Seroepidemiology of Chronic Fatigue Syndrome: A Case-Control Study

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We performed serological testing for a large number of infectious agents in 26 patients from Atlanta who had chronic fatigue syndrome (CFS) and in 50 controls matched by age, race, and sex. We did not find any agent associated with CFS. In addition, we did not find elevated levels of antibody to any of a wide range of agents examined. In particular, we did not find elevated titers of antibody to any herpesvirus, nor did we find evidence of enteroviral exposure in this group of patients.

Chronic fatigue syndrome (CFS) is an illness of unknown etiology, characterized by debilitating fatigue lasting longer than 6 months and a variety of nonspecific symptoms, including myalgia, arthralgia, lymphadenopathy, low-grade fever, sleep disorders, and inability to concentrate [1]. An infectious etiology has been suggested for CFS, although the evidence is not compelling [2]. Many patients report the sudden onset of a flulike illness that presaged their fatiguing illness, and a number of infectious agents are known to cause postinfection fatigue. Reports that viral antibody titers are elevated in CFS cases has led to the speculation that latent viruses may be reactivated in this illness as a result of an underlying perturbation of immune function, and that elevated titers of antibody to common agents may be a reflection of this disturbance.

We conducted serological tests for a large number of infectious agents as part of a case-control study assessing risk factors for CFS. Our goals were (1) to determine whether we could detect a common exposure history among CFS patients who met a stringent research case definition and (2) to determine whether levels of antibody to any of the agents examined were elevated in CFS cases.

Materials and Methods

Case and Control Selection

Patients with CFS were recruited from the Atlanta component of the Centers for Disease Control and Prevention's (CDC's) surveillance study of CFS [3]. Cases of CFS in Atlanta who currently met the 1988 CFS research case definition [4] and who had been sick for $\leq$10 years were eligible for the study; 26 cases were recruited to participate in the study. We selected two controls for each case. They were matched for age ($\pm$5 years), race, and sex. Controls were selected by random-digit dialing in the five-county area of Atlanta covered by the surveillance system. All controls were screened to eliminate those with medical conditions that could bias the results (Reyes et al., unpublished data).

Specimen Collection

We collected blood and stool samples from each participant. Serum and stool specimens were stored at -70°C. Each laboratory was requested to perform serological testing of samples as a single batch to minimize interassay variation. If that was not possible, samples from a case and two matched controls were tested in the same batch. All samples were coded so that the tests were performed in a blinded fashion.

Laboratory Testing

Retroviruses. Serological testing for HIV-1 and HIV-2 was performed with use of a U.S. Food and Drug Administration (FDA)–licensed ELISA kit (Genetic Systems, Redmond, WA). Confirmatory testing was performed with an FDA-licensed western blot test (Cambridge Biotech, Rockville, MD). Antibodies against human T-lymphotrophic virus types I and II were detected with an ELISA, and confirmatory testing was performed by western blotting [5]. To detect the presence of a retrovirus in peripheral blood lymphocytes (PBLs) from cases, we cultured 2×10^6 PBLs with allogeneic phytohemagglutinin-stimulated PBLs for 4 weeks. Cultures were fed weekly and culