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Primary Infection with the Epstein-Barr Virus and Risk of Multiple Sclerosis

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Abstract

To determine whether multiple sclerosis (MS) risk increases following primary infection with the Epstein-Barr virus (EBV), we conducted a nested case-control study including 305 individuals who developed MS and 610 matched controls selected among the over 8 million active-duty military personnel with serum stored in the Department of Defense Serum Repository. Time of EBV infection was determined by measuring antibody titers in serial serum samples collected before MS onset among cases, and on matched dates among controls. Ten (3.3%) cases and 32 (5.2%) controls were initially EBV negative. . All of the 10 EBV-negative cases became EBV positive before MS onset ; in contrast, only 35.7 % (10) of the 28 controls with follow-up samples seroconverted (exact p value = 0.0008). We conclude that MS risk is extremely low among individuals not infected with EBV, but it increases sharply in the same individuals following EBV infection.

INTRODUCTION

A link between the Epstein-Barr virus (EBV) and MS is supported by the increased risk of MS, in individuals with history of mononucleosis ^{1,2} or with elevated serum titers of antibodies against EBV nuclear antigens (EBNA) ³⁻⁶, and by the higher prevalence of EBV infection in MS cases than controls. ⁷⁻¹⁰ There are no longitudinal studies, however, estimating MS risk in EBV negative individuals or the temporal relation between EBV infection and MS. We therefore addressed these questions prospectively in a large population of healthy young adults

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Conflict of Interest Statement

None of the authors have any conflicts of interest to disclose.

METHODS

Study population

Active-duty US Army, Navy, and Marines personnel who have at least one serum sample in the Department of Defense Serum Repository (DoDSR), which stores approximately 46 million serum samples originally collected from over 8 million individuals for HIV testing.^{11, 12}

Case and Control Ascertainment

Cases were identified by searching the electronic databases of the Physical Disability Agencies of the US Army and US Navy for the diagnostic code corresponding to MS reported between 1992 and 2004, and then reviewing hard copy medical records. Overall, 515 cases were reviewed, of which 315 had definite (n=237) or probable (n=78) MS according to previously described criteria¹² and had at least one pre-clinical serum sample, i.e. a sample collected prior to the development of neurological symptoms (date of MS onset), as attested from the medical record. For each case, we obtained up to three pre-clinical samples (the earliest and latest available, as well as a third sample collected between those two).¹² Two controls for each case were randomly selected from the DoDSR population, matched by branch of service, age, sex, race/ethnicity, and dates of blood collection, as previously reported.¹² Ten cases could not be matched, leaving 305 cases and 610 matched controls in the analyses. By design, the controls in this study provide an unbiased estimate of the distribution of exposure and covariates among the millions of individuals who comprise the source population of the cases, and the odds ratios are unbiased estimates of the corresponding rate ratios that would be obtained by testing for EBV positivity all the individuals in the source population.¹³

Laboratory Analyses

Serum samples were sent to the laboratory ordered in triplets, each triplet including a case sample and the corresponding matched control samples in random order and without identification of case-control status. Blind quality control triplets consisting of multiple aliquots of the same serum samples were randomly interspersed amongst the study samples to monitor the reproducibility of the assays. EBV serology was performed using indirect immunofluorescence (VCA) or anticomplement immunofluorescence (EBNA complex, EBNA-1, EBNA-2) for the detection of IgG antibodies.^{5, 14-16} IgG antibodies against cytomegalovirus (CMV), used here as a control herpes virus, were determined using an ELISA.¹⁷ The serological assays for a first set of 83 cases and their 166 matched controls were performed at Virolab Inc., CA, USA.⁵ Due to the closure of Virolab, the remaining samples (222 cases and 444 matched controls) were assayed at the Karolinska Institute in Stockholm, Sweden, under the supervision of one of the authors (KF); to reduce costs, antibodies to EBNA-1 and EBNA-2 were determined only in a subset of 166 cases and 332 matched controls.

Statistical analyses

Individuals were considered EBV negative (i.e. not infected with EBV) if they had no detectable anti-EBV antibodies (VCA < 20; EBNA complex, EBNA-1 or EBNA-2 < 5); a primary EBV infection was deemed to have occurred in these individuals if any of their subsequent samples became positive for anti-VCA antibodies, because these antibodies appear soon after infection and remain present indefinitely.¹⁸ Exact logistic regression, which calculates unbiased estimates when data are very sparse, was used to estimate the relative risk of MS following EBV infection. All P values are 2-tailed. The statistical software SAS v9.1 (SAS Institute Inc, Cary, NC) was used for all analyses.

The research protocol was approved by the institutional review boards of the Walter Reed Army Institute of Research and the Harvard School of Public Health, both of which determined a waiver of informed consent was appropriate for the use of already existing medical record data and biological samples

RESULTS

The main characteristics of cases and controls are shown in Table 1. The relatively high proportions of males (66%) and blacks (30%) among MS cases and their matched controls reflect the demographic composition of the source population.

At baseline, 10 (3.3%) of the 305 MS cases and 32 (5.2%) of the 610 controls were EBV seronegative ($p=0.2$). At least one follow-up blood sample was available for all the EBV-seronegative cases and for 28 of the 32 seronegative controls. During the follow-up, all of the 10 initially seronegative cases became EBV positive before the onset of MS, while only 10 of the 28 controls seroconverted (p , exact test = 0.0008); results adjusted for duration of follow-up and serum levels of 25(OH) vitamin D were similar (p , exact test = 0.005). The seroconversion rate among controls was 11.2% per year, which is similar to that in previous longitudinal studies of military recruits or university students.^{19, 20} The relation between time of seroconversion and MS onset is shown in Figure 1, and demographic characteristics and antibody titers in cases and controls who were EBV negative at baseline are shown in Table 2. In all cases, the anti-VCA titers increased to 320 or higher before MS onset. The mean interval between the date of the first EBV positive serum sample and MS onset was 3.8 yrs (range: 1.7 years to 7.0 years). Based on the assumption that EBV infection occurred, on average, at the mid-point between the last seronegative sample and the first seropositive sample, the mean interval between primary EBV infection and MS onset would be 5.6 years (range 2.3 years to 9.4 years). Because none of the EBV-negative individuals developed MS, the relative risk of MS following EBV infection cannot be directly estimated; the estimator involves a division by zero and the relative risk would tend to infinity. In addition, a statistically nonsignificant relative risk of 2.0 (95% confidence interval: 0.8 to 5.0) for MS was found for individuals who seroconverted during the follow-up as compared with individuals who were already EBV positive at baseline.

Seropositivity to CMV at baseline was lower in cases (49%) than in controls (58%), but there was no significant association between CMV seroconversion and MS risk (relative risk = 0.94; $p=0.9$).

DISCUSSION

In this large prospective investigation, we found a total absence of incident MS among individuals without detectable serum antibodies to EBV. About one third of these individuals seroconverted during the follow-up, and after seroconversion, manifested a rate of MS similar to that of individuals of the same age and sex who were already EBV positive at the study baseline.

These findings suggest that EBV infection increases the risk of MS, but several alternative interpretations need to be considered, including laboratory errors, reverse causation, and confounding. The detection of anti-EBV antibodies by indirect immunofluorescence provides a highly sensitive and specific marker of EBV infection, as demonstrated by observations in longitudinal studies that seronegative individuals have a high risk of EBV-induced acute infectious mononucleosis, from which EBV seropositive individuals are completely protected.^{21, 22} The occurrence of EBV infection is confirmed by the observation that in all cases the anti-VCA antibodies, undetectable in the initial sample,

increased to titers of 320 and above before MS onset, and anti-EBNA complex or anti-EBNA-1 titers also became positive in all cases. Further, the annualized rate of EBV seroconversion that we observed among controls during the follow-up (11.2%) is remarkably similar to that in previous studies in the U.S. Military Academy (12.3%) and college students (12.0 – 15 %).^{19, 20} In those studies, seroconversion was associated with a high frequency of heterophile antibody-positive clinical mononucleosis, a marker of primary EBV infection. Thus, the appearance of anti-EBV antibodies in previously seronegative individuals is a robust marker of primary EBV infection. In contrast, seroconversion to CMV occurred at a similar rate in cases and controls, and was thus unrelated to MS risk.

