exanthem subitum (primary HHV-6 B infection) were used for the assay. Acute phase serum did not react with p100 ((C) lane 1) and 101K ((C) lane 2). Meanwhile, convalescent phase serum reacted with only 101K ((C) lane 4). A healthy adult serum (Pt.6) reacted with only 101K (D), and another healthy adult serum (Pt. 26) reacted with both p100 and 101k (E). Molecular mass standards are indicated in kDa on the left. White arrow head indicates specific band reacted to p100 antigen. Black arrow head indicates specific band reacted to 101K antigen.

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Table.1 Demographics, clinical features, serological results based on immunoblot using recombinant p100 and 101K, and HHV-6 immunofluorescence assay (IFA) titer.

Patient	Diagnosis[a]	Sex[b]	Age[c]	Sampling Days	IB[d]		IFA titer	
					p100	101K	6A	6B
1	CI-HHV-6A	m	60 y		-	-	16	8
2	CI-HHV-6A	m	13 y		+	+	64	64
3	CFS	m	56 y		+	+	128	128
4	ES	m	13 m	0	-	-	< 8	< 8
				9	-	+	64	> 256
5	ES	m	9 m	6	-	-	< 8	< 8
				14	-	+	128	128
6	ES	f	15 m	4	-	-	< 8	< 8
				32	-	+	128	128
7	ES	m	13 m	3	-	-	< 8	< 8
				11	-	-	32	32
8	ES	m	17 m	1	-	-	< 8	< 8
				10	-	-	8	64
9	ES	m	12 m	4	-	-	< 8	< 8
				21	-	+	32	> 256
10	ES	f	10 m	0	-	-	< 8	< 8
				12	-	+	> 256	32
11	ES	f	11 m	2	-	-	< 8	< 8
				18	-	+	64	64
12	ES	m	9 m	3	-	-	< 8	< 8
				17	-	+	128	128
13	ES	f	8 m	2	-	-	< 8	< 8
				12	-	+	64	64
14	ES	f	7 m	2	-	-	< 8	< 8
				12	-	+	32	64
15	ES	m	8 m	3	-	-	< 8	< 8
				15	-	+	128	> 256
16	DIHS	m	29 y	12	-	+	< 8	8
				37	+	+	256	>256
17	DIHS	m	34 y	9	-	+	< 8	8
				29	+	+	16	64

18	DIHS	m	66 y	7	-	-	< 8	8
				43	-	+	16	128
19	DIHS	m	64 y	19	-	-	< 8	16
				45	-	+	128	>256
20	DIHS	f	59 y	21	-	+	< 8	8
				38	-	+	32	128
21	DIHS	m	73 y	9	-	-	< 8	< 8
				27	-	+	8	128
22	HSCT	m	3 y	8	-	+	32	16
				43	-	+	64	256
23	HSCT	m	10 m	13	-	+	< 8	8
				62	-	+	256	256
24	HSCT	f	1 y	3	-	+	16	128
				31	-	+	64	128
25	HSCT	m	11 y	6	-	+	< 8	16
				26	-	+	32	128
26	HSCT	m	3 y	15	-	+	32	32
				50	-	+	256	256

[a] CIHHV-6A, Chromosomally integrated HHV-6A; CFS, Chronic Fatigue Syndrome; ES, exanthem subitum; DIHS, Drug induced hypersensitivity syndrome; HSCT, Hematopoietic stem cell transplant.

[b] m, Male; f, Female.

[c] y, years; m, months.

[d] IB, Immunoblot assay using p100 and 101K. —, No reactivity; +, reactivity.

The detection limit of immunofluorescence assay (IFA) was <8.

Table.2 Healthy Adults: Demographics, clinical features, serological results based on immunoblot using recombinant p100 and 101K, HHV-6 immunofluorescence assay (IFA) titer, and PCR genotyping.

Healthy adult	Sex[a]	Age (years)	IB[b]		IFA titer		nested PCR[c]	
			p100	101K	6A	6B	PBMCs	Saliva
1	m	47	-	-	< 8	16	-	-
2	f	44	-	+	32	32	-	B
3	m	23	-	+	16	32	-	B
4	m	46	-	+	16	16	B	B
5	f	20	+	+	32	32	-	B
6	f	23	-	+	< 8	8	-	B
7	f	24	-	+	32	32	B	B
8	f	22	-	-	8	16	B	B
9	m	22	-	-	16	16	-	B
10	m	22	-	+	16	32	-	B
11	f	22	-	+	8	16	-	-
12	m	24	-	+	64	64	-	B
13	f	23	-	+	16	32	B	B
14	m	23	-	+	8	16	-	B
15	f	24	-	+	32	32	-	B
16	m	23	-	+	16	16	-	B
17	f	23	-	+	64	64	B	B
18	m	24	-	+	16	16	B	B
19	m	25	-	+	< 8	8	B	B
20	f	30	-	+	16	64	B	B
21	m	38	-	-	< 8	8	-	-
22	f	23	-	+	8	16	-	B
23	m	24	-	+	8	32	B	B
24	m	32	-	+	8	32	-	-
25	f	78	+	+	8	16	NS	-
26	f	75	-	-	< 8	8	NS	B
27	f	73	-	+	8	16	NS	B
28	m	86	+	+	< 8	8	NS	B
29	m	69	+	+	16	32	NS	B
30	f	62	-	+	< 8	8	NS	-

31	f	73	-	+	< 8	8	NS	-
32	f	88	-	+	32	64	NS	-
33	f	78	-	-	< 8	8	NS	B
34	f	82	-	-	< 8	16	NS	-
35	f	72	-	+	8	32	NS	B
36	m	60	-	+	< 8	< 8	NS	-
37	m	68	-	+	< 8	< 8	NS	B
38	m	72	-	+	< 8	16	NS	-

[a] m, Male; f, Female.

[b] Samples were tested by immunoblot assay using p100 and 101K. —, No reactivity; +, reactivity.

[c] —, negative result; B, HHV-6 variant B; NS, not determined because of no sample.

The detection limit of immunofluorescence assay (IFA) was <8.

Figure 1

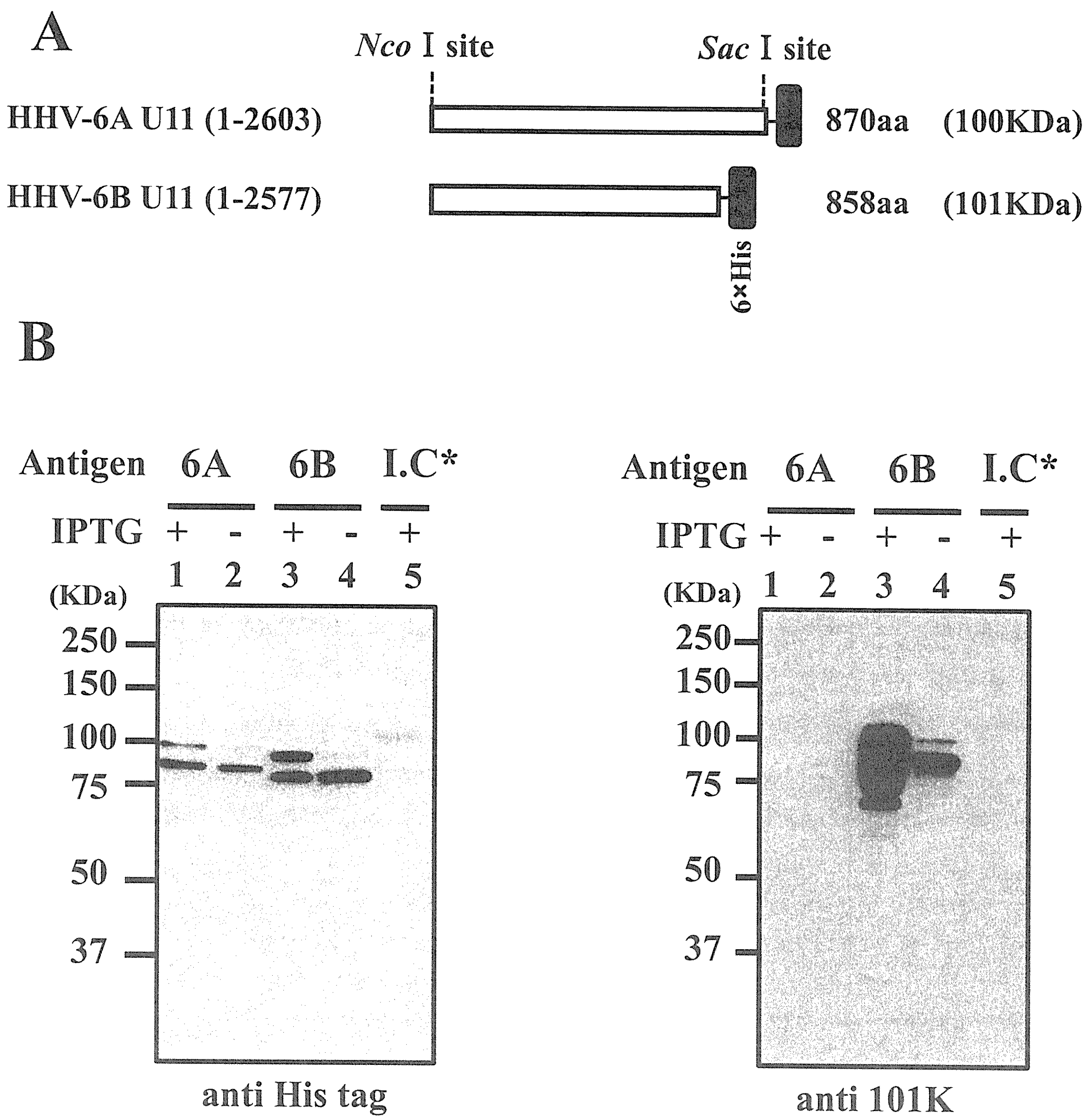
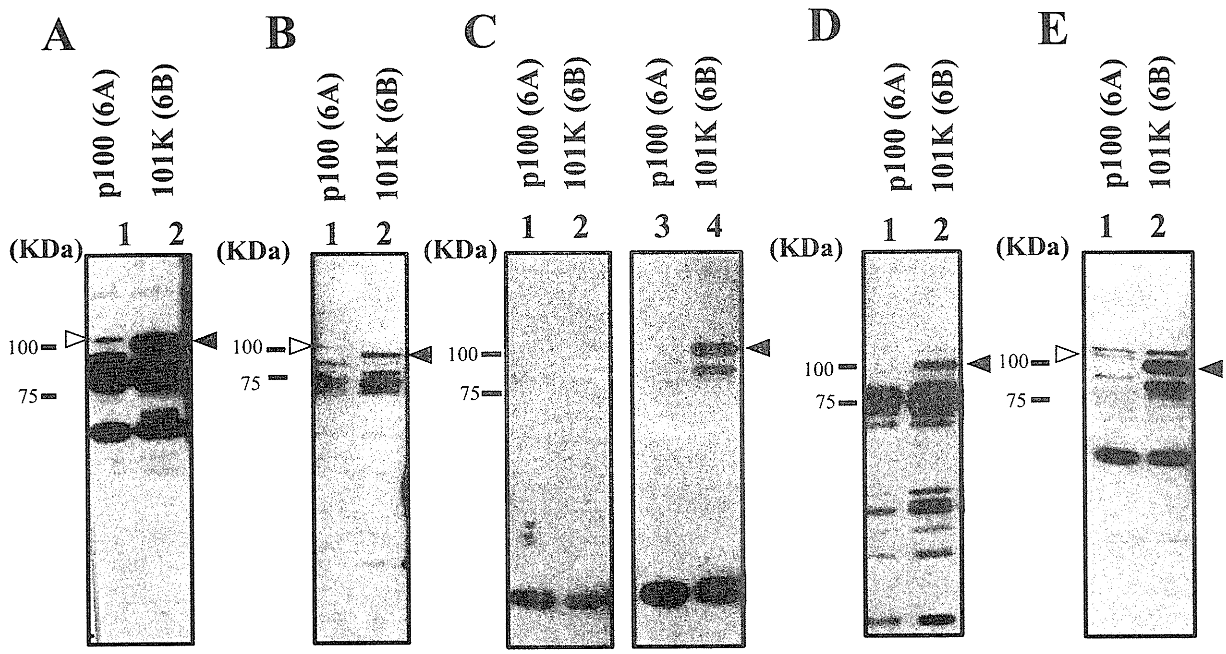
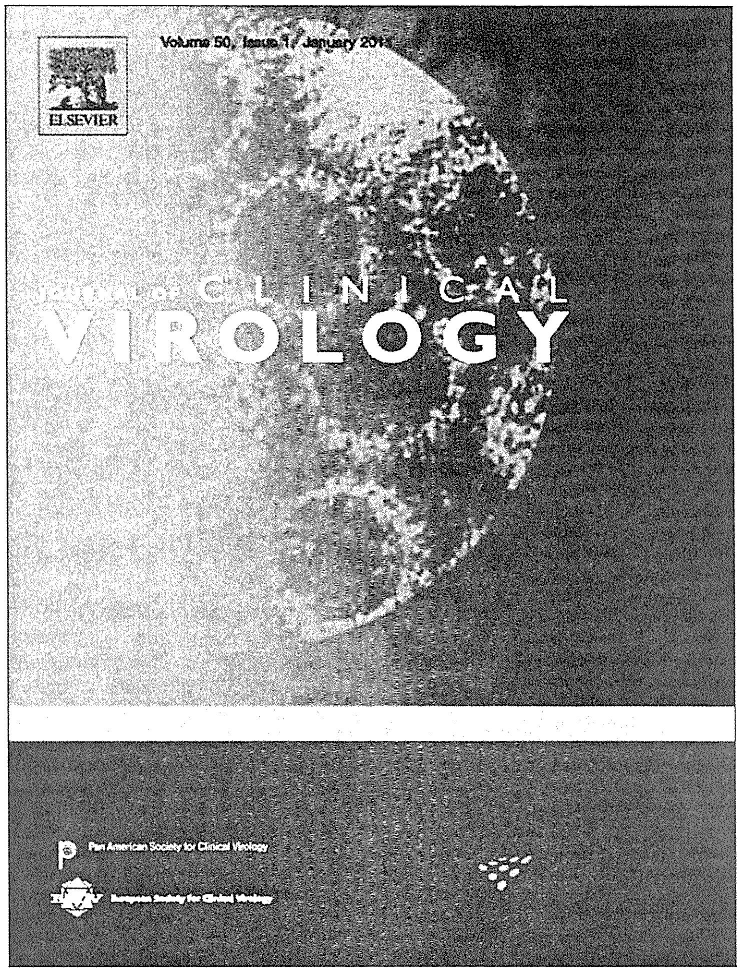

A
Nco I site
Sac I site
HHV-6A U11 (1-2603)
870aa (100KDa)
HHV-6B U11 (1-2577)
858aa (101KDa)
6×His
B
Antigen 6A 6B I.C*
IPTG + - + - +
1 2 3 4 5
(KDa)
250
150
100
75
50
37
anti His tag
Antigen 6A 6B I.C*
IPTG + - + - +
(KDa) 1 2 3 4 5
250
150
100
75
50
37
anti 101K

Figure 2

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Short communication

Kinetics of cytokine and chemokine responses in patients with primary human herpesvirus 6 infection☆

Tetsushi Yoshikawa[a,*], Yuri Kato[a], Masaru Ihira[b], Naoko Nishimura[c], Takao Ozaki[c], Takuji Kumagai[d], Yoshizo Asano[a]

[a] *Department of Pediatrics, Fujita Health University School of Medicine, Toyoake, Aichi 4701192, Japan*
[b] *Faculty of Clinical Engineering, Fujita Health University School of Health Sciences, Toyoake, Aichi, Japan*
[c] *Department of Pediatrics, Konan Kosei Hospital, Konan, Aichi, Japan*
[d] *Kumagai Pediatric Clinic, Sapporo, Hokkaido, Japan*

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ABSTRACT

Background: Cytokines and chemokines induced by human herpesvirus 6 (HHV-6) infection may play an important role in the observed HHV-6-associated clinical complications. However, basic data for cytokine and chemokine synthesis in primary HHV-6 infected patient without complication is lacking.
Objective: Aim of this study was to elucidate basic kinetic data for expressions of cytokines and chemokines in patients with primary HHV-6 infection without complication.
Study design: Twenty-six patients suffering from fever were enrolled in this study. Fourteen biomarkers were measured in 74 serially collected sera samples from 26 patients. Additionally, serum samples obtained from 14 healthy children were used for control.
Results: Twenty of the 26 patients were diagnosed with primary HHV-6 infection based on viral isolation and serological analysis. The mean age ($P = 0.1289$) and proportion of males to females ($P = 0.9999$) between the patients with and without primary HHV-6 infection were not statistically different. At the acute phase of the disease, three cytokines (IFN-γ; $P = 0.0046$, IL-2; $P = 0.0366$, and IL-4; $P = 0.0255$) and one chemokine (MCP-1; $P = 0.0019$) were significantly higher in patients with primary HHV-6 infection compared to those without infection. Interleukin-5 levels during the convalescent period were significantly higher in patients with HHV-6 infection ($P = 0.0205$). By 1 month post-infection, cytokine and chemokine expression had returned to almost basal levels.
Conclusion: As suggested by the previous in vitro studies, present in vivo analysis also suggests that HHV-6 has potency for induction of cytokines and chemokines.

1. Background

Primary human herpesvirus 6 (HHV-6) infection can cause exanthem subitum in infants and young children.[1] Although the disease is generally a benign febrile illness with self-limiting clinical course,[2] several severe complications, such as encephalitis,[3] have been reported. It has been suggested that several cytokines might be involved in causing HHV-6 encephalitis.[4] Additionally, our recent studies suggested that several inflammatory cytokines were upregulated by HHV-6 reactivation and these may contribute to the associated clinical manifestations in transplant recipients[5,6] and patients with drug induced hypersensitivity syndrome.[7] Moreover, in vitro studies demonstrated that HHV-6 can modulate host immunity by several mechanisms, including killing lymphocytes and controlling cytokine synthesis.[8–12] Thus, the cytokines and chemokines induced by HHV-6 infection may play an important role in the observed HHV-6-associated clinical manifestations.

2. Objective

Aim of this study was to elucidate basic kinetic data for expressions of cytokines and chemokines in patients with primary HHV-6 infection without complication.

3. Study design

3.1. Patients and sample collection

Twenty-six patients suffering from fever, who visited Kumagai Pediatric Clinic, were enrolled in this study. Patients were consented by their guardians for participation in this study. This study

Abbreviations: HHV-6, human herpesvirus 6; IL, interleukin; IFN, interferon; TNF, tumor necrosis factor; MCP, monocyte chemotactic protein.

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* Corresponding author. Tel.: +81 562 939251; fax: +81 562 952216.
E-mail address: tetsushi@fujita-hu.ac.jp (T. Yoshikawa).

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Table 1
Cytokine levels in serially collected sera samples in patients with and without primary HHV-6 infection.

