaguchi H, Miyashita T, Herbst H, et al. Epstein-Barr virus-infected T lymphocytes in Epstein-Barr virus-associated hemophagocytic syndrome. *J Clin Invest*. 1993;92:1444-1450.
35. Su IJ, Hsu YH, Lin MT, Cheng AL, Wang CH, Weiss LM. Epstein-Barr virus-containing T-cell lymphoma presents with hemophagocytic syndrome mimicking malignant histiocytosis. *Cancer*. 1993;72: 2019-2027.
36. Dolezal MV, Kamel OW, van de Rijin M, Cleary ML, Sibley RK, Warnke RA. Virus-associated hemophagocytic syndrome characterized by clonal Epstein-Barr virus genome. *Am J Clin Pathol*. 1995;103:189-194.
37. Lay JD, Tsao CJ, Chen JY, Kadin ME, Su IJ. Upregulation of tumor necrosis factor-α gene by Epstein-Barr virus and activation of macrophages in Epstein-Barr virus-infected T cells in the pathogenesis of hemophagocytic syndrome. *J Clin Invest*. 1997;100: 1969-1979.
38. Ohshima K, Haraoka S, Harada N, et al. Hepatosplenic gamma delta T-cell lymphoma: relation to Epstein-Barr virus and activated cytotoxic molecules. *Histopathology*. 2000;36:127-135.
39. Ohno T, Kanoh T, Arita Y, et al. Fulminant clonal expansion of large granular lymphocytes: characterization of their morphology, phenotype, genotype, and function. *Cancer*. 1988;62:1918-1927.
40. Kawa-Ha K, Ishihara S, Ninomiya T, et al. CD3-negative lymphoproliferative disease of granular lymphocytes containing Epstein-Barr viral DNA. *J Clin Invest*. 1989;84:51-55.
41. Watari K, Asano S, Shirafuji N, et al. Serum granulocyte colony-stimulating factor levels in healthy volunteers and patients with various disorders as estimated by enzyme immunoassay. *Blood*. 1989;73:117-122.
42. Heaney ML, Dolde DW. Soluble cytokine receptors. *Blood*. 1996; 87:847-857.
43. Luoghnan MS, Sanderson CJ, Nossal GJV. Soluble interleukin 2 receptors are released from the cell surface of normal murine B lymphocytes stimulated with interleukin 5. *Proc Natl Acad Sci U S A*. 1988;85:3115-3119.
44. de Totero D, di Celle PF, Cignetti A, Foa R. The IL-2 receptor complex: expression and function on normal and leukemic B cells. *Leukemia*. 1995;9:1425-1431.
45. Imashuku S, Hibi S, Tabata Y, Todo S. Hemophagocytic syndrome in five patients with Epstein-Barr virus-negative B-cell lymphoma. *Cancer*. 1999;85:2298-2300.
46. Abken H, Fluck J, Willecke. Four cell-secreted cytokines act synergistically to maintain long term proliferation of human B cell lines in vitro. *J Immunol*. 1992;149:2785-2794.
47. Boussiotis VA, Nadler LM, Strominger JL, Goldfeld E. Tumor necrosis factor α is an autocrine growth factor for normal human B cells. *Proc Natl Acad Sci U S A*. 1994;91:7007-7011.
48. Genestier L, Bonnefoy-Berard N, Rouault JP, Flacher M, Revillard JP. Tumor necrosis factor-α up-regulates Bcl-2 expression and decreases calcium-dependent apoptosis in human B cell lines. *Int Immunol*. 1995;7:533-540.
49. Kato H, Kinoshita T, Suzuki S, et al. Elevated serum interleukin-6 (IL-6) is derived from neoplastic lymphoid cells in patients with B-cell non-Hodgkin's lymphoma: correlation with extent of IL-6 expression and serum concentration. *Br J Haematol*. 1996;92: 1014-1021.
50. Voorzanger N, Touitou R, Garcia E, et al. Interleukin (IL)-10 and IL-6 are produced in vivo by non-Hodgkin's lymphoma cells and act as cooperative growth factors. *Cancer Res*. 1996;56:5499-5505.
51. Benjamin D, Park CD, Sharma V. Human B cell interleukin 10. *Leuk Lymphoma*. 1994;12:205-210.
52. Cortes J, Kurzrock R. Interleukin-10 in non-Hodgkin's lymphoma. *Leuk Lymphoma*. 1997;26:251-259.
53. Khatri VP, Caligiuri MA. A review of the association between interleukin-10 and human B-cell malignancies. *Cancer Immunol Immunother*. 1998;46:239-244.
54. Ip M, Chan KW, Chan IKL. Systemic inflammatory response syndrome in intravascular lymphomatosis. *Intensive Care Med*. 1997; 23:783-786.
55. Deusch E, Mayer A, Hobisch-Hagen P, et al. Angiotropic large B-cell lymphoma misdiagnosed as urosepsis with multiple organ dysfunction syndrome. *Acta Anaesthesiol Scand*. 1999;43:100-103.
56. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med*. 1992;20:864-874.
57. Romagnani S. Cytokines and the Th1/Th2 paradigm. In: Balkwill F, ed. *The Cytokine Network and Immune Functions*. Oxford, UK: Oxford University Press; 2000:73-93.
58. Kallio R, Surcel HM, Bloigu A, Syrjala. Balance between interleukin-10 and interleukin-12 in adult cancer patients with or without infections. *Eur J Cancer*. 2001;37:857-861.