The pathological process leading to MS starts before the clinical symptoms.²³ The results of our study could therefore be explained if pre-clinical MS resulted in increased susceptibility to EBV infection. While we cannot exclude this possibility, it should be noted that such an increase should be selective for EBV, because the prevalence of CMV infection was not higher among MS cases than controls, and rather extreme to result in a 100% prevalence of EBV infection before MS onset, neither of which seems likely.

Finally, a positive association between EBV infection and MS could be explained if both were affected by a common factor or confounder. A genetic resistance to both EBV infection and MS is not a plausible explanation, because many EBV negative individuals eventually became EBV infected and, following infection, susceptible to MS. Environmental factors, such as latitude, vitamin D, or cigarette smoking, being less strong risk factors for MS than EBV infection itself²⁴, and at most only weakly associated with EBV infection, are unlikely to explain the EBV-MS association. Confounding by an infectious agent that is co-transmitted with EBV cannot be excluded, but there is no evidence supporting this.

The most likely interpretation of the main results of our study, therefore, is that EBV infection itself increases the risk of MS.

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Figure 1.

Time of EBV seroconversion and MS onset in the 10 cases who were seronegative at baseline. The vertical lines within each bar represent the time of blood collections after the initial sample, which was taken at time zero for each individual. The arrows and bar darkening mark the time of the first EBV positive serum. The black circles are drawn at the time of MS onset.

Table 1

Characteristics of cases and controls

	Cases (n=305)	Controls (n=610)
Sex, N (%)		
Male	202 (66)	404 (66)
Female	103 (34)	206 (34)
Age at blood draw, yr		
Mean (SD)	23.5 (5.4)	23.5 (5.4)
Range	16 - 40	17 - 41
Race/Ethnicity, N (%)		
White	175 (57)	350 (57)
Black	93 (30)	186 (30)
Hispanic	27 (9)	54 (9)
Other	10 (3)	20 (3)
Education, N (%)		
High school	239 (78)	497 (81)
Some college	18 (6)	33 (5)
Completed college	32 (10)	62 (10)
Graduate school	14 (5)	15 (2)
Age at onset, yr		
Mean (SD)	28.2 (6.0)	
Range	18 - 48	
Time between first blood draw and MS onset, yrs		
Mean (SD)	4.8 (3.0)	
Range	0 - 13	

Percentages may not total to 100% due to missing data.

Time of seroconversion for EBV and serum titers of anti-EBV IgG antibodies of 38 individuals without detectable EBV antibodies at baseline and at least one follow-up serum sample. Date of onset of first neurological symptoms reported for individuals who developed MS.

Table 2

Case / Control Number	Age ¹ , Gender	Race ²	Date of Blood Collection or MS Onset	Time from Baseline (Months)	EBV Infection	EBV VCA	EBNA	EBNA 1	EBNA 2
Cases									
1	19 M	W	1 st sample 1/96 2 nd sample 2/98	0 25	- +	<20 640	<5 640	<5 320	<5 40
			MS onset 10/99	45					
2	20 F	W	1 st sample 6/90 2 nd sample 1/95 3 rd sample 2/96	0 55 68	- + +	<20 640 640	<5 80 80	<5 80 80	<5 80 80
			MS onset 2/97	80					
3	18 M	B	1 st sample 1/93 2 nd sample 7/96 3 rd sample 4/97	0 42 51	- + +	<20 640 640	<5 640 1280	<5 320 640	<5 <5 <5
			MS onset 6/98	65					
4	30 M	W	1 st sample 11/90 2 nd sample 5/93 3 rd sample 8/98	0 30 93	- + +	<20 2560 5120	<5 80 80		
			MS onset 2/00	111					
5	18 M	H	1 st sample 3/93 2 nd sample 6/98 3 rd sample 3/00	0 63 84	- + +	<20 80 640	<5 160 160	<5 160 320	<5 <5 <5
			MS onset 3/01	96					
6	17 M	W	1 st sample 12/92	0	-	<20	<5	<5	<5

