ORIGINAL ARTICLE

Epstein-Barr Virus Infection in Childhood May Precipitate Atopic Diseases

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ABSTRACT

Background: Epstein Barr virus (EBV) has been suspected of being involved in the development of atopy. There are several studies suggesting a positive as well as negative association between EBV infection and atopic diseases. Here, we carried out a large-scale, systematic investigation to address the issue of the possible association between EBV infection and atopic diseases.

Methods: Anti-EBV-viral capsid antigen (VCA) antibody titer, anti-EBV nuclear antigen (EBNA) antibody titer, atypical lymphocyte (AtLy) count and EBV-DNA copy number in 10^6 WBC were examined as evidence for EBV infection, and characteristic parameters of atopic disease such as total serum immunoglobulin E (IgE) level, highest antigen-specific IgE antibody titer (h-RAST) and peripheral blood eosinophil (Eos) count were measured and compared among atopic subjects and non-atopic controls, and correlations between parameters of atopy and EBV infection were subjected to statistical analysis.

Results: Anti-EBV, in particular anti-EBNA antibody titer and AtLy count in peripheral blood were markedly higher in patients with bronchial asthma (BA) and/or atopic dermatitis (AD) than in non-atopic controls, especially in early childhood. No similar findings were obtained for antibodies to cytomegalovirus (CMV). EBV-DNA copy numbers in WBC were elevated in atopic subjects. Correlations between EBV-DNA copy number and other parameters of EBV infection (anti-EBV antibody titer and AtLy count) but those with cytomegalovirus (CMV) infection and markers of atopic disease (IgE, h-RAST level, and Eos count) were demonstrated. It was found that anti-EBNA seronegative atopics have higher copy numbers of EBV DNA in WBC and more elevated levels of IgE and h-RAST than anti-EBNA seropositive atopics. Anti-EBV VCA antibody titer in individuals aged 15 years and younger and anti-EBNA antibody titer among Japanese were suggested to have declined considerably in the past 15 years.

Conclusions: The present study suggests that EBV infection in early childhood could precipitate atopic diseases.

INTRODUCTION

In the past several decades, the prevalence of atopic diseases has rapidly increased, especially in so-called developed countries. Of the viruses thought to play a role in the development of atopy, Epstein Barr virus (EBV) is particularly worthy of attention.[1,2] EBV infection mainly takes place in the first year of life, and more commonly develops in younger people in so-called developing countries.[3]

Nordbring et al.[4] and Bahna et al.[5] described an elevation of serum immunoglobulin E (IgE) during acute EBV infection, although this was not confirmed by a more recent study.[6] Olson et al.[7] reported a high frequency of IgE-mediated allergic disease in chronic mononucleosis syndrome, which is thought to be

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caused by EBV. There are several studies suggesting a positive as well as negative association between elevated EBV antibodies and atopic diseases.[1,2,4-11]

Therefore, we carried out a large-scale study. In this investigation, in addition to serum IgE level, h-RAST and peripheral blood Eos count were employed as markers of atopic manifestation. As markers of preceding EBV infection, anti-EBV viral capsid antigen (VCA) antibody[1,2] and anti-EBV nuclear antigen (EBNA) antibody, peripheral blood atypical lymphocyte (AtLy) count and finally, EBV-DNA copy numbers in 10^6 WBC (EBV-DNA) were employed.

METHODS

SUBJECTS
Japanese people living in the Tokyo area (Kanto district) who attended the institutes to which the authors of this report belong and were referred to the Department of Allergy and Rheumatology of The University of Tokyo were enrolled in the present study. Blood samples of bronchial asthma (BA) and / or atopic dermatitis (AD) patients were obtained when their diseases were well controlled and stable. The control subjects were healthy age-matched individuals who attended the institutes for health check, vaccination or preoperative examination before minor surgery. A diagnosis of BA was made according to the criteria of the National Institutes of Health, USA with slight modification.[12] Patients with AD, fulfilling the criteria of Hannifin and Rajka,[13] took part in the study. When several atopic diseases coexisted in an individual, the patient was included in an appropriate disease group according to his/her chief complaints. This study was approved by the Committee for the Protection of the Rights of Human Subjects of the University of Tokyo, Graduate School of Medicine. Written informed consent was obtained from the subjects themselves or from their parents in the case of minors.

All atopic subjects had an elevated total IgE level (>160 IU / ml) and allergen-specific IgE antibody (ies) h-RAST ; (>0.70 UA/ml) to at least one of the following allergens : house dust mite (*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*), Japanese cedar pollen, cat and dog dander. Children aged ten years or below were also examined for IgE antibodies to egg white, cow's milk and soy bean, which are the major food allergens in Japan. Clinically non-atopic individuals with no history of atopic disease (AD, BA, allergic rhinitis, rhinoconjunctivitis, or urticaria) and no symptoms of atopic disease upon examination by at least one of the investigators, but with an elevated serum IgE (>160 IU/ml) and clearly positive h-RAST (>0.70 UA/ml) were classified as asymptomatic atopic subjects (Asym). The normal control subjects (Normal) were healthy individuals with no symptoms or history of AD, BA or other atopic disease, low IgE level ($\leq$160 IU / ml), and negative RAST (<0.35 UA/ml).

AGE MATCHING
Age matching was statistically checked to confirm that the inter-group age-difference was not significant. When a sufficient number of blood samples was available, more strict age matching was performed in which patient/control pairs whose age difference was less than one year (in the case of subjects aged 5 or under, less than 6 months) were selected and the most suitable pairs were enrolled in the study.

ANTI-VIRAL ANTIBODY TITERS
Anti-EBV VCA IgG, IgM and anti-EBNA were assayed by a fluorescent antibody (FA) technique.[14] Anti-EBV VCA IgM antibody titer measured by the FA method was below the detection limit in all cases, and were not subjected to analysis. There were no significant gender differences in anti-EBV antibody titers (anti-EBV VCA ; $P = 0.185^{ns}$, anti-EBNA ; $P = 0.200^{ns}$; Mann-Whitney U-test). Anti-CMV IgG and IgM were examined using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol (Denka Seiken, Tokyo, Japan).

MEASUREMENT OF TOTAL SERUM IGE LEVEL AND ANTIGEN-SPECIFIC IGE ANTIBODY TITER
Total serum IgE level and h-RAST titers were determined using a Pharmacia CAP-RAST System (Uppsala, Sweden).

ANALYSIS OF LEUKOCYTES
Cytologic examination of peripheral WBC was performed using May Grünwald Giemsa stain. The relative proportions of the various leukocyte subpopulations were determined by differential cell counting of 1000 cells.

EBV-DNA COPY NUMBER IN 10^6 WBC
EBV-DNA in 10^6 WBC was quantified using a real-time RT-PCR assay.[15]

DATA ANALYSIS
Data were analyzed using SPSS® (SPSS Inc., Chicago, IL, USA). The titers of anti-EBV (anti-VCA and anti-EBNA) antibodies, serum total IgE level h-RAST, Eos count and AtLy count were confirmed to distribute normally after logarithmic transformation. Geometric means (GM) and standard deviations (SD) were obtained after logarithmic transformation. The age of enrolled subjects was expressed as arithmetic means (AM)±standard errors of the mean (SEM). The non-parametric Mann-Whitney U-test was used for inter-group comparisons. Correlations between two parameters were examined by Kendall's τ-test. Statistical significance was expressed by symbols : * * * = highly significant ($P < 0.001$), * * = highly significant ($P < 0.01$), * = significant ($P < 0.05$),†= not sig-

Table 1 Comparison of markers of EBV infection and atopic manifestations in different age groups

(1)−4 (years)

	N	Age AM ± SEM	Anti-EBV VCA GM ± SD	Anti-EBNA GM ± SD	AtLy GM ± SD	IgE GM ± SD	h-RAST GM ± SD	Eos GM ± SD
Healthy	8	2.25 ± 0.49	0.85 ± 0.32	0.77 ± 0.21	1.61 ± 0.58	1.56 ± 0.71	-0.02 ± 0.66	2.26 ± 0.61
vs								
BA/AD patients	10	3.00 ± 0.37	1.75 ± 0.79	1.18 ± 0.55	1.77 ± 0.75	2.58 ± 0.45	1.70 ± 0.58	0.83 ± 0.19
P (two tailed)		0.259ns	0.020 *	0.035 *	0.574ns	0.006 **	0.001 **	0.006 **

