Simultaneous Measurement of Antibodies to Epstein-Barr Virus, Human Herpesvirus 6, Herpes Simplex Virus Types 1 and 2, and 14 Enteroviruses in Chronic Fatigue Syndrome: Is There Evidence of Activation of a Nonspecific Polyclonal Immune Response?

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As a test of the hypothesis that elevated titers of viral antibodies in patients with chronic fatigue syndrome (CFS) are due to a nonspecific polyclonal immune response, antibodies to Epstein-Barr virus (EBV), human herpesvirus 6 (HHV-6), and 14 enteroviruses in 20 patients with CFS and 20 age- and gender-matched controls were simultaneously measured. Similarly, titers of IgG to herpes simplex virus (HSV) types 1 and 2 were measured in 18 of these cases and in the respective controls. IgG to EBV viral capsid antigen (VCA) was present at titers ≥1:320 in 55% of cases vs. 15% of controls (P = .02). The geometric mean titers of early antigen antibody to EBV, HHV-6 IgG, and HSV-1 and HSV-2 IgG were not significantly different among cases and controls. Of the 14 enteroviral antibodies tested for, only those to coxsackieviruses B1 and B4 were present at significant titers (≥1:8) in cases vs. controls (P = .02 and P = .001, respectively). Of the cases, 19 (95%) had either an EBV VCA IgG titer ≥1:320 or a coxsackievirus B1 or B4 antibody titer ≥1:8, a percentage significantly higher than that of controls (40%; P = .0004). Titers of EBV VCA IgG and coxsackievirus B1 and B4 antibodies were simultaneously elevated in only 20% of cases. There was no correlation between elevated titers of EBV VCA IgG and IgG to HHV-6, HSV-1, and HSV-2 or antibody to coxsackieviruses B1 and B4 in the cases. The prevalence of reported allergies to medications or other substances was identical in both groups (60%). These findings suggest that in the majority of cases of CFS, elevation of viral antibody titers is not due to a nonspecific polyclonal immune response.

The chronic fatigue syndrome (CFS) is a disease of unclear origin. Some studies have found a correlation between high titers of antibody to Epstein-Barr virus (EBV) and this condition [1–4], while others have noted its association with antibodies to human herpesvirus 6 (HHV-6) [4], herpes simplex virus type 2 (HSV-2) [2], and enteroviruses [5–7]. Although the exact cause of elevated titers of viral antibodies in patients with CFS has not been determined, activation of a nonspecific polyclonal immune response has been postulated as a possible mechanism [2, 8]. To test this hypothesis, I simultaneously measured the titers of antibodies to 18 common viruses (EBV, HHV-6, HSV-1, HSV-2, and 14 enteroviruses) in patients with CFS and compared them with those of age- and gender-matched controls.

Materials and Methods

Patients. During the period 1989–1992, 51 patients were evaluated for chronic fatigue by the author, an infectious disease physician in private practice in suburban St. Louis. Of these, 19 (37%) met the diagnostic criteria for CFS as proposed by the Centers for Disease Control and Prevention [8] and underwent viral serological testing, performed primarily by the author (i.e., testing had not been previously performed by other physicians through other laboratories). One other patient with unexplained chronic fatigue met the criteria for CFS except that the fatigue reduced her daily activities to 75% (instead of ≤50%) of the baseline. This patient was also included in the study, bringing the total number of cases to 20.

Controls. Twenty controls were selected from among healthy individuals working at St. John's Mercy Medical Center (St. Louis) and their acquaintances and were matched with the cases by age (±5 years) and gender. All controls were personally interviewed by the author and specifically denied having chronic fatigue or any other significant symptoms; the results of physical examination were normal. In addition, reported allergies to medications or other substances were recorded.

Serologies. Serological evaluation for EBV was performed in all except three cases by the Clinical Virology Laboratory of the Children's Hospital in Philadelphia by immunofluorescence assay (IFA). Tests for IgM and IgG antibodies to HHV-6 were performed by the Microbiology Reference Laboratory (Cyprus, CA) with IFA. Titers of IgG antibody to HSV-1 and HSV-2 were measured by the St. John's Mercy Medical Center Immunology Laboratory by IFA. Coxsackievirus A (types 7, 9, 10, and 21), coxsackie-

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Informed consent was obtained from all volunteers. The study protocol was approved by the St. John's Mercy Medical Center Human Research Committee.

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virus B (types 1–6), and echovirus (types 4, 9, 11, and 16) serologies were performed by the Nichols Institute Reference Laboratories (San Juan Capistrano, CA) by complement fixation. All serum samples from controls were sent to the respective labs simultaneously. The sera from cases were usually obtained and tested by these laboratories at the time of each patient's initial visit. No attempt was made to independently confirm the reproducibility of test results of the various laboratories.

Statistical considerations. For calculation of the geometric means, antibody titers less than the threshold for detection were assigned a value of 1, and those reported as greater than or equal to the maximal titer tested were assigned the maximal titer. Statistical analyses included Welch's alternate *t*-test (two-tailed), Fisher's exact test, and Pearson correlation test and were performed with the use of GraphPad InStat software (San Diego, CA). A P value of $<.05$ was considered statistically significant.

Results

Profile of cases and controls. Twenty patients with CFS underwent serological testing for EBV, HHV-6, and 14 enteroviruses; all except two also underwent testing for HSV-1 and -2 IgG. The age range of the patients at the time of testing was 16–53 years, with a mean age of 36.2 years. Of the cases, 19 (95%) were White and one was Asian. Seventeen (85%) were female. The frequency of CFS signs and symptoms in the patients (according to CDC criteria) is shown in table 1. The median duration of symptoms before testing was 27 months (range, 5–240 months). All controls were White and were 18–51 years of age, with a mean age (36.9 years) similar to that of the cases. Although this characteristic was not specifically matched for, all cases and controls belonged to middle or upper socioeconomic classes and were thus representative of the population served by the investigator.

EBV serology. The distribution of EBV viral capsid antigen (VCA) antibody and early antigen (EA) antibody (restricted) in cases and controls is shown in figure 1. The geometric mean titer of EBV VCA IgG in cases was higher than that in controls (544 vs. 180), and this difference reached statistical significance ($P = .01$). The geometric mean titer of EA antibody (restricted) in cases was not significantly different than that in controls (56 vs. 23; $P = .15$). EA antibody (diffuse) was present at titers ≥1:10 in none of the cases and five (25%) of the controls; this difference was statistically significant ($P = .047$).

Of the cases, 11 (55%) had EBV VCA IgG titers ≥1:320, compared with three (15%) of the controls; this difference was statistically significant ($P = .02$). Similarly, nine (45%) of the cases had EBV VCA IgG titers ≥1:640, compared with one control (5%); this difference also was statistically significant ($P = .008$). The difference in the geometric mean titers of EBV EA antibody for the two groups was of marginal statistical significance (33.8 for cases vs. 48.6 for controls; $P = .08$). EBV nuclear antigen antibody was found at levels ≥1:40 in 12 (60%) of the cases and 16 (80%) of the controls; this difference was not statistically significant ($P = .30$).

Table 1. Profile of patients with CFS as defined by CDC criteria [8].

Criterion	No. (%) of patients ($n = 20$)
Persistent/relapsing fatigue	
Does not resolve with bed rest	20 (100)
Causes ≥50% reduction in daily activity	19 (95)*
Exclusion of other chronic conditions	20 (100)
Mild fever	16 (80)
Sore throat	14 (70)
Lymph node pain	14 (70)
Unexplained muscle weakness	15 (75)
Myalgia	17 (85)
Prolonged fatigue after exercise	16 (80)
New, generalized headaches	15 (75)
Migratory arthralgia	12 (60)
Photophobia	11 (55)
Transient visual scotomata	2 (10)
Forgetfulness	12 (60)
Excessive irritability	10 (50)
Confusion	8 (40)
Difficulty thinking	16 (80)
Inability to concentrate	19 (95)
Depression	11 (55)
Sleep disturbance	19 (95)
Initial onset acute or subacute	17 (85)
Physical criteria	
Low-grade fever	3 (15)
Nonexudative pharyngitis	3 (15)
Palpable or tender lymph nodes	9 (45)

* One patient reported a 25% reduction in daily activity.