Sampling days	Cytokines (pg/ml)	Normal control	With primary HHV-6 inf.		W/O primary HHV-6 inf.		*P*-Value[a]
		Mean ± SD	Median (25–75%)		Median (25–75%)		
Days 0–5 (acute)	IL-1β	0.0 ± 0.0	0.0	(0.0–0.0)	0.0	(0.0–0.0)	0.3921
	IL-2	0.0 ± 0.0	**24.1**	**(15.7–26.8)**	**8.4**	**(0.0–17.6)**	**0.0366**
	IL-4	0.0 ± 0.0	**13.5**	**(0.0–23.0)**	**0.0**	**(0.0–0.0)**	**0.0255**
	IL-5	0.0 ± 0.0	11.5	(8.9–12.2)	9.5	(2.3–10.1)	0.2227
	IL-6	1.1 ± 4.2	14.2	(0.0–21.2)	7.8	(0.0–22.5)	0.8001
	IL-10	3.9 ± 4.8	40.3	(18.9–64.0)	9.0	(0.0–25.5)	0.0513
	IL12p70	0.0 ± 0.0	0.0	(0.0–0.0)	0.0	(0.0–0.0)	0.3237
	IFN-γ	0.0 ± 0.0	**168.2**	**(119.1–291.7)**	**66.7**	**(38.0–85.5)**	**0.0046**
	TNF-α	0.0 ± 0.0	0.0	(0.0–9.3)	0.0	(0.0–6.6)	0.0874
Days 6–15 (convalescent)	IL-1β	0.0 ± 0.0	0.0	(0.0–12.4)	0.0	(0.0–0.0)	0.1264
	IL-2	0.0 ± 0.0	23.6	(6.9–33.5)	7.8	(0.0–16.4)	0.0701
	IL-4	0.0 ± 0.0	18.5	(0.0–21.7)	0.0	(0.0–0.0)	0.0535
	IL-5	0.0 ± 0.0	**10.9**	**(8.4–13.4)**	**0.0**	**(0.0–6.2)**	**0.0205**
	IL-6	1.1 ± 4.2	0.0	(0.0–17.5)	4.2	(0.0–9.0)	0.4503
	IL-10	3.9 ± 4.8	0.0	(0.0–15.4)	0.0	(0.0–0.0)	0.0653
	IL12p70	0.0 ± 0.0	0.0	(0.0–0.0)	0.0	(0.0–0.0)	0.4173
	IFN-γ	0.0 ± 0.0	45.8	(35.2–52.0)	13.4	(0.0–45.2)	0.3033
	TNF-α	0.0 ± 0.0	15.9	(6.6–19.7)	8.8	(0.0–17.7)	0.6276
1 month after onset of illness	IL-1β	0.0 ± 0.0	0.0	(0.0–0.0)	0.0	(0.0–0.0)	0.8195
	IL-2	0.0 ± 0.0	22.2	(9.6–29.5)	17.2	(4.2–17.8)	0.6367
	IL-4	0.0 ± 0.0	0.0	(0.0–13.9)	0.0	(0.0–7.8)	0.7676
	IL-5	0.0 ± 0.0	10.8	(0.0–14.1)	9.6	(1.7–13.5)	0.9729
	IL-6	1.1 ± 4.2	0.0	(0.0–0.0)	0.0	(0.0–0.0)	0.6859
	IL-10	3.9 ± 4.8	0.0	(0.0–1.8)	0.0	(0.0–4.7)	0.8627
	IL12p70	0.0 ± 0.0	0.0	(0.0–0.0)	0.0	(0.0–0.0)	0.4945
	IFN-γ	0.0 ± 0.0	31.1	(9.8–43.7)	16.1	(3.3–21.2)	0.3625
	TNF-α	0.0 ± 0.0	5.6	(0.0–16.9)	0.0	(0.0–11.4)	0.8853

Inf.: infection, W/O: without.
Bold value indicates the biomarkers, in which there were significant difference between the 2 groups.
[a] Comparison between patients with and without primary HHV-6 infection.

was approved by the review boards of Fujita Health University. EDTA-treated peripheral blood and serum were serially collected from the patients at the initial visit to the clinic (days 0–5, acute), days 6–15 (convalescent), and 1 month after onset of the illness. Meanwhile, 1 ml of serum was collated from 14 healthy controls in Konan Kosei Hospital.

3.2. Virus isolation and serological analysis

The procedures for the isolation and identification of HHV-6 have been described elsewhere.[2] IgG antibody titers to HHV-6 were measured using an indirect-immunofluorescence assay as described previously.[2] We defined the patients with either seroconversion or HHV-6 viremia as the patients with primary HHV-6 infection.

3.3. Measurements of cytokines and chemokines

Serially collected serum samples were processed immediately after collection and stored at −70 °C for the subsequent measurement of cytokines and chemokines. The quantification of nine cytokines (interleukin 1β (IL-1β), IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α)) and five chemokines (IL-8, regulated on activation normal T-cell expressed and secreted (RANTES), monokine induced by interferon-γ (MIG), monocyte chemotactic protein-1 (MCP-1), interferon inducible protein-10 (IP-10)) in serum were determined by the cytometric bead array kit (BD Biosciences, CA, USA). Assays were carried out according to the manufacturer's instructions.

3.4. Statistical analysis

Demographic factors and clinical data were compared between patients with and without primary HHV-6 infection by Fisher's exact test or Student's *t*-test. Mean peak cytokine and chemokine levels were compared between the two groups using the Mann–Whitney *U*-test. The statistical analysis was performed with StatView software, version J-5.0.

4. Results

4.1. Virological analysis and demographic factors

Seroconversion of HHV-6 IgG antibody titers was observed in 20 of the 26 (76.9%) patients. Additionally, HHV-6 was isolated from 13 of the 20 patients that had seroconverted. All of the patients with primary HHV-6 infection showed the typical clinical course of exanthem subitum. None of the patients had complications such as febrile seizure and hepatitis. No skin rash was observed in the patients without primary HHV-6 infection. In these patients, initial hematological examinations revealed normal range of leukocyte counts and C-reactive protein. The mean age of the patients with and without primary HHV-6 infection was 9.1 months (ranged between 6 and 16 months) and 12 months (ranged between 7 and 16 months), respectively ($P = 0.1289$). The proportion of males to females was not statistically different between the two categories (with primary HHV-6 infection, 6/14; without primary HHV-6 infection; 2/4, $P = 0.9999$).

4.2. Cytokine and chemokine levels

A total of 74 serially collected sera samples (days 0–5, 25 samples; days 6–15, 24 samples; 1 month after the onset of the illness, 25 samples) were analyzed in this study. The cytokine levels for the patients with and without primary HHV-6 infection are reported in Table 1. The expression of three cytokines (IFN-γ, IL-2, and IL-4) at days 0–5 were significantly higher in patients with primary HHV-6 infection than those without primary HHV-6 infection. The level of

Table 2
Chemokine levels in serially collected sera samples in patients with and without primary HHV-6 infection.

Sampling days	Chemokines (pg/ml)	Normal control	With primary HHV-6 inf.		W/O primary HHV-6 inf.		P-Value[a]
		Mean ± SD	Median (25–75%)		Median (25–75%)		
Days 0–5 (acute)	IL-8	12.3 ± 4.2	30.7	(23.0–44.2)	31.1	(24.6–36.0)	0.8313
	RANTES	19,238.3 ± 6592.1	2745.4	(724.3–19,822.6)	3513.3	(2302.2–18,470.3)	0.5839
	MIG	648.9 ± 499.4	859.9	(534.9–1204.8)	850.1	(539.0–1569.6)	0.6701
	MCP-1	148.1 ± 72.7	**974.7**	**(732.1–1385.0)**	**296.6**	**(250.6–519.7)**	**0.0019**
	IP-10	660.1 ± 333.5	3130.8	(2560.3–3985.5)	1977.6	(1448.5–3678.1)	0.2733
Days 6–15 (convalescent)	IL-8	12.3 ± 4.3	28.6	(17.7–43.0)	28.0	(18.1–30.3)	0.4836
	RANTES	19,238.3 ± 6592.1	3506.0	(2059.8–22,030.1)	12,459.2	(3229.5–22,535.5)	0.6057
	MIG	648.9 ± 499.4	339.0	(189.9–842.5)	615.8	(450.6–1195.8)	0.2267
	MCP-1	148.1 ± 72.7	402.1	(310.4–455.5)	315.6	(274.8–353.0)	0.1046
	IP-10	660.1 ± 333.5	792.6	(489.7–1066.4)	517.7	(268.6–832.1)	0.1815
1 month after onset of illness	IL-8	12.3 ± 4.3	25.9	(9.7–40.8)	18.0	(15.9–21.3)	0.4611
	RANTES	19,238.3 ± 6592.1	3893.5	(858.0–22,256.7)	12,463.3	(1524.4–24,455.6)	0.3412
	MIG	648.9 ± 499.4	325.7	(202.7–482.7)	503.0	(440.0–561.6)	0.2849
	MCP-1	148.1 ± 72.7	469.1	(333.1–611.8)	394.8	(288.5–440.8)	0.2040
	IP-10	660.1 ± 333.5	548.7	(425.2–687.8)	444.8	(384.6–505.1)	0.5475

Inf.: infection, W/O: without.
Bold value indicates the biomarkers, in which there were significant difference between the 2 groups.
[a] Comparison between patients with and without primary HHV-6 infection.

IL-5 during the convalescent period (days 6–15) was significantly higher in patients with primary HHV-6 infection than those without primary HHV-6 infection (10.0 ± 5.2 pg/ml vs. 3.2 ± 5.0 pg/ml, $P = 0.0205$).

The chemokine levels are shown in Table 2. MCP-1 expression was significantly higher in patients with primary HHV-6 infection than those without primary HHV-6 infection (1028.2 ± 459.3 pg/ml vs. 385.2 ± 224.0 pg/ml, $P = 0.0019$). No other chemokine levels were statistically different between the patients with primary HHV-6 infection and those without primary HHV-6 infection.

5. Discussion

At the time of the acute phase of disease (days 0–5), the expression of 3/9 cytokines (IFN-γ, IL-2, and IL-4) and 1/5 chemokines (MCP-1) were significantly higher in patients with primary HHV-6 infection than those without HHV-6 infection, which might contain various type of viral infections. Additionally, IL-5 levels during the convalescent period (days 6–15) was significantly higher in patients with primary HHV-6 infection. Moreover, as shown in Table 1, all of the cytokines and chemokines analyzed in this study were returned to normal levels by 1 month after onset of the illness, such that there was no statistical difference between the patients with and without primary HHV-6 infection. Therefore, the upregulation of cytokines and chemokines observed during the acute and convalescent periods of the disease are considered to be specific finding. Many in vitro analyses have been carried out to determine whether HHV-6 can modulate with cytokine and chemokine production,[8–12] however, to our knowledge, this is the first study, which analyzed cytokine and chemokine expression in vivo, specifically in patients with primary HHV-6 infection.

Interpretation of increased levels of cytokines or chemokines in patients with HHV-6 infection might be difficult because of the complex cytokine and chemokine networks in vivo. It has been demonstrated that IL-2, which is strong T-cell activator, is necessary for efficient HHV-6 replication in cultured lymphocytes.[13] Therefore, upregulation of IL-2 in patients with primary HHV-6 infection may enhance viral replication in vivo. However, a previous in vitro study reported reduced IL-2 production by HHV-6 infected T-cells, which may contribute to immune suppression.[14] These conflicting data suggest that the in vitro findings may not fully recapitulate the cytokine and chemokine responses to primary HHV-6 infection in vivo. Meanwhile, an increase in production of IL-4 and IL-5, which are associated with allergic reaction, has been demonstrated in patients with drug induced hypersensitivity syndrome with HHV-6 reactivation.[15] However, no remarkable observation with regard to these cytokines has been reported on the basis of in vitro experiments. Thus, further in vitro and in vivo analysis is needed to the functional biological relevance of the expression of these cytokines and chemokines during HHV-6 infection.