(2) 5−15 (years)

	N	Age AM ± SEM	Anti-EBV VCA GM ± SD	Anti-EBNA GM ± SD	AtLy GM ± SD	IgE GM ± SD	h-RAST GM ± SD	Eos GM ± SD
Healthy	12	9.25 ± 0.70	1.28 ± 0.74	0.93 ± 0.37	1.30 ± 0.00	1.69 ± 0.65	0.17 ± 0.92	1.88 ± 0.40
vs								
BA/AD patients	12	9.67 ± 0.73	1.35 ± 0.56	1.15 ± 0.47	1.49 ± 0.47	2.91 ± 0.49	1.78 ± 0.49	2.70 ± 0.18
P (two tailed)		0.661ns	0.436ns	0.210ns	0.149ns	0.000 ***	0.000 ***	0.000 ***

(3) 16−30 (years)

	N	Age AM ± SEM	Anti-EBV VCA GM ± SD	Anti-EBNA GM ± SD	AtLy GM ± SD	IgE GM ± SD	h-RAST GM ± SD	Eos GM ± SD
Healthy	17	22.1 ± 1.29	1.92 ± 0.43	1.14 ± 0.32	1.30 ± 0.00	1.67 ± 0.53	0.10 ± 0.76	2.04 ± 0.29
vs								
BA/AD patients	17	22.2 ± 1.29	1.92 ± 0.64	1.23 ± 0.38	1.31 ± 0.02	3.08 ± 0.62	1.96 ± 0.46	2.84 ± 0.21
P (two tailed)		0.890ns	0.710ns	0.474ns	0.317ns	0.000 ***	0.000 ***	0.000 ***

(4) 31−45 (years)

	N	Age AM ± SEM	Anti-EBV VCA GM ± SD	Anti-EBNA GM ± SD	AtLy GM ± SD	IgE GM ± SD	h-RAST GM ± SD	Eos GM ± SD
Healthy	8	36.6 ± 1.53	2.09 ± 0.36	1.11 ± 0.28	1.30 ± 0.00	1.86 ± 0.50	0.25 ± 0.81	1.82 ± 0.39
vs								
BA/AD patients	8	36.4 ± 1.51	2.09 ± 0.22	1.49 ± 0.22	1.30 ± 0.00	3.30 ± 0.99	2.18 ± 1.23	2.70 ± 0.39
P (two tailed)		0.784ns	0.749ns	0.014 *	1.000ns	0.016 *	0.008 **	0.002 **

(5) 46−(years)

	N	Age AM ± SEM	Anti-EBV VCA GM ± SD	Anti-EBNA GM ± SD	AtLy GM ± SD	IgE GM ± SD	h-RAST GM ± SD	Eos GM ± SD
Healthy	10	63.0 ± 3.56	2.11 ± 0.59	1.18 ± 0.25	1.30 ± 0.00	1.79 ± 0.74	-0.23 ± 0.69	2.04 ± 0.39
vs								
BA/AD patients	10	62.9 ± 3.54	1.93 ± 1.48	1.27 ± 0.39	1.44 ± 0.30	2.35 ± 0.49	0.74 ± 0.91	2.58 ± 0.22
P (two tailed)		0.939ns	0.538ns	0.479ns	0.147ns	0.031 *	0.024 *	0.002 **

nificant ($P < 0.1$), ns = not significant ($P > 0.1$).

RESULTS

ANTI-EBV, ESPECIALLY ANTI-EBNA ANTIBODY TITERS ARE ELEVATED IN ATOPIC PATIENTS

In the first set of studies, anti-EBV antibody titers were measured in blood samples from 204 individuals (52 Normal, 40 Asympt, 58 BA and 54 AD).

