

## Epstein-Barr Virus Antibodies in Kawasaki Disease

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The prevalent ages at onset for Kawasaki Disease (KD) and Epstein-Barr virus (EBV) infection are known to be similar in Korea and Japan. We evaluated the correlation between EBV infection and KD. The antibodies to EBV such as anti-viral capsid antigen (VCA) IgG and IgM, anti-diffuse and restricted early antigen IgG (anti-EADR IgG), and the anti-EBV determined nuclear antigen IgG (anti-EBNA IgG) were examined in 29 KD patients at five separate times sequentially during a period of one year, and also in 14 other children with a past history of KD. The results of each group were compared with those of age-matched controls. The positive rates of anti-VCA IgG and IgM at presentation in the KD patients were 41.4% (12/29) and 0% (0/29), respectively. Only one patient was found to be anti-VCA IgM-positive within two months. There were no cases of anti-VCA IgG except one, anti-EADR IgG and anti-EBNA IgG positive to negative seroconversion during the year. The children with a past history of KD showed higher anti-EBNA IgG-positive rates than the controls ( $p = 0.04$ ). There was no difference in the seropositive rates of the antibodies to EBV, cytomegalovirus, herpes simplex virus and herpes zoster virus. In conclusion, children with KD were noted to have normal immune responses to EBV infection. Children with a past history of KD seemed to be infected with EBV at a later age than children with no history of KD.

**Key Words:** Epstein-Barr virus, herpes virus, Kawasaki disease

### INTRODUCTION

Kawasaki Disease (KD) is an acute multisystem vasculitis with an unknown etiology that afflicts mostly young children.<sup>1</sup> Clinical and epidemiolo-

gical studies have suggested that KD is closely related to an infectious disease.<sup>2,3</sup> The acute onset of a self-limiting course, the prevalent population (rare in children < 6 months and > 5 years of age) and the existence of clusters or epidemics with a wave-like spread all suggest that KD is related to infectious agents, particularly those of a viral origin. The recent incidence of KD in Korea is approximately 90 per 100,000 children younger than five years of age. This is similar to that of Japan because of the geographic, racial and environmental similarities between the two countries.<sup>4</sup> The recurrence rate of KD is known to be approximately 2-3%.<sup>4,5</sup>

The Epstein-Barr virus (EBV) is an ubiquitous virus that is usually asymptomatic in young children, although it is the primary cause of infectious mononucleosis in some older children and young adults. The seroprevalence of EBV is known to differ among developed countries. In Japan as well as in Korea most children by five years of age are considered to be infected and seroconverted to EBV.<sup>6,7</sup> Furthermore, EBV is associated with some immunological disorders such as hemophagocytic syndrome, lymphoproliferative disorders (e.g., Duncan Syndrome) and Burkitt's Lymphoma.<sup>8</sup> The EBV also has the characteristics of a latent infection as one of the human herpes viruses, and sometimes provokes reactivation.

There have been several studies on the relationship between KD and EBV. Several studies in Japan have reported that the prevalence of the EBV antibody in KD patients and in children with a past history of KD is significantly lower than that of age-matched control children.<sup>9-13</sup> However, other studies including a serologic study have reported that EBV is not the pathologic agent of KD.<sup>14,15</sup> The purpose of this study was to evaluate

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the relationship between KD and the atypical presentation of an EBV infection.

## MATERIALS AND METHODS

We performed three examinations in this study. In the first examination, the subjects were 29 children who had been diagnosed with KD (17 boys). All the children met the criteria for KD and were treated with intravenous immunoglobulin (IVIG) at a dose of 2 g/kg over 12 hours and with aspirin (30-40 mg/kg) during the febrile period. For coronary artery lesions (CAL), echocardiography was performed within two weeks of the onset of the illness, during the fourth week, and then repeated depending on the initial findings. There were five children with CAL (17.2%) but none had a giant aneurysm. The mean age of the patients was  $2.1 \pm 1.1$  years, with a range from 4 months-5 years. After obtaining parental consent, serial sera were collected from the patients at five different intervals as follows: before, 1-2 weeks after, 1-2 months after, 6 months after and 1 year after IVIG treatment. Thirty-four healthy children of the same ages (mean age  $2.2 \pm 1.0$  years, 6 months-5 years) were used as the control group. In the second examination, a serologic study of other herpes viruses including the herpes simplex virus (HSV), the varicella-zoster virus (VZV), and the cytomegalovirus (CMV) were examined using the sera from the KD patients at presentation. In the third examination, the subjects were 14 children who had experienced KD in the past at their mean age of  $2.8 \pm 1.5$  years. The mean age of the subjects at presentation was  $8.3 \pm 2.7$  years. Fifteen age-matched healthy children with a mean age of  $8.4 \pm 2.6$  years were used as the control group. The collected samples were frozen at  $-20^{\circ}\text{C}$  before being examined for EBV antibodies. Measurements of antibodies were done using commercially available kits. Anti-viral capsid antigens IgG and IgM (anti-VCA IgG and IgM) were examined by indirect fluorescent antibody assay (Bion Enterprises Ltd., Park Ridge, IL, USA), the anti-diffuse and restricted early antigen IgG (anti-EADR IgG) was evaluated by immunofluorescence assay (Granbio Inc., Temecula, CA, USA), and the anti-EBV determined nuclear antigen IgG (anti-EBNA