Upregulation of MCP-1 has been demonstrated in HHV-6 infected human peripheral blood monocytes in vitro.[11] Thus, our observation of increased MCP-1 levels in patients with primary HHV-6 infection is consistent with the previous in vitro study. MCP-1 is strong mediator of monocyte chemoattraction. As it has been suggested that HHV-6 can latently infect monocytes/macrophage cells after primary infection,[16] upregulation of this chemokine might be beneficial for efficient establishment of HHV-6 latency. Furthermore, it has been demonstrated that HHV-6 encodes viral chemokines,[17] which can mediate monocyte chemoattraction. Collectively, these data suggest that HHV-6 might use various mechanisms to establish latency in monocytes/macrophage.

Conflict of interest

The authors do not have any commercial or other associations that might pose a conflict of interest.

Ethical approval

This study was approved by the review boards of Fujita Health University.

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High Incidence of Cytomegalovirus, Human Herpesvirus-6, and Epstein–Barr Virus Reactivation in Patients Receiving Cytotoxic Chemotherapy for Adult T Cell Leukemia

Masao Ogata,[1,2]* Takako Satou,[2] Rie Kawano,[2] Tetsushi Yoshikawa,[3] Junji Ikewaki,[2] Kazuhiro Kohno,[2] Takeaki Ando,[2] Yasuhiko Miyazaki,[4] Eiichi Ohtsuka,[4] Yoshio Saburi,[4] Hiroshi Kikuchi,[5] Tetsunori Saikawa,[1] and Jun-ichi Kadota[2]

[1]*Blood Transfusion Center, Oita University Hospital, Oita, Japan*
[2]*Department of Hematology, Oita University Faculty of Medicine, Oita, Japan*
[3]*Department of Pediatrics, Fujita Health University School of Medicine, Aichi, Japan*
[4]*Department of Hematology, Oita Prefectural Hospital, Oita, Japan*
[5]*Nishibeppu National Hospital, Oita, Japan*

The etiology of cytomegalovirus (CMV), human herpesvirus-6 (HHV-6), and Epstein–Barr virus (EBV) reactivation and the potential for complications following cytotoxic chemotherapy in the absence of allogeneic transplantation are not clearly understood. Patients with adult T cell leukemia (ATL) are susceptible to opportunistic infections. In this study, the incidence, kinetics and clinical significance of reactivation of CMV, HHV-6, and EBV in ATL patients were investigated. Viral DNA in a total of 468 plasma samples from 34 patients was quantified using real-time PCR. The probability of CMV, HHV-6, and EBV reactivation by 100 days after the start of chemotherapy was 50.6%, 52.3%, and 21.6%, respectively. Although most CMV reactivations were self-limited, plasma CMV DNA tended to persist or increase if the CMV DNA levels in plasma reached $\geq 10^4$ copies/ml. CMV reactivation was negatively associated with survival, but the *P*-value for this association was near the borderline of statistical significance ($P = 0.052$). One patient developed fatal interstitial pneumonia concomitant with peak CMV DNA accumulation (1.6×10^6 copies/ml plasma). Most HHV-6 and EBV reactivations were self-limited, and no disease resulting from HHV-6 or EBV was confirmed. HHV-6 and EBV reactivation were not associated with reduced survival ($P = 0.35$ and 0.11, respectively). These findings demonstrated that subclinical reactivation of CMV, HHV-6, and EBV were common in ATL patients receiving chemotherapy. There were differences in the viral reactivation patterns among the three viruses. A CMV load $\geq 10^4$ copies/ml plasma was indicative of subsequent exacerbation of CMV reactivation and developing serious clinical course. *J. Med. Virol. 83:702–709, 2011.*

KEY WORDS: ATL; CMV; HHV-6; EBV; real-time PCR

INTRODUCTION

Cytomegalovirus (CMV), human herpesvirus-6 (HHV-6), and Epstein–Barr virus (EBV) are ubiquitous in the human population. More that 90% of Japanese individuals over the age of 50 have antibodies against CMV or EBV [Takeda et al., 2001]. HHV-6 infects virtually all individuals during childhood [Zerr et al., 2005a]. These herpesviruses establish latency after primary infection and can reactivate under immunosuppressive condition. Reactivation of CMV, HHV-6, and/or EBV is common after solid-organ or hematopoietic stem cell transplantation and is linked to various serious clinical diseases [Boeckh et al., 1996; Shapiro et al., 1999; Yoshikawa et al., 2002; Zerr et al., 2005b; Ogata et al., 2006], and it is common practice to treat CMV or EBV reactivation in recipients of stem cell transplants [Meerbach et al., 2008; Boeckh and

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*Correspondence to: Masao Ogata, Blood Transfusion Center, Oita University Hospital, Hasama-machi, Oita 879-5593, Japan. E-mail: mogata@med.oita-u.ac.jp

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Ljungman, 2009; Omar et al., 2009]. CMV is increasingly recognized as a significant pathogen in patients receiving only chemotherapy without transplants. Han [2007] reported that a high portion of non-transplant patients with lymphoid malignancies (13.6%) were positive for CMV antigenemia. Nguyen et al. [2001] reported that the frequency of CMV pneumonia has been increasing in adults with leukemia who have not undergone transplantation.