The non-parametric Mann-Whitney U-test for strictly age-matched inter-group (healthy = Normal/Asym *vs* BA/AD) comparison of anti-EBV antibody levels in all subjects[1] or in anti-EBV seropositive individuals[2] were carried out. No statistically significant difference in anti-EBV VCA titer was detected between healthy and atopic (BA/AD) patients ($P \geq 0.099$), but anti-EBNA titer was considerably elevated in atopic patients (all subjects : $P = 0.010*$, EBV seropositives : $P = 0.020*$).

INTER-GROUP COMPARISON OF PARAMETERS OF EBV INFECTION AND ATOPIC MANIFESTATION IN DIFFERENT AGE GROUPS

Characteristic parameters of EBV infection (anti-EBV VCA, anti-EBNA, AtLy) and atopic manifestations (IgE, h-RAST, Eos) at different ages were compared in healthy and atopic (BA/AD) subjects. As shown in Table 1, IgE, h-RAST and Eos in the atopic (BA/AD) groups were significantly higher than those in the healthy groups in all age groups tested. A rise of anti-EBV VCA and anti-EBNA in atopic (BA/AD) patients compared with healthy subjects was observed clearly in subjects aged 4 years or younger.

Table 2 Correlation between markers of EBV infection and atopic manifestations

		IgE	h-RAST	Eos
Anti-EBV VCA	Correlation coefficient	0.123	0.073	0.048
	P value (two tailed)	0.109^{ns}	0.365^{ns}	0.536^{ns}
	N	90	90	90
Anti-EBNA	Correlation coefficient	0.182	0.178	0.213
	P value (two tailed)	0.019 *	0.029 *	0.006 * *
	N	90	90	90
AtLy	Correlation coefficient	0.097	0.123	0.144 *
	P value (two tailed)	0.256^{ns}	0.171^{ns}	0.092 †
	N	90	90	90

In the course of the study, we noticed that healthy (clinically non-atopic) subjects are not always non-atopic serologically ; that is, they could be hyper-IgE responsive and / or hyper-eosinophilic. Therefore, they were grouped as asymptomatic (Asym : clinically non-atopic but serologically atopic), while subjects who are non-atopic both clinically and serologically were grouped as Normal. With the new grouping, a rise of anti-EBV VCA antibody titer in atopics seemed to become more evident (Healthy *vs* BA/AD patients : $P = 0.610^{ns}$, Normal *vs* Atopics = Asym/BA/AD : $P = 0.078^{†}$).

CORRELATION BETWEEN PARAMETERS OF EBV INFECTION AND ATOPIC MANIFESTATION

Parameters of previous EBV exposure (anti-EBV VCA and anti-EBNA antibodies, AtLy) and characteristic markers of atopic disease (IgE, h-RAST, Eos) were examined (Table 2). Significant correlation was often observed between markers of previous EBV infection and markers of atopic manifestation, further suggesting a close association between EBV infection and the development of atopic diseases.

EBV-DNA COPY NUMBER IN WBC AND ANTI-CMV IN ATOPIC SUBJECTS AND NORMAL CONTROLS

In the second set of studies, individuals were recruited and in addition to the factors examined in the above studies, EBV-DNA copy number/10^6 WBC and anti-CMV antibody levels were assayed.

Inter-group comparison between 11 normal controls and 11 strictly age-matched atopic subjects (Asym/BA/AD) was carried out. As shown in Table 3, EBV-DNA copy numbers in WBC were clearly increased in atopic subjects compared with normal controls ($P = 0.002 * *$). Anti-EBV VCA and anti-EBNA antibody levels were higher in atopic subjects ($P = 0.039 *$ and $P = 0.067^{†}$, respectively), while anti-CMV IgG and IgM were not significantly different between atopic subjects and normal controls ($P = 0.469^{ns}$ and $P = 0.843^{ns}$, respectively).

CORRELATION BETWEEN EBV-DNA IN WBC AND MARKERS OF ATOPY OR VIRAL (EBV / CMV) INFECTION

The correlation between markers of atopy (IgE, h-RAST and Eos) and markers of viral (EBV/CMV) infection was examined in 58 unselected and in 33 EBV seropositive subjects and the results are shown in Table 4.