HHV-6 serology. The distribution of HHV-6 IgG in cases and controls is shown in figure 2. All cases and controls had a detectable (≥1:10) titer of HHV-6 IgG (geometric means, 211 and 164, respectively); this difference was not statistically significant ($P = .54$). Of the cases, 12 (60%) had HHV-6 IgG titers ≥1:160; an identical number of controls had such titers. HHV-6 IgM antibody was undetectable (titer, <1:20) in all cases and controls.

HSV-1 and HSV-2 IgG. HSV-1 IgG was present (at titers ≥1:10) in 10 cases (56%) vs. eight controls (44%); this difference was not statistically significant ($P = .74$). Titers of HSV-1 IgG were elevated (≥1:320) in 10 (56%) of the cases vs. seven (39%) of the controls ($P = .51$). The geometric mean titers of HSV-1 IgG for the two groups were not significantly different (516 for cases vs. 334 for controls; $P = .27$). HSV-2 IgG was present (at titers ≥1:10) in 11 (61%) of the cases vs. six (33%) of the controls; this difference was not statistically significant ($P = .18$). HSV-2 IgG was present at titers ≥1:320

in seven cases (39%) vs. two controls (11%); this difference was not statistically significant (P = .12). The geometric mean titers of HSV-2 IgG for the two groups were not significantly different (316 for cases vs. 162 for controls; P = .28).

Enteroviral serology. A comparison of the titers of enteroviral antibody in cases and controls is shown in figure 3. Except for titers of antibody to coxsackieviruses B1 and B4, there was no statistically significant difference in the antibody titers among cases and controls. Serum antibodies to coxsackieviruses B1 and B4 were present at titers ≥1:8 in 15 (75%) and 9 (45%) of the cases vs. 7 (35%) and none of the controls, respectively (figure 4); these differences were statistically significant (P = .02 and P = .001, respectively).

Serum antibody to echovirus 16 was present at titers ≥1:8 in 12 (60%) of cases vs. five (25%) of controls; this difference was of marginal statistical significance (P = .054). There was no statistical significance in the frequency of higher (≥1:16) titers of specific enteroviral antibodies in cases vs. controls (data not shown).

Combination serology. The prevalence of significant serum levels of antibody to viruses statistically associated with the cases in this study is shown in table 2, in which data regarding individual viruses as well as combinations are detailed. Of the cases, 19 (95%) had an EBV VCA IgG titer of ≥1:320 *or* a positive (≥1:8) titer of antibody to coxsackievirus B1 or coxsackievirus B4. This rate was significantly higher than that among the controls (40%; P = .0004). Similarly, 17 (85%) of the cases had EBV VCA IgG at a titer ≥1:320 or a positive titer of coxsackievirus B4 antibody, whereas only three (15%) of controls had such antibody titers; this difference was highly significant (P < .0001). Antibodies to coxsackieviruses B1 and B4 *and* EBV VCA IgG were simultaneously present at indicated titers (EBV VCA IgG, ≥1:320, and coxsackieviruses B1 and B4, ≥1:8) in 20% of cases, a proportion not significantly different than that of controls. Serologies were positive for EBV VCA IgG (at titers ≥1:320) and antibodies to coxsackievirus B1 (at titers ≥1:8) in 40% of cases and 10% of controls (P = .03).

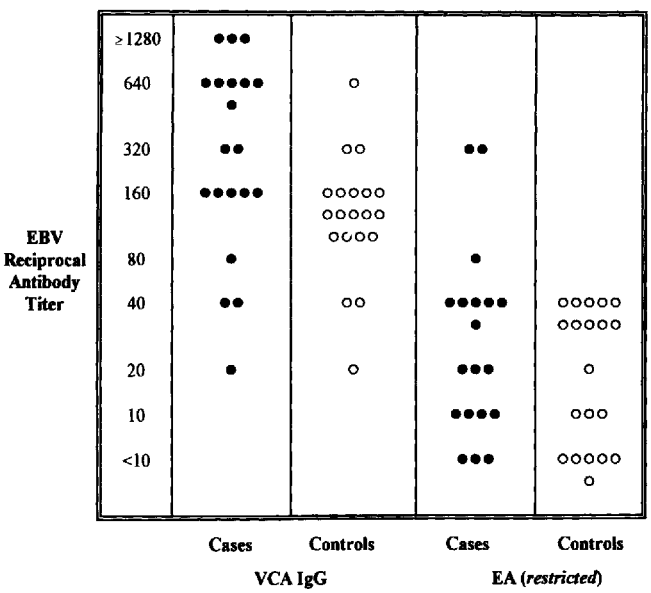

Figure 1. Serum levels of EBV VCA and EA antibody in 20 patients with CFS and 20 controls (n = 19 for titers of EA antibody in cases because one patient was inadvertently omitted).