Adult T cell leukemia (ATL) is an aggressive T cell malignancy caused by a retrovirus, human T-cell leukemia virus type I (HTLV-I), which is endemic in Japan, Melanesia/Australia, the Caribbean, parts of South America, and part of Africa [Van Brussel et al., 1999]. Prognosis for patients with ATL is extremely poor due to multidrug resistance of malignant cells and frequent complications due to opportunist infections. ATL patients are susceptible to various opportunistic infections, including pneumocyctis pneumonia, fungal infections, and herpesvirus disease due to defective cellular immunity [Uchiyama, 1997; Yasunaga et al., 2001; Chen et al., 2006]. Suzumiya et al. [1993] reported that CMV was involved in 35 of 47 (74.5%) autopsied cases of ATL and that CMV pneumonia is a significant cause of death in ATL patients. EBV-associated B cell lymphoproliferative disorder [Tobinai et al., 1991; Tanaka et al., 2008] and HHV-6 encephalitis [Idutsu et al., 2007] has also been reported in patients with ATL. Development of these herpesvirus diseases may indicate that subclinical reactivations of herpesviruses are common in ATL patients. To date, two studies evaluated CMV reactivation in ATL patients using an antigenemia assay [Fujiwara et al., 2000, 2001]. However, in both studies, antigenemia was assessed only on admission and when a patient exhibited a fever. Furthermore, less is known about incidence or significance of HHV-6 or EBV reactivation.

Understanding the dynamics of reactivation of these herpesviruses in ATL patients may facilitate the prevention of CMV-, HHV-6-, or EBV-related diseases. Presence of plasma viral DNA can be a good indicator of active CMV [Boeckh and Ljungman, 2009] or HHV-6 [Zerr, 2006b] infection. There is debate over which sample type, whole blood versus plasma or serum is most suitable for monitoring EBV reactivation; nevertheless, the detection of EBV DNA genomes in plasma that does not contain B-cells is more likely the result of a lytic EBV infection and indicates that the patients has an active EBV infection [Ljungman, 2010]. The present study used real-time polymerase chain reaction (PCR) to detect viral DNA genomes in plasma to specifically evaluate the epidemiology and kinetics of CMV, HHV-6, and EBV reactivation in ATL patients receiving chemotherapies.

MATERIALS AND METHODS

Patients

Thirty-four patients who were admitted to Oita University Hospital or Oita Prefectural Hospital and who had acute/lymphoma-type ATL that met the Japan Lymphoma Study Group criteria [Shimoyama, 1991] were enrolled in this study. The diagnosis of ATL was made based on seropositivity for HTLV-I and a histologically and/or cytologically proven peripheral T-cell malignancy. Study protocols were approved by the Ethical Committee of the Oita University Faculty of Medicine, and patients participating in this study signed an informed consent form for study protocols. Initial treatment regimens included cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP), cyclophosphamide, pirarubicin, vincristine, and prednisolone (THP-COP), cyclophosphamide, pirarubicin, etoposide, and prednisolone (THP-CEP), or mLSG15 (sequential therapy by VCAP-AMP-VECP) [Tsukasaki et al., 2007]. Salvage treatments were selected at the discretion of the physicians for management of refractory disease. No patients were treated with prophylactic anti-viral agents. Patients who received hematopoietic stem cell transplantation were censored from this study upon beginning preconditioning for transplantation.

Real-Time PCR

Blood sampling to monitor viral DNA was started after initiation of chemotherapy and performed during the period in which patients were hospitalized in order to receive chemotherapy. Interval of sampling was at baseline, once every 7–14 days. Plasma samples were separated from EDTA-treated whole blood by centrifugation (1,750*g* for 10 min) and filtration through a 0.22-μm pores filter. The design of the PCR primers (5′-TCACCAGTGTCGTGTATGCCA-3′ and 5′-CACACAGCGCTCGTTGTAATC-3′) and a TaqMan probe (5′-[FAM]CCCATGAACGTGCTCATCGAC GTGA[TAMRA]-3′) for CMV quantitation were based on the UL97 open reading frame of CMV, and quantitation was performed as previously described [Ikewaki et al., 2005]. Primers and probe for evaluation of HHV-6 DNA load were based on sequences from U67 according to the methods originally described by Locatelli et al. [2000] and performed as previously described [Ogata et al., 2006]. For EBV, PCR primers were complementary to sequences in the BALF5 gene and performed as previously described [Kimura et al., 1999].

Analyses

Viral reactivation was defined as the detection of viral DNA in a sample of plasma. Duration of the reactivation event was defined as the period of consecutive positive results. The statistical significance of differences between the groups was assessed by Fisher's exact test or the Mann–Whitney *U* test as appropriate. The cumulative incidence of viral reactivation and survival was calculated according to the Kaplan–Meier method, and comparisons of survival were made using the log-rank test. Statistical analyses were performed using Prism 5 for Macintosh software (GraphPad Software,