In the unselected population, moderate correlation between EBV DNA and atopic markers (IgE : $P = 0.019 *$, h-RAST : $P = 0.055^{†}$, Eos : $P = 0.062^{†}$) was observed, while its correlation with markers of EBV infection seemed more significant (anti-EBV VCA : $P = 0.021 *$, anti-EBNA : $P = 0.048 *$). In EBV seropositive subjects, the correlation between EBV DNA and atopic marker was more evident (IgE : $P = 0.021 *$, h-RAST : $P = 0.038 *$ and Eos : $P = 0.028 *$), while correlation between anti-EBV antibody titers was less clear (anti-EBV VCA : $P = 0.250^{ns}$, anti-EBNA : $P = 0.024 *$). In both subject groupings, EBV DNA never correlated with anti-CMV antibody levels ($P > 0.1^{ns}$). Such findings strongly suggest that the increased anti-viral antibody titer in atopic patients was specific for EBV.

ANTI-EBNA NEGATIVE ATOPICS HAVE HIGHER EBV DNA IN WBC AND HIGHER HYPER-IGE RESPONSIVENESS THAN ANTI-EBNA POSITIVE ATOPICS

Subsequently, various markers were compared in 18 anti-EBNA negative and 24 anti-EBNA positive atopics (Asym/BA/AD) aged 5 to 38 years. It was found that all ($N = 14$) anti-EBV VCA negative subjects were also anti-EBNA negative. As shown in Table 5, anti-EBNA negative atopic subjects had higher numbers of EBV DNA copies in WBC, and higher levels of IgE and h-RAST than anti-EBNA positive atopics suggesting that their previous EBV load was heavier compared with anti-EBNA positive atopics.

AGE DISTRIBUTION OF ANTI-EBV ANTIBODIES IN HEALTHY INDIVIDUALS IN 2002 COMPARED WITH THAT IN 1987

Anti-EBV VCA and anti-EBNA titers of clinically

Table 3 EBV DNA copy number in WBC is higher in atopics compared to normal subjects

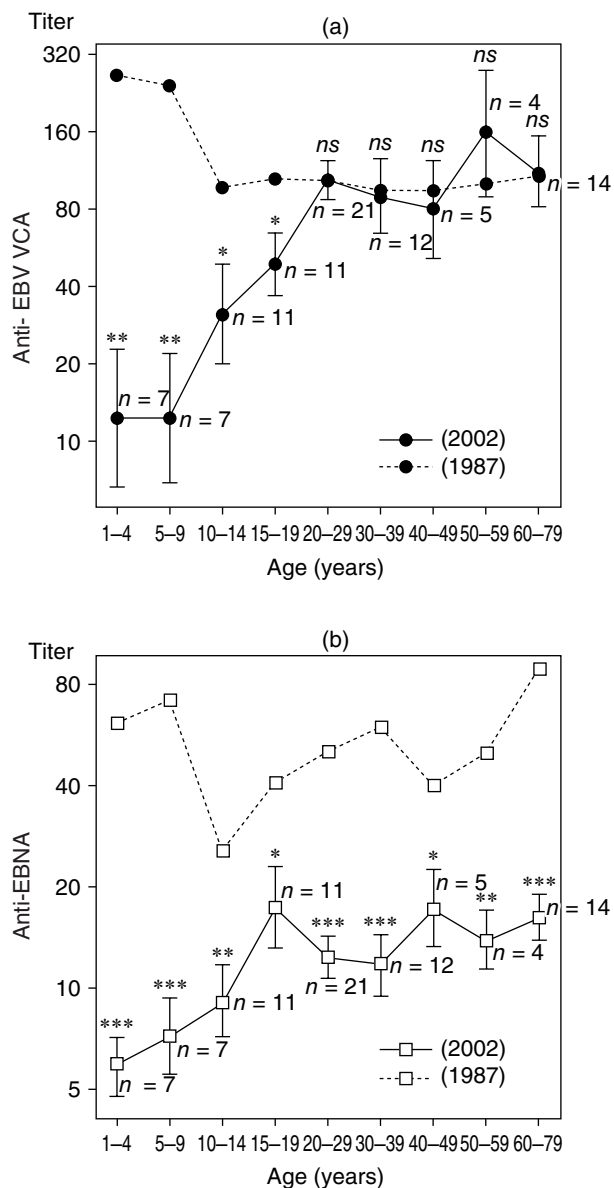|	N	Age	EBV DNA	Anti-EBV VCA	Anti-EBNA	IgE	h-RAST	Eos	Anti-CMV IgG	Anti-CMV IgM
		AM ± SEM	GM ± SD	GM ± SD	GM ± SD	GM ± SD	GM ± SD	GM ± SD	GM ± SD	GM ± SD
Normal	11	29.5 ± 6.51	1.00 ± 0.00	1.63 ± 0.48	1.16 ± 0.39	1.14 ± 0.57	-0.46 ± 0.00	1.97 ± 0.55	0.76 ± 0.53	-0.50 ± 0.16
vs										
Atopics (Asym/BA/AD)	11	29.1 ± 6.50	1.83 ± 0.88	2.09 ± 0.43	1.49 ± 0.49	2.79 ± 0.95	1.66 ± 1.01	2.73 ± 0.27	0.97 ± 0.69	-0.53 ± 0.51
P (two tailed)		0.869ns	0.002 **	0.039 *	0.067 †	0.000 ***	0.000 ***	0.001 ***	0.469ns	0.843ns