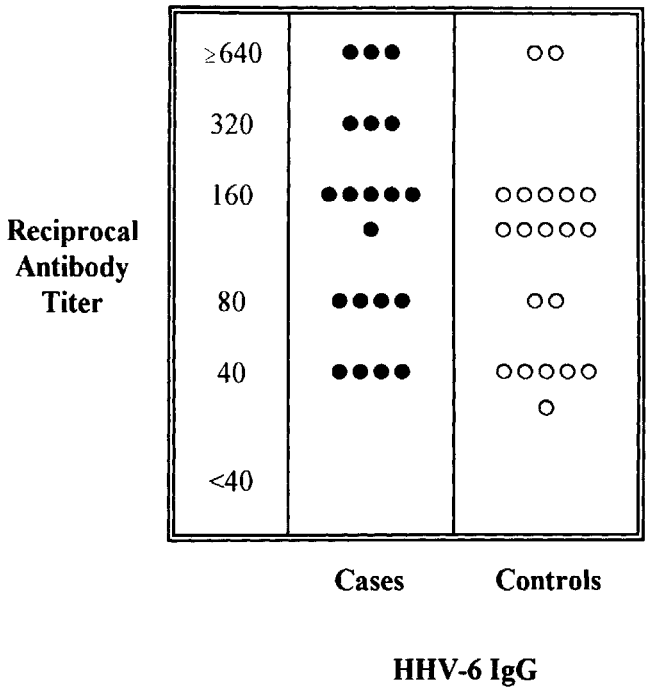

Figure 2. Comparison of titers of HHV-6 IgG antibody in patients with CFS and control subjects.

There was no correlation between EBV VCA IgG and coxsackievirus B1 or B4 antibody titers in the cases (r = −0.1 [P = .69] and r = −0.01 [P = .94], respectively). There was also no correlation between EBV VCA IgG and HHV-6 IgG titers (r = −0.2 [P = .36]) or HSV-1 or HSV-2 antibody titers in the cases (r = −0.08 [P = .75] and r = 0.02 [P = .94], respectively).

Reported allergies. Six (30%) of the cases vs. five (25%) of the controls reported a drug allergy (table 3); this difference was not statistically significant. There was also no significant difference between rates of allergies to substances other than drugs reported by cases and controls. The rate of allergies to drugs and other substances was identical in both groups (60%).

Discussion

As has been reported by other investigators [1, 2], EBV VCA IgG was present at higher titers in patients with CFS than in controls in this study. However, in contrast to the findings of previous studies [1–3], the geometric mean titer

