Epstein-Barr Virus (EBV) and the Chronic Fatigue Syndrome: Normal Virus Load in Blood and Normal Immunologic Reactivity in the EBV Regression Assay

Caroline M. A. Swanink, Jos W. M. van der Meer,
Jan H. M. M. Vercoulen, Gijs Bleijenberg,
Jan F. M. Fennis, and Joep M. D. Galama

*From the Departments of Medical Microbiology, Internal Medicine, and
Medical Psychology, University Hospital Nijmegen, Nijmegen,
the Netherlands*

The etiology of chronic fatigue syndrome (CFS) is unknown. Some patients have high antibody titers to viral capsid antigen (VCA) and early antigen (EA) of Epstein-Barr virus (EBV), suggesting that reactivation of EBV is involved. We investigated virus load (spontaneous transformation) and immunologic regression of EBV-induced transformation in peripheral blood mononuclear cells (PBMCs) from 10 selected patients with CFS who had high antibody titers to VCA and EA. The outcome was compared with that for nine healthy controls and one patient with severe chronic active EBV infection (SCAEBV). There were no significant differences in viral load between patients and healthy controls. Immunologic regression of in vitro-transformed PBMCs was also equally efficient in patients and controls. The SCAEBV-infected patient and two controls, who were all seronegative for EBV, showed impaired regression. In conclusion, we were unable to demonstrate a role for reactivation of EBV in CFS, even in selected patients with high titers of antibody to VCA and EA of EBV.

Chronic fatigue syndrome (CFS) is claimed to be often precipitated by an acute infection. Research into the potential viral causes of CFS has concentrated on herpesviruses, enteroviruses, and retroviruses. Of the herpesviruses, Epstein-Barr virus (EBV) has received particular attention. Higher-than-expected IgG titers to the viral capsid antigen (VCA) and early antigen (EA) of EBV (EB-VCA and EB-EA) and low titers or absence of antibodies to the nuclear antigen of EBV (EB-NA) have been described [1, 2]. However, similar antibody profiles have occasionally been found for healthy adults [3–5], but this would still not rule out a role for EBV in a subgroup of patients with these antibody profiles. Persistent viral activity or reactivation may be the consequence of impaired cellular immunity, as has been reported in patients with CFS [6–9]; impairment of cellular immunity provides the opportunity for viral reactivation, boosting virus-specific responses. Sumaya [4] and Jones [5] have both noted that spontaneous EBV-induced proliferation of peripheral blood mononuclear cells (PBMCs) occurs more readily in patients with chronic fatigue (27% and 30%, respectively) than in healthy controls (6% and 5%, respectively). This would indicate that suppression of circulating EBV-transformed B cells may be diminished in patients with

CFS. To further explore the potential role of EBV, we investigated the load of replicating EBV and regression of EBV-transformed colonies in a selected group of patients with CFS who had high antibody titers to EB-VCA or EB-EA.

From a cohort of 88 patients with CFS and 76 controls matched for gender, age, and geographical area, we selected 10 patients with high titers of EB-VCA IgG (≥ 128) or EB-EA IgG (≥ 32). Such titers were found for <5% of 200 healthy blood donors (figure 1) but for 12% of the CFS patients. All patients fulfilled the criteria for CFS described by Sharpe et al. [10]. Patients were compared with their matched controls. Characteristics of patients and controls are summarized in table 1. For comparison, a 23-year-old woman with severe chronic active EBV infection (SCAEBV) was simultaneously tested. Her disease was in a stable phase (4 years after onset), and she was receiving maintenance treatment with acyclovir at a dose of 800 mg/day. This patient had persistently high antibody titers to EB-VCA and EB-EA (table 1).