Fig. 1 Comparison of anti-EBV antibodies in Japanese healthy individuals in 2002 and in 1987. (**a**) Anti-EBV VCA antibody. (**b**) Anti-EBNA antibody. GM ± SEM are plotted against age. Anti-EBV-VCA and anti-EBNA antibody titers in 2002 and 1987 are represented as closed and open symbols, respectively.

* * * P < 0.001

* * P < 0.01

* P < 0.05

† P < 0.1 ns (not significant)

ns P > 0.1 ns (not significant)

healthy (non-atopic) individuals obtained in the present (2002) study were plotted against age and compared with the Japanese data reported in 1987.[16] The methods of anti-EBV VCA and anti-EBNA titration were exactly the same (FA) in the two studies. Neither the exact number (N) nor SD of the data of the

Table 4 Correlation between EBV DNA in WBC and markers of atopy or viral (EBV / CMV) infection

(1) unselected population

		Anti-EBV VCA	Anti-EBNA	IgE	h-RAST	Eos	Anti-CMV IgG	Anti-CMV IgM
EBV DNA	Correlation Coefficient	0.266	0.231	0.245	0. 207	0.203	0.176	0.075
	P value (two tailed)	0.021 *	0.048 *	0.019 *	0.055 [†]	0.062 [†]	0.111[ns]	0.478[ns]
	N	56	56	58	57	54	58	58

(2) EBV seropositive subjects

		Anti-EBV VCA	Anti-EBNA	IgE	h-RAST	Eos	Anti-CMV IgG	Anti-CMV IgM
EBV DNA	Correlation Coefficient	0.183	0.407	0.318	0.297	0.302	-0.003	0.005
	P value (two tailed)	0.250[ns]	0.024 *	0.021 *	0.038 *	0.028 *	0.984[ns]	0.969[ns]
	N	28	23	33	33	33	33	33

1987 survey are available at present, but, since the scale of the survey was very large (total N = 1086 and each age group contained more than 60 samples), one sample τ-test was carried out using the GM of each age group in the 1987 survey as a test value. As shown in Figure 1(a), anti-EBV VCA titer in subjects before adolescence was lower in 2002 than in 1987, while anti-EBNA titers surveyed in 2002 were considerably lower in all age groups examined than those surveyed in 1987(Fig. 1(b)).

DISCUSSION

EBV, a B lymphocytotropic herpesvirus, is associated with several benign and malignant diseases. Most people become latently infected with the virus before adulthood.[17] Seroconversion in adults is rare, and about 5% of adults remain seronegative for life (EBV-non-seroconverters).