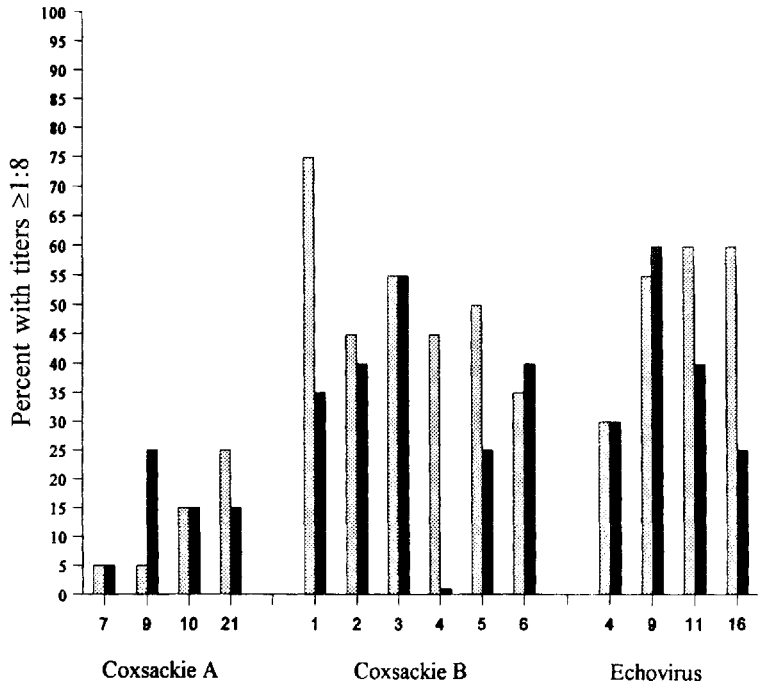

Figure 3. Frequency of significant (≥1:8) titers of antibody to various types of coxsackievirus (coxsackie) A, coxsackievirus B, and echoviruses in 20 patients with CFS (□) and 20 controls (■). ($P > .05$ for all comparisons of cases vs. controls, except as follows: coxsackievirus B1 antibodies, $P = .02$; coxsackievirus B4 antibodies, $P = .001$; and echovirus 16 antibodies, $P = .054$.)

of EBV EA antibody was not statistically higher in patients with CFS than in controls. In fact, EBV EA antibody (diffuse) was more likely to be present in the controls. Similarly, this study revealed no significant association between HHV-6 IgG levels and CFS, a finding supported by some investigators [2] but not others [4]. There was also no significant association between HSV-1 and HSV-2 antibody levels and CFS. It is of interest that of the 14 enteroviral antibodies tested for, only antibodies to coxsackieviruses B1 and B4 were significantly associated with CFS; such an association between these viruses and CFS has been previously suggested [7].

Although none of the antibodies for which serological tests were performed (EBV VCA IgG and coxsackievirus B1 and B4 antibodies) was present in >75% of cases at significantly elevated titers, 95% of patients with CFS (vs. 40% of controls) had a significantly elevated titer of antibody to at least one of these viruses. Similarly, 85% of patients with CFS (vs. 15% of controls) had elevated levels of antibody to EBV VCA or coxsackievirus B4. Simultaneously elevated levels of antibody to EBV VCA and coxsackieviruses B1 and B4 were found in no more than 20% of cases, however.

The relative infrequency of simultaneously high levels of antibody to the above viruses and the general lack of a positive correlation between various titers do not support the hypothesis that a nonspecific polyclonal immune response is the explanation for high levels of antibody to these viruses in many patients with CFS. Rather, the data from this study suggest that in the majority of patients, the titers of antibod-

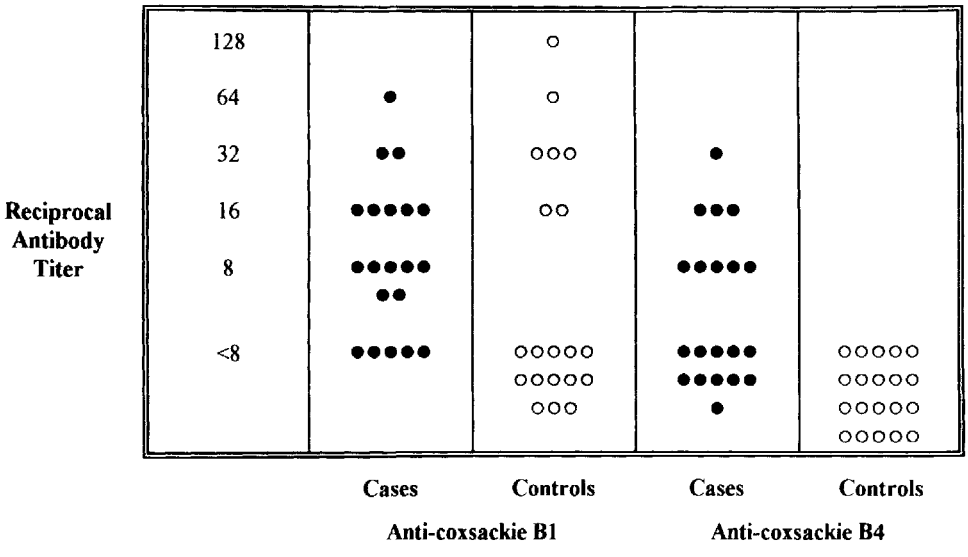

Figure 4. Comparison of titers of antibody to coxsackieviruses (anti-coxsackie) B1 and B4 in patients with CFS and control subjects.

Table 2. Comparison of significant titers of antibody to EBV VCA IgG (≥1:320) and coxsackieviruses B1 and B4 (≥1:8) in patients with CFS vs. controls.