Case / Control Number	Age/ Gender	Race ²	Date of Blood Collection or MS Onset	Time from Baseline (Months)	EBV Infection	EBV VCA	EBNA	EBNA 1	EBNA 2	
			2 nd sample	3/97	51	+	5120	320	160	20
			3 rd sample	4/02	112	+	5120	320	160	20
			MS onset	6/03	121					
7	16 M	B	1 st sample	8/89	0	–	<20	<5	<5	<5
			2 nd sample	2/93	42	–	<20	<5	<5	<5
			3 rd sample	3/96	79	+	5120	40	160	<5
			MS onset	7/98	107					
8	18 M	H	1 st sample	9/89	0	–	<20	<5	<5	<5
			2 nd sample	4/94	55	+	640	80	160	<5
			3 rd sample	10/99	121	+	5120	640	320	<5
			MS onset	6/00	129					
9	20 M	W	1 st sample	3/99	0	–	<20	<5	<5	<5
			2 nd sample	10/00	19	–	<20	<5	<5	<5
			3 rd sample	9/01	30	+	5120	160	20	<5
			MS onset	7/03	52					
10	20 M	W	1 st sample	12/89	0	–	<20	<5	<5	<5
			2 nd sample	10/94	58	+	320	80	20	<5
			3 rd sample	7/99	115	+	320	40	80	<5
			MS onset	10/01	142					
Controls										
1	19 F	W	1 st sample	6/93	0	–	<20	<5	<5	<5
			2 nd sample	2/95	20	–	<20	<5	<5	<5
2	19 M	W	1 st sample	11/89	0	–	<20	<5	<5	<5
			2 nd sample	5/90	6	–	<20	<5	<5	<5

Case / Control Number	Age ¹ , Gender	Race ²	Date of Blood Collection or MS Onset	Time from Baseline (Months)	EBV Infection	EBV VCA	EBNA	EBNA 1	EBNA 2
3	17 F	W	1 st sample 8/89	0	-	<20	<5	<5	<5
			2 nd sample 8/94	60	-	<20	<5	<5	<5
4	19 F	W	1 st sample 6/90	0	-	<20	<5	<5	<5
			2 nd sample 1/95	55	+	320	20	<5	20
			3 rd sample 2/96	68	+	640	20	10	20
5	21 M	W	1 st sample 10/88	0	-	<20	<5	<5	<5
			2 nd sample 2/93	52	+	320	320	160	<5
6	18 M	W	1 st sample 3/96	0	-	<20	<5	<5	<5
			2 nd sample 8/96	5	-	<20	<5	<5	<5
			3 rd sample 10/96	7	-	<20	<5	<5	<5
7	21 M	W	1 st sample 1/90	0	-	<20	<5		
			2 nd sample 5/95	64	+	1280	20		
			3 rd sample 9/96	80	+	1280	10		
8	21 M	W	1 st sample 8/88	0	-	<20	<5		
			2 nd sample 10/94	74	+	1280	5		
			3 rd sample 5/00	141	+	1280	40		
9	19 M	W	1 st sample 8/93	0	-	<20	<5		
			2 nd sample 8/96	36	-	<20	<5		
			3 rd sample 6/99	70	-	<20	<5		
10	19 M	H	1 st sample 6/92	0	-	<20	<5	<5	<5
			2 nd sample 3/93	9	-	<20	<5	<5	<5
			3 rd sample 5/94	23	-	<20	<5	<5	<5
11	19 M	W	1 st sample 8/97	0	-	<20	<5	<5	<5
			2 nd sample 8/98	12	-	<20	<5	<5	<5
			3 rd sample 7/99	23	-	<20	<5	<5	<5
12	20 F	W	1 st sample 2/91	0	-	<20	<5	<5	<5
			2 nd sample 4/93	26	-	<20	<5	<5	<5
			3 rd sample 12/94	46	-	<20	<5	<5	<5