Infectious mononucleosis (IM) is an acute and usually self-limiting lymphoproliferative disease caused mostly by primary EBV infection.[14,18] The hallmark of acute IM is appearance of AtLy in the peripheral blood. IgE level is increased mostly during the initial stage of IM, and at that time, a strong correlation is noted between the IgE response and the number of circulating AtLy.[5]

In 1981, it was reported that seropositive children aged 5 to 18 years with atopy have higher serum levels of anti-EBV VCA antibody than non-atopic children of similar ages. The increase in EBV VCA titer was most pronounced in children with asthma (BA).[1] This observation was confirmed in adults with atopic eczema (AD) by the same group.[2]

On the basis of the well-known finding that EBV almost selectively infects B lymphocytes and induces hyperproduction of immunoglobulins, it has been strongly suspected that EBV infection may result in an aberrant IgE response to any antigen and in this way may precipitate atopic diseases.[1,2] However, the results of a recent study showing a higher prevalence of high IgE levels in EBV-seronegative children appear to be inconsistent with the above hypothesis.[6] This apparent discrepancy has never been re-evaluated until now. Thus, we carried out an investigation employing recent technology and advanced computer software for statistical analysis. In this study, in addition to the inter-group comparison of anti-EBV VCA antibody titers between atopic patients and healthy individuals, the correlation between parameters that reflect previous EBV exposure (EBV DNA copy number in WBC, anti-EBNA, anti-EBV VCA antibody titers and AtLy count in blood) and markers associated with atopic disease (serum total IgE, h-RAST level and Eos count in blood) were examined.

It was found that anti-EBV VCA titer was not elevated in atopic (BA/AD) patients compared with age-matched healthy individuals; however, anti-EBNA antibody titer was markedly increased in atopic subjects compared with healthy (clinically non-atopic) individuals (Table 6).

An elevated anti-EBV VCA antibody titer in atopic subjects became evident when EBV-seropositive atopics (Asym/BA/AD) were compared with normal control subjects (Table 6).

Inter-group comparison of parameters of atopic manifestation and EBV infection in different age groups, carried out subsequently, demonstrated that a rise of anti-EBV VCA and anti-EBNA in atopic subjects (BA/AD) compared with healthy subjects was observed most clearly in individuals aged 4 years or younger(Table 1). These data suggest that EBV infection in childhood increases the risk of development of atopic diseases (BA/AD).

Correlation between parameters of previous EBV exposure (anti-EBV VCA and anti-EBNA antibodies, AtLy) and characteristic markers of atopic disease (IgE, h-RAST, Eos) were examined and the results

Table 5 Anti-EBNA-negative atopics have higher EBV DNA in WBC and are more hyper-IgE responsive compared to anti-EBNA-positive atopics

	N	Age AM ± SEM	EBV DNA GM ± SD	Anti-EBV VCA GM ± SD	Anti-EBNA GM ± SD	IgE GM ± SD	h-RAST GM ± SD	Eos GM ± SD	Anti-CMV IgG GM ± SD	Anti-CMV IgM GM ± SD
EBNA (−) atopics	18	20.2 ± 2.74	1.25 ± 0.46	0.93 ± 0.48	0.70 ± 0.00	3.22 ± 1.13	2.13 ± 1.16	2.87 ± 0.47	1.00 ± 0.69	−0.42 ± 0.19
vs										
EBNA (+) atopics	24	18.6 ± 2.27	1.04 ± 0.18	1.99 ± 0.49	1.54 ± 0.34	2.57 ± 0.81	1.37 ± 0.86	2.63 ± 0.31	0.90 ± 0.56	−0.47 ± 0.23
P (two tailed)		0.760 ns	0.035 *	0.000 ***	0.000 ***	0.031 *	0.012 *	0.184 ns	0.627 ns	0.258 ns

are summarized in Table 2. The clear correlation between markers of previous EBV infection and markers of atopic manifestation further suggests a close association between EBV infection and the development of atopic diseases.

Such findings were in agreement with the results shown in Tables 4, 5, which demonstrated that anti-EBV antibodies, but not anti-CMV antibody titers, were significantly different between the normal and atopic subjects (Asym/BA/AD) and no significant correlation was found between anti-CMV antibody levels and parameters of atopy (IgE, h-RAST). Furthermore, there was no statistically significant correlation between anti-EBV and anti-CMV antibody titers. These results suggest that an assumed link between atopic manifestation and EBV infection is specific for EBV infection, and is not merely a reflection of viral infection in general.