Antibodies to EB-VCA and EB-EA were detected by indirect immunofluorescence on HH514 cells, a subclone of P3HR1, donated by Dr. G. Miller (Yale University School of Medicine, New Haven, CT). In our laboratory, HH514 cells are used for routine diagnostic tests on EBV-VCA and EB-EA, and we find that the results of immunofluorescence tests on these cells are easy to interpret, although antibody titers are usually 10 times lower than those obtained by immunofluorescence on P3HR1 cells. We tested serum samples of 200 healthy blood donors to obtain reference titers, which are depicted in figure 1. Raji cells were used for anti-complement immunofluorescence determination of antibodies to EB-NA as described previously [3]. Venous heparinized

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Reprints or correspondence: Dr. Caroline M. A. Swanink, Department of Medical Microbiology, University Hospital Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, the Netherlands.

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Figure 1. A: Distribution of titers of IgG to viral capsid antigen of Epstein-Barr virus among 200 healthy blood donors determined with use of indirect immunofluorescence on HH514 cells. B: Distribution of titers of IgG to early antigen of Epstein-Barr virus among 200 healthy blood donors determined with use of indirect immunofluorescence on HH514 cells.

blood (40 mL) was drawn from patients and controls, and peripheral blood mononuclear cells (PBMCs) were isolated by buoyant density centrifugation on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). The transformation assay was performed essentially as described by Rickinson et al. [11] with use of cyclosporin A to inhibit T cell function. Six repetitive wells of a 96-well flat-bottomed microtest plate (Costar Europe, Cambridge, United Kingdom) were seeded with PBMCs at twofold dilutions ranging from 1×10^6 to 1.5×10^4 cells per well. Plates were incubated at 37°C in a humidified atmosphere containing 5% CO_2 . At weekly intervals, half of the medium was replaced by fresh culture medium and the cultures were observed for the occurrence of transformation.

The regression assay was also performed as described by Rickinson et al. [11]. PBMCs were incubated with 2 mL of supernatant from B95-8 cells containing 10^4 transforming units of EBV per milliliter. Cultures were maintained by regular refeeding during a 6-week period, and they were observed throughout that period for the incidence of virus-induced transformation and subsequent regression. The regression was expressed in terms of the minimum initial cell number required per well for a 50% incidence of regression, as calculated by the Reed and Muench formula [11a].

The results of the transformation assay of samples from patients and controls are depicted in figure 2, A. In contrast to earlier reports in the English-language literature, PBMCs from patients with CFS did not show increased transformation compared with those of healthy controls (median, <1 per 10^6 cells for both patients and controls). However, high numbers of transforming PBMCs were found in the patient with SCAEBV infection (1 per 3.6×10^4 cells). The inherent problem that leads to SCAEBV is unknown, but the manifes-

Table 1. Characteristics of patients with CFS vs. those of healthy controls.

Characteristics	All patients (n = 88)	All controls (n = 76)	Selected patients (n = 10)	Selected controls (n = 9)	Patients with SCAEBV (n = 1)
Sex					
Male	23	17	3	2	0
Female	65	60	7	7	1
Mean age (y)	40	41	42	44	23
Median duration of symptoms (y)	7	...	6	...	4
No. of patients with indicated symptoms					
Acute onset of symptoms	58	...	9	...	1
Myalgia and muscle fatigue	80	3	10	0	1
Neuropsychological complaints	63	2	7	0	1
Tender lymph nodes	12	0	3	0	1
Geometric mean (median) titer for indicated antibodies					
EB-VCA IgG	39.5 (32)	38.0 (32)	97.2 (128)	32.3 (32)	1,024
EB-VCA IgM	<10	<10	<10	<10	<10
EB-EA IgG	10.3 (8)	7.4 (8)	22.8 (16)	7.1 (8)	256
EB-NA antibodies	32.1 (16)	40.2 (32)	34.8 (32)	18.3 (16)	32

NOTE. SCAEBV = severe chronic active EBV infection, EB = Epstein-Barr, VCA = viral capsid antigen, EA = early antigen, NA = nuclear antigen.