Case / Control Number	Age ¹ , Gender	Race ²	Date of Blood Collection or MS Onset	Time from Baseline (Months)	EBV Infection	EBV VCA	EBNA	EBNA 1	EBNA 2
13	20 M	W	1 st sample 2 nd sample	0 15	- +	<20 1280	<5 20	<5 20	<5 5
14	32 M	W	1 st sample 2 nd sample 3 rd sample	0 27 60	- - -	<20 <20 <20	<5 <5 <5	<5 <5 <5	<5 <5 <5
15	19 M	W	1 st sample 2 nd sample 3 rd sample	0 66 89	- - -	<20 <20 <20	<5 <5 <5	<5 <5 <5	<5 <5 <5
16	17 M	W	1 st sample 2 nd sample 3 rd sample	0 24 56	- + +	<20 1280 1280	<5 5 20	<5 10 20	<5 <5 <5
17	23 M	W	1 st sample 2 nd sample	0 2	- -	<20 <20	<5 <5	<5 <5	<5 <5
18	17 M	W	1 st sample 2 nd sample 3 rd sample	0 41 101	- + +	<20 1280 1280	<5 160 80	<5 20 80	<5 5 20
19	20 M	O	1 st sample 2 nd sample 3 rd sample	0 49 93	- - -	<20 <20 <20	<5 <5 <5	<5 <5 <5	<5 <5 <5
20	18 M	H	1 st sample 2 nd sample 3 rd sample	0 11 23	- - -	<20 <20 <20	<5 <5 <5	<5 <5 <5	<5 <5 <5
21	27 M	W	1 st sample 2 nd sample 3 rd sample	0 13 40	- - -	<20 <20 <20	<5 <5 <5	<5 <5 <5	<5 <5 <5
22	18 M	W	1 st sample 2 nd sample 3 rd sample	0 30 109	- - +	<20 <20 640	<5 <5 160	<5 <5 20	<5 <5 <5

Case / Control Number	Age ¹ , Gender	Race ²	Date of Blood Collection or MS Onset	Time from Baseline (Months)	EBV Infection	EBV VCA	EBNA	EBNA 1	EBNA 2
23	23 F	W	1 st sample 4/91	0	—	<20	<5	<5	<5
			2 nd sample 6/93	26	—	<20	<5	<5	<5
			3 rd sample 6/94	38	—	<20	<5	<5	<5
24	17 M	W	1 st sample 11/89	0	—	<20	<5	<5	<5
			2 nd sample 6/93	43	—	<20	<5	<5	<5
			3 rd sample 10/96	83	—	<20	<5	<5	<5
25	28 M	W	1 st sample 9/89	0	—	<20	<5	<5	<5
			2 nd sample 9/92	36	—	<20	<5	<5	<5
			3 rd sample 9/93	48	—	<20	<5	<5	<5
26	24 M	W	1 st sample 10/97	0	—	<20	<5	<5	<5
			2 nd sample 2/99	16	—	<20	<5	<5	<5
			3 rd sample 6/00	32	—	<20	<5	<5	<5
27	21 M	H	1 st sample 7/94	0	—	<20	<5	<5	<5
			2 nd sample 6/97	35	—	<20	<5	<5	<5
			3 rd sample 8/01	85	+	1280	80	10	20
28	18 M	W	1 st sample 8/97	0	—	<20	<5	<5	<5
			2 nd sample 2/00	30	+	320	5	<5	5
			3 rd sample 3/01	43	+	640	5	<5	5

¹ = Age at baseline

² Race/ethnicity: W = White, B = Black, H = Hispanic, O = Other