	Cases		Controls		
Viral antibody	No. (%)	95% CI (%)	No. (%)	95% CI (%)	*P* value
CV B1	15/20 (75)	50–90	7/20 (35)	15–60	.02
CV B1 and B4	8/20 (40)	20–60	0/20 (...)	0–17	.03
CV B1 and EBV VCA IgG	8/20 (40)	20–60	2/20 (10)	1.2–32	.06*
CV B1 and B4 *and* EBV VCA IgG	4/20 (20)	6–40	0/20 (...)	0–17	.11†
CV B4	9/20 (45)	20–70	0/20 (...)	0–17	.001
CV B4 and EBV VCA IgG	4/20 (20)	6–40	0/20 (...)	0–17	.11†
EBV VCA IgG	11/20 (55)	30–80	3/20 (15)	3–38	.02
CV B1 or B4 *or* EBV VCA IgG	19/20 (95)	80–100	8/20 (40)	20–60	.0004
CV B1 or B4	16/20 (80)	60–90	7/20 (35)	15–60	.01
CV B1 or EBV VCA IgG	18/20 (90)	70–100	8/20 (40)	20–60	.002
CV B4 or EBV VCA IgG	17/20 (85)	62–97	3/20 (15)	3–38	<.0001

NOTE. CI = confidence interval; CV = coxsackievirus.
* Marginally significant.
† Not significant.

ies to only one or a few of the viruses associated with CFS are elevated. This finding is in contrast to that of another study [2], in which investigators noted that titers of antibodies to several non-EBV viruses such as cytomegalovirus, HSV-2, and measles viruses were higher in patients with CFS than in controls. However, the patients in that study had been ill for a shorter period (median, 4 months), and their characteristics might have been less homogeneous since the definition of CFS had not yet been standardized. Furthermore, the percentage of patients with *concurrently* elevated viral antibody titers was not reported in that study.

As has been noted in other investigations [9, 10], a high percentage (60%) of patients with CFS in this study reported an allergy to drugs or other substances. However, a similar percentage of the controls also reported such allergies, a circumstance which further emphasizes the importance of including a control population when performing studies involving patients with CFS.

Several limitations of this study deserve further discussion. First, even though sera from cases and controls were tested by the same laboratories with only a few exceptions, they were not tested concurrently, thus raising the possibility of day-to-day intralaboratory variations (which would account for at least some of the differences observed in the results between the two groups). However, this would not affect characterization of the simultaneous antibody response against multiple viruses in each group. Second, because of the large number of comparisons and analyses performed, the possibility of significant findings occurring by chance alone should be considered. Third, the small sample size of the study allowed the detection of only large differences in antibody titers between the cases and the controls. For example, with the study sample size of 40, the probability (power) of detecting a minimal difference of 15 between reciprocal geometric means of EBV EA antibody at an estimated standard deviation of 20 is no more than 80%. To improve the power of the study to 95% for detecting the same reciprocal geometric mean difference between the two groups while maintaining an alpha error of 0.05, a study sample size of 94 would have been necessary. It is noteworthy, however, that other investigators [11] have found significant differences in EBV EA antibody titers in patients with CFS and controls with use of a study sample similar in size to that in this study. Last, since this was not a longitudinal study, the antibody profiles described above may have changed because of fluctuations in antibody titers over time.

Table 3. Comparison of reported drug allergies and other allergies in CFS cases vs. healthy controls.

	Cases (n = 20)		Controls (n = 20)	
Allergy*	No. (%)	95% CI (%)	No. (%)	95% CI (%)
Drug(s)	6 (30)	12–54	5 (25)	9–49
Other(s)†	6 (30)	12–54	9 (45)	24–68
Drug(s) and other(s)	12 (60)	36–80	12 (60)	36–80

* *P* value (Fisher's exact test) was not significant (>.05) in any comparison between cases and controls.
† Includes food and inhalation allergies.

In conclusion, although this was a small study, the lack of simultaneously high levels of antibody to many common viruses does not support the theory that a polyclonal immune reaction is the cause of elevated titers of viral antibodies in patients with this condition. Further studies are needed to establish the cause of elevation of titers of viral antibodies to specific viruses in patients with CFS.

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