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Case Report Open Access

Six Cases of Infectious Mononucleosis by Cytomegalovirus as Diagnosed by Multiplex Virus PCR Assay

Nagao M[1], Yoshioka Y[1], Saito T[1], Tsunemine H[2], Ito K[2], Kodaka T[2], Tsuji G[3], Watanabe K[4], Shimizu N[5]* and Takahashi T[2]*

[1]Departments of Laboratory of Cell Therapy, Tokyo Medical and Dental University, Japan
[2]Department of Hematology, Shinko Hospital, Tokyo Medical and Dental University, Japan
[3]Departments of Rheumatology, Shinko Hospital, Kobe, Tokyo Medical and Dental University, Japan,
[4]Departments of Pediatrics and Developmental Biology, Tokyo Medical and Dental University, Japan
[5]Center for Stem Cell and Regenerative Medicine, Tokyo Medical and Dental University, Tokyo, Japan

Abstract

Infectious mononucleosis (IM) is mostly caused by Epstein-Barr virus (EBV) while IM by cytomegalovirus (CMV) is rather rare. In our retrospective clinical research with multiplex virus PCR analysis, we encountered 6 cases of CMV-IM and analyzed the clinical characteristics including diagnostic problems. The diagnosis of CMV-IM was made when the CMV genome was solely detected from the patient's peripheral blood by multiplex virus PCR analysis. Then viral load was determined by quantitative PCR. Viral serological examinations were performed by a laboratory company as routine laboratory tests. Specific PCR signal for CMV genome was obtained by multiplex virus PCR in 6 patients, and blood CMV load ranged from 102 to 104 copies/mL. Clinical pictures and laboratory findings of these patients with CMV-IM were similar to those of EBV-IM in terms of fever, fatigue, morphology and number of atypical lymphocyte, and liver dysfunction. On serological examination, an IgM antibody against CMV was positive in all 6 patients; however, a VCA-IgM antibody against EBV was also positive in all patients examined, compromising serological differential diagnosis of IM. To make an exact diagnosis of CMV-IM, direct detection of the virus genome is important, and our multiplex virus PCR assay may be very useful in terms of quick performance and good specificity.

Keywords: Infectious mononucleosis; Cytomegalovirus; Multiplex virus PCR assay; Cross reaction; IgM antibody

Introduction

Infectious mononucleosis (IM) is characterized by fever, pharyngitis, cervical lymphadenopathy, skin rash, liver dysfunction, and leukocytosis consisting of many atypical lymphocytes following initial infection by certain viruses [1-3]. Approximately 90% of IM is caused by Epstein-Barr virus (EBV) and the remaining is by cytomegalovirus (CMV) [3]. CMV-IM was first reported by Klemola and Kääriäinen [4], and was characterized by mild pharyngitis, skin rash, and cervical lymphadenopathy compared with symptoms in EBV-IM [1,4]. In addition, human herpes virus type 6 (HHV-6) occasionally causes IM, and this type of IM was first reported in 1983 [5]. Clinically, differential diagnosis of CMV-IM from EBV-IM is performed with serological tests; however, cross-reaction between CMV-IgM and EBV-IgM antibodies often occurs [6,7], causing a confusion in the differential diagnosis of CMV-IM from EBV-IM. Another problem of the serological tests is that it takes a few days to obtain the results. To detect a single virus genome, a polymerase chain reaction (PCR) assay system has been employed [8-11]. However, this assay is available in limited institutes and is mostly performed commercially by the laboratory company. The PCR assay is therefore expensive and takes time. In recent years, we developed an assay method that enables us to simultaneously detect 12 kinds of viral DNA genomes, including those of CMV and EBV, with multiplex PCR in the blood and to subsequently determine the viral load with combined real-time PCR [12]. With this assay, it takes only 3 hours to obtain the results [12]; therefore, this prompt diagnosis may be clinically important because CMV infection can be managed by antiviral agents, for example when a patient has severe liver dysfunction. In our clinical study with this PCR assay, we encountered 6 cases of CMV-IM and analyzed the clinical characteristics including diagnostic problems between CMV- and EBV-IM.