IgG) was examined using an anticomplement immunofluorescence test (Bion Enterprises Ltd., Park Ridge, IL, USA). Antibodies to CMV and VZV (RADIM, Pomezia, Italy) and HSV (Nova Tec Immunodiagnostica GmbH, Dietzenbach, Germany) were measured by an enzyme-linked immunosorbent assay. The Ethics Committee on Clinical Research of The Catholic University of Korea at Daejeon St. Mary's Hospital approved this study.

### Statistical analysis

To analyze the relationships between the KD group and the control group, a chi-square test and a Fischer's exact test were used (SPSS version 10.0). A *p* value of  $< 0.05$  was considered statistically significant.

## RESULTS

### Sequential profiles of EBV antibodies in KD patients

The seropositive rates of the anti-VCA IgG and IgM antibodies in the 29 KD patients at presentation were 41.4% (12/29) and 0% (0/29), respectively. Seropositive rates of almost 100% of anti-VCA IgG and anti-EBNA IgG were observed at 1-2 weeks and at 1-2 months after the IVIG treatment due to the effect of the infused IVIG. During one year, five seronegative anti-VCA IgG patients converted to positive and one seropositive patient converted to negative status. Only one patient showed the anti-VCA IgM at 1-2 weeks. There was no case of seroconversion from positive to negative in the anti-VCA IgG except for one, an anti-EADR IgG and anti-EBNA IgG. There was no significant difference in the seropositive rates of the EBV antibodies except for an anti-EBNA IgG between the KD patients at presentation and the controls (Table 1).

### Seropositive rates of herpes viruses in KD patients

The seropositive rates of antibodies to EBV (41% vs. 65%), CMV (45% vs. 59%), HSV (21% vs. 24%),

**Table 1.** Sequential Seropositivity for Epstein-Barr Virus Antibodies in Kawasaki Disease (KD) Patients after Intravenous Immunoglobulin Treatment and in Controls

Antibodies	Before n (%)	KD patients (n = 29)*				Controls (n = 34)	p value <sup>†</sup>
		1-2 wk	1-2 mo	6 mo	1 yr		
Anti-VCA IgG	12 (41)	29 (100)	29 (100)	21 (72)	16 (55)	22 (65)	0.06/0.44
Anti-VCA IgM	0	1	0	0	0	0	–
Anti-EADR IgG	8 (28)	13 (45)	15 (52)	16 (55)	13 (45)	16 (47)	0.11/0.86
Anti-EBNA IgG	5 (17)	26 (90)	28 (97)	10 (34)	8 (28)	14 (41)	0.04/0.26

\* The same numbers of samples were collected serially five times before and after intravenous immunoglobulin treatment.

<sup>†</sup> Comparison between KD patients before and one year after intravenous immunoglobulin treatment, and controls.

VCA, viral capsid antigen; EADR, diffuse and restricted early antigen; EBNA, EBV determined nuclear antigen.

**Table 2.** Seropositivity for Herpes Viruses Antibodies in Kawasaki Disease (KD) Patients and in Controls

Herpes viruses	KD Patients (n = 29)*	Controls (n = 34)	p value
Anti-VCA IgG	12 (41%)	22 (65%)	0.06
Anti-CMV IgG	13 (45%)	20 (59%)	0.27
Anti-HSV IgG	6 (21%)	8 (24%)	0.79
Anti-HZV IgG	10 (34%)	9 (26%)	0.49

\*Samples were collected before intravenous immunoglobulin treatment.

VCA, viral capsid antigen; CMV, cytomegalovirus; HSV, herpes simplex virus; HZV, herpes zoster virus.

**Table 3.** Epstein-Barr Virus Antibodies in Children with a Past History of Kawasaki Disease (KD) and in Controls

	KD Patients (n = 14)	Controls (n = 15)	p value
Anti-VCA IgG	10 (71%)	14 (93%)	0.17
Anti-VCA IgM	0	0	–
Anti-EADR IgG	8 (57%)	3 (20%)	0.04
Anti-EBNA IgG	9 (64%)	14 (93%)	0.08

VCA, viral capsid antigen; EADR, diffuse and restricted early antigen; EBNA, EBV determined nuclear antigen.

and HZV (34% vs. 26%) were similar in the patients and controls. In the controls, the prevalence of HSV and HZV was lower than that of EBV and CMV (Table 2).

#### EBV antibodies in the children with a past history of KD

Children with a past history of KD were less likely to have anti-VCA IgG antibodies than the controls (10/14 vs. 14/15), but the difference was not statistically significant. There was a difference

in the rates of anti-EADR IgG positive patients (8/14, 57% vs. 3/15, 20%,  $p = 0.04$ ) between the groups, and there was also a more significant difference when the subjects and controls that were anti-VCA IgG-positive were selected (8/10, 80% vs. 3/14, 21%,  $p = 0.01$ , Table 3).

#### DISCUSSION

EBV has been an attractive candidate as the primary agent of KD for several reasons. However,

results of studies on KD and EBV have had some limitations. Iwanaga et al. first suggested that an abnormal immune response to the EBV may be responsible for KD.<sup>9</sup> They found that only two out of 69 (3%) KD patients were EBV seropositive. In sharp contrast, 84% of the age- and sex-matched controls were seropositive. Since the publication of this report, several studies have produced similar results in Japan.<sup>10-13</sup> One study reported that more than 80% of 57 KD patients showed serologic evidence of a primary EBV infection within one month of the illness as measured by a sensitive anti-complement immunofluorescence test. The serologic response to the EBV was significantly low and transient, and it disappeared 1-2 weeks after the initial detection.<sup>13</sup> In addition, Okano et al.<sup>11</sup> reported that 14 of 34 (41%) children with a past history of KD (with a mean age of 8.1 years) and 73 out of 88 (83%) of age- and sex-matched controls were seropositive for the EBV. In contrast, Marchette et al.<sup>14</sup> discovered that 61 of 278 (22%) KD patients (at a mean age of 28 months) and 110 of 242 (45%) controls (with a mean age of 44 months) were anti-VCA IgG-positive ( $p < 0.01$ ). However, there was no difference in prevalence when partitioning according to age and ethnicity. These studies indirectly indicate that EBV seroprevalence in children is different in Japan and in Hawaii.

In order to evaluate whether the seropositivity of the EBV antibodies changes, we used the serial sera of KD patients. Because the prevalence of EBV is closely related to age, we carefully selected the same-aged controls. Since all KD patients were treated with IVIG (2 g/kg), the data at 1-2 weeks, 1-2 months, and 6 months were not reliable for interpreting the EBV antibodies except for data concerning anti-VCA IgM. We found that after receiving IVIG (2 g/kg) the antibodies contained in the IVIG (anti-hepatitis A IgG) could be detected in approximately 30% of KD patients at 6 months and in 0% of the patients at 1 year (unpublished observation). We could not identify any patients except for one who was anti-VCA IgM-positive in the acute stage (within 1-2 weeks). One child who was anti-VCA IgM-positive at one week seroconverted to anti-VCA IgG- and anti-EADR-positive after one year. While five anti-VCA IgG-negative patients seroconverted to posi-

tive after one year, only one anti-VCA IgG-positive child seroconverted to negative after one year. However, considering the child's age of six months, this conversion may be the result of maternal antibodies. In this study, there was no difference in the anti-VCA IgG seropositivity between KD patients and age-matched controls. EBNA antibodies are known to appear after the anti-VCA IgG is detected, and they persist for life. In this study, there was a difference in the anti-EBNA IgG seropositivity between KD patients and controls (17% vs. 41%,  $p = 0.04$ , Table 1). However, when the patients and controls with anti-VCA IgG positive status were selected (5/12, 42% vs. 14/22, 64%,  $p = 0.22$ ), there was no difference. These findings suggest that KD patients experience normal immune responses to the EBV. In keeping with previous studies,<sup>12,14</sup> we did not find any differences in seropositivity to HSV, HZV and CMV (Table 2). EADR antibodies are detected after anti-VCA IgM and IgG antibodies appear, and they disappear within 6 months to 2-3 years after initial infection. The earlier finding of a lower anti-EBV-positive rate<sup>11</sup> and our finding of a higher anti-EADR-positive rate suggest that children who have recovered from KD are infected with EBV later than other children of the same age. This finding may be affected by the immunological changes that occur after KD or IVIG treatment. Another postulation is that the humoral immunity caused by an unknown agent that is partly related to EBV may protect the children from EBV infection to some extent.

In conclusion, our results indicate that children with KD have normal immune responses to an EBV infection. Children with a past history of KD seem to be infected with EBV later than normal children of the same age.

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