As shown in Table 3 in which normal controls and atopics (Asym/BA/AD) were compared for anti-EBV VCA ($P = 0.039*$), anti-EBNA ($P = 0.067†$) and atopic markers (IgE : $P = 0.000***$, h-RAST : $P = 0.000***$, Eos : $P = 0.001**$), EBV DNA copy numbers in WBC were clearly elevated ($P = 0.002**$) in the atopics.

It was a kind of mystery to find that mean anti-EBV antibody levels were higher in atopics than controls, but on the other hand, many EBV seronegative atopic (BA/AD) patients whose anti-EBV antibody levels are clearly lower than those in normal subjects do exist.

Helminen *et al.* reported a study of the resistance to EBV infection, and speculated that the persistent seronegativity of some adults may be explained by eradication of the virus at the beginning of infection.[19]

However, this speculation seemed not to be compatible with the results shown in Table 5, which demonstrated that anti-EBNA seronegative atopics have higher copy numbers of EBV DNA in WBC and more elevated levels of IgE and h-RAST than anti-EBNA seropositive atopics.

The reality of anti-EBV (especially anti-EBNA) non-seroconversion may be an immunological unresponsiveness which abrogates possibly protective anti-EBNA antibody responses needed for limiting symptomatic EBV infection to make anti-EBNA non-seroconverters more sensitive to atopic diseases (BA/AD) (Table 5).

In 1969, Hinuma and colleagues examined the age distribution of anti-EBV antibody in Japanese, and reported that the prevalence of seropositivity was considerably high in infancy and persisted for life.[20]

Anti-EBNA antibody titer in Japanese individuals and anti-EBV VCA IgG antibody titer in childhood are suggested to have decreased over the past 15 years (Fig. 1). If low anti-EBV antibody titers in early childhood are a considerable risk factor for symptomatic EBV infection, and if EBV is actually associated with atopic diseases, this may explain why atopic patients have abruptly increased recently in Japan as well as

Table 6 Anti-EBV antibody titers, especially anti-EBNA, are elevated in atopic patients

(1) All subjects

	N	Age AM ± SEM	Anti-EBV VCA GM ± SD	Anti-EBNA GM ± SD	AtLy GM ± SD	IgE GM ± SD	h-RAST GM ± SD	Eos GM ± SD
Healthy	45	30.4 ± 3.13	1.81 ± 0.63	1.10 ± 0.33	1.30 ± 0.00	1.67 ± 0.61	-0.02 ± 0.72	1.97 ± 0.38
vs								
BA/AD patients	45	30.4 ± 3.11	1.92 ± 0.55	1.30 ± 0.39	1.45 ± 0.42	2.90 ± 0.72	1.66 ± 0.93	2.76 ± 0.26
P (two tailed)		0.994^{ns}	0.610^{ns}	0.010 *	0.006 * *	0.000 * * *	0.000 * * *	0.000 * * *

(2) EBV-seropositive subjects

	N	Age AM ± SEM	Anti-EBV VCA GM ± SD	Anti-EBNA GM ± SD	AtLy GM ± SD	IgE GM ± SD	h-RAST GM ± SD	Eos GM ± SD
Healthy	28	36.3 ± 3.62	2.12 ± 0.37	1.24 ± 0.28	1.30 ± 0.00	1.69 ± 0.65	-0.01 ± 0.70	1.93 ± 0.34
vs								
BA/AD patients	28	36.3 ± 3.61	1.95 ± 0.40	1.41 ± 0.29	1.38 ± 0.23	3.00 ± 0.76	1.72 ± 1.03	2.78 ± 0.24
P (two tailed)		0.987^{ns}	$0.099^{†}$	0.020 *	$0.078^{†}$	0.000 * * *	0.000 * * *	0.001 * *

in developed countries throughout the world. Low maternal anti-EBV antibody titers would result in low titers of anti-EBV antibodies in the infant, which may increase the risk of symptomatic EBV infection, one of which may be an atopic disease.

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