Patients and Methods

All 6 patients were referred to the Laboratory of Cell Therapy by their attending physicians for multiplex virus PCR analysis because of possible viral infection between August 2011 and December 2015. EDTA-2Na-chelated whole blood (200 L) was obtained from individual patients who provided written informed consent. The present report is a part of a single institutional retrospective study designated the "Multiple Virus-Analytic Study by Multiplex PCR for Patients with Immune Dysfunction", which had been approved by the institutional review board. The methods for both qualitative multiplex PCR and quantitative real-time PCR were previously described in detail [12]. The multiplex PCR was designed to qualitatively detect the genome of 12 DNA viruses including 8 herpes family viruses , namely, CMV, EBV, HHV-6, varicella-zoster virus (VZV), BK virus (BKV), JC virus (JCV), parvovirus B19 (ParvoB19), human herpes virus type 7 (HHV-7) and type 8 (HHV-8), herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), and hepatitis B virus (HBV). When a specific PCR signal was obtained, quantitative real-time PCR was performed to determine

***Corresponding authors:** Takahashi T, Department of Hematology, Shinko Hospital, 1-4-47, Wakinohamacho, Chuo-ku, Kobe 651-0072, and Norio Shimizu, Center for Stem Cell and Regenerative Medicine, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai Chiyoda-ku, Tokyo 101-0062, Japan, Tel: 81-78-261-6711; Fax: 81-78-261-6726; E-mail: takahashi.takayuki@shinkohp.or.jp

Shimizu N, Center for Stem Cell and Regenerative Medicine, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai Chiyoda-ku, Tokyo 101-0062, Japan, Tel: 81-35-280-8080; Fax: 81-35-280-8079; E-mail: nshivir@tmd.ac.jp

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the viral load. Viral serological examinations were commercially performed with the enzyme immunoassay by a laboratory company as routine laboratory tests (SRL, Inc. Hachiohji, Tokyo, Japan).

Statistical Analysis

Student T-test was used to compare mean values of laboratory tests between CMV-IM and EBV-IM.

Clinical characteristics and symptoms in 6 cases of CMV-IM

As shown in Table 1, all patients except for Patient 4 were young. Their median age was 26.5, ranging from 24 to 64. All patients but one were febrile and had general fatigue and headache (except for Patient 1). Half of these patients had throat pain and skin rash, while cervical lymphadenopathy was observed only in one patient (Table 1).

Laboratory findings of CMV-IM patients

The WBC counts of these 6 patients ranged from 4.6 to 11.5 × 10^9/L with a median count of 7.6 × 10^9/L. Regarding platelet count, Patient 3 showed marked thrombocytopenia of 2 × 10^9/L, possibly being immune thrombocytopenic purpura as previously reported [13], which was later resolved with immunosuppressive therapy. The median percentage of atypical lymphocytes was 21.3%, ranging from 19.0 to 36.9%. The morphology of these atypical lymphocytes was monocyte-like as seen in EBV-IM (Figure 1). All 6 patients had liver dysfunction without jaundice, and 2 of them showed marked transaminases (Table 2). Serum concentrations of LDH were elevated in all 6 patients, while those of CRP were slightly elevated or within normal limits. For comparison, Table 3 shows laboratory data from patients with EBV-IM in our hospital during the same period when CMV-IM patients were examined. However, mean WBC count values, absolute number of atypical lymphocytes, AST, or ALT were not significantly different between CMV-IM and EBV-IM. In addition, all 7 patients with EBV-IM also provided written informed consent before the multiplex virus PCR assay.

Patient	Age	Sex	BT	Fatigue	Headache	Pharyngitis	Skin rash	LN swelling
1	35	F	39	+	-	+	-	-
2	24	F	38	+	+	-	+	-
3	29	M	sub fever	+	+	-	+	-
4	64	F	38	+	+	+	-	-
5	24	F	40	+	+	-	+	+
6	24	F	38	+	+	+	-	-

CMV-IM: Cytomegalovirus-Infectious Mononucleosis; BT: Body Temperature; LN: Lymph Node.

BT is expressed in ℃.

Table 1: Clinical picture of 6 patients with CMV-IM.

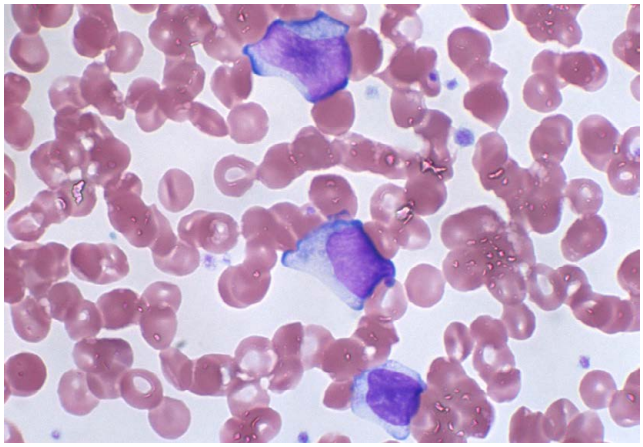

Figure 1: Atypical lymphocytes from Patient 2.

Phenotype of typical lymphocytes in CMV-IM

Flow cytometric analysis of atypical lymphocytes was performed in Patient 5. Double positive cells for CD3/CD4 and CD3/CD8 cells were 13.4% and 66.7%, respectively, in CD45-gated cells indicating that the atypical lymphocytes were $CD8^+$ T-cells.

Multiplex virus PCR analysis in CMV-and EBV-IM patients

With the multiplex virus PCR assay, CMV, but not EBV, was detected in all 6 patients. As for other viruses, HHV-6 was simultaneously detected in Patient 2. The median blood CMV copy number was 1.4 × 10^4/copies/mL ranging from 1.8 × 10^2 to 5.7 × 10^4 copies/mL. In addition, in 7 patients with EBV-IM CMV was not detected (Table 4).

Other assays to detect CMV

A CMV pp65 antigenemia assay was performed by a laboratory company in Patients 2 and 3 yielded positive results in these 2 patients (LSI Medience Corp., Tokyo, Japan). In this examination, the number of CMV pp65 antigen-positive WBC was counted with indirect enzyme immunoassay.

Serological examination for CMV and EBV in patients with CMV-IM

As shown in Table 5, the IgM antibody against CMV was detected in all 6 patients supporting the results of multiplex virus PCR as shown in Table 4. The IgG antibody against CMV was also detected in all patients but Patient 6, in whom the result was faintly positive (+/-). Interestingly, EBV-viral capsid antigen (VCA)-IgM and VCA-IgG antibodies were detected in all 5 patients examined. EBV-Epstein-Barr nuclear antigen (EBNA), however, was already positive in all 5 patients

Patient	WBC	Atyp.lym (%)	Atyp.lym (No)	PLT	AST	ALT	ALP	T-Bil	LDH	CRP
1	7.3	33	2.4	147	410	361	326	0.7	705	0.9
2	4.6	21	0.9	121	89	86	300	0.7	377	1.1
3	11.5	37	4.2	2	88	144	233	0.6	550	1.7
4	10.2	23	2.3	140	58	88	281	0.6	399	0.2
5	7.1	22	1.5	115	337	367	511	0.7	663	0.5
6	7.9	19	1.5	183	137	166	669	0.5	549	1.7

EBV: Epstein-Barr Virus; WBC: White Blood Cell; PLT: Platelet.

Normal range in our institution of AST, ALT, ALP, T-Bil, LDH and CRP are 5-40 IU/L, 3-40 IL/L, 115-360 IU/L, 0.2-1.3 mg/dL, 120-230 IU/L and below 0.3 mg/dL, respectively.

Table 2: Laboratory data of 6 patients with CMV-IM at presentation; The EBV, WBC and PLT are shown × 10^9/L.

Patient	WBC	Atyp.lym (%)	Atyp.lym (No)	PLT	AST	ALT	ALP	T-Bil	LDH	CRP
1	14	69	9.6	197	331	368	572	0.5	648	0.5
2	10	45	4.5	154	217	285	706	0.7	506	0.8
3	9.5	72	6.8	96	426	666	###	0.8	568	1.2
4	8.7	24	2.1	193	180	280	769	0.8	484	1.2
5	6.5	15	1	202	346	330	175	0.4	773	0.3
6	13.1	55	7.1	210	157	342	360	0.8	341	1.3
7	10.5	14	1.5	26.8	250	440	###	0.9	387	0.2

EBV: Epstein-Barr virus; WBC: White Blood Cell; PLT: Platelet.

Normal range in our institution of AST, ALT, ALP, T-Bil, LDH and CRP are 5-40 IU/L, 3-40 IL/L, 115-360 IU/L, 0.2-1.3 mg/dL, 120-230 IU/L and below 0.3 mg/dL, respectively.

Table 3: Laboratory data of 7 patients with EBV-IM at presentation. The EBV, WBC and PLT are shown × 10^9/L.

Patient	CMV	EBV	CMV or EBV viral load (copy/mL)	HHV-6(copy/mL)	CMV antigenemia
1	+	-	1.7×10^4	-	N.D.
2	+	-	5.7×10^4	3.6×10^4	7, 9/15 × 10^4 WBC
3	+	-	1.0×10^4	-	1, 4/15 × 10^4 WBC
4	+	-	1.4×10^3	-	N.D.
5	+	-	2.0×10^4	-	N.D.
6	+	-	1.8×10^2	-	N.D.
7	-	+	1.3×10^4	-	N.D.
8	-	+	1.6×10^3	-	N.D.
9	-	+	2.5×10^3	-	N.D.
10	-	+	7.6×10^3	-	N.D.
11	-	+	1.7×10^2	-	N.D.
12	-	+	4.6×10^3	-	N.D.
13	-	+	2.4×10^5	-	N.D.

CMV: Cytomegalovirus; EBV: Epstein-Barr virus-viral; HHV-6: human herpes virus 6; CMV antigenemia is expressed by the number of CMV pp65 antigen-positive cells/150,000 white blood cells; Normally, the antigen-positive cell is zero; N.D.: Not Done.

Table 4: Blood multiplex virus PCR analysis and viral load in patients with CMV- and EBV-IM.

Patient	CMV-IgM	CMV-IgG	EBV-VCA-IgM	EBV-VCA-IgG	EBV-EA-IgG	EBNA-IgG
1	+	+	+	+	-	+
2	+	+	+	+	+/-	+
3	+	+	+	+	+/-	+
4	+	+	N.D	N.D	N.D	N.D
5	+	+	+	+	+/-	+
6	+	+/-	+	+	+/-	+

VCA: Viral capsid antigen; EBNA: Epstein-Barr nuclear antigen; EA: early antigen; N.D: Not done.

Table 5: Serological tests in 6 patients with CMV-IM.

examined, while the EBV-early antigen (EA)-IgG antibody was faintly positive (+/-) in these patients (Table 5).

Clinical course of 6 patients with CMV-IM

Four of 6 CMV-IM patients, had to be hospitalized for fluid therapy because of poor general conditions, and 2 of them received treatment with ganciclovir because of severe liver dysfunction. Furthermore, Patient 3 developed severe thrombocytopenia as low as 2×10^9/L as previously described. The symptoms of these 4 patients resolved within 2 to 3 weeks with improved abnormal laboratory data. The remaining 2 patients spontaneously recovered from the symptoms described as above with normalized laboratory data.

Discussion

The positive detection rate of anti-CMV antibody in Japanese people, most of whom were infected with the virus during infancy was high being 80 to 90% [14], and higher than that among people in the United States [15]. In recent years, however, positive detection rate of the antibody among young Japanese people has decreased to around 60%, possibly because of decreased horizontal infection in infancy [14]. Therefore, the incidence of CMV-IM, which was low when compared with that of EBV-IM, is assumed to have increased in recent years [14]. This situation may be similar in many countries in which CMV prevalence was high in the past. Thus, we have to precisely distinguish between CMV-IM and EBV-IM.

Generally, the clinical picture of CMV-IM developed in immune-competent adults has been reported to be milder compared with that of EBV-IM [1,4]. However, in this series of CMV-IM patients, 4 of them had to be hospitalized. Although a small patient cohort of this report, CMV-IM appears to sometimes cause serious complications; therefore, prompt and proper diagnosis is important because CMV-IM can be successfully treated with antiviral agents such as ganciclovir. In addition to similar clinical pictures, there was no difference between CMV-IM and EBV-IM in terms of the number of WBC/atypical lymphocytes or degree of liver dysfunction in the present study. The morphology of atypical lymphocytes in CMV-IM was monocyte-like as seen in EBV-IM (Figure 1). Therefore, it appears to be difficult to distinguish CMV-IM from EBV-IM with clinical pictures and

laboratory findings. Furthermore, double-positivity of IgM antibodies against CMV and EBV, as observed in individual patients in this series of CMV-IM, makes it more difficult to make a differential diagnosis between both IMs. This double-positivity is considered to be a cross-reaction of IgM antibody to CMV and EBV antigens except for in a rare dual infection with both viruses. The cross-reaction has been thought to be caused by the similarity of CMV and EBV antigens, which are used in ELISA assays to detect IgM antibodies with respective viruses [6]. In this situation, the IgM antibody against CMV in CMV-IM also reacts with the EBV-reference antigen in the assay resulting in false positive EBV-VCA-IgM antibody [6,16]. In EBV-IM, the incidence of false positivity of CMV-IgM antibody has been reported to be 20 to 40% in EBV-IM cases [16]. In the present CMV-IM series, however, EBV-EA-IgG tests were negative or faintly positive (±), while EBNA was positive in all patients examined. In EBV-IM, EA-IgG is mostly positive, but becomes positive during late phases of EBV-IM. Therefore, negative EA-IgG and positive EBNA may be useful in distinguishing CMV-IM from EBV-IM. However, these results depend on the timing of the serological tests; therefore, EA-IgG and EBNA do not play a definite role in differentiation. To resolve this problem, a direct assay of CMV and EBV genomes appears to be useful. Real-time PCR assay to detect CMV or EBV has been available in laboratory medicine [8-11]. However, this method is able to assay only a single virus and is not suitable for the screening of multiple candidate viruses. Although the CMV pp65 antigenemia assay is useful, this method has the same limitations in terms of being a single virus assay and slow performance. On the other hand, our multiplex virus PCR assay is capable of screening 12 species of DNA viruses in a short time. Its sensitivity and specificity have already been established [12,15,16]. We have applied this assay to clinical research for screening candidate viruses in various morbid states and we are currently trying to make this assay a routine test in the future in laboratory medicine.

Disclosure

The authors disclose that we have no conflicts of interest with any individuals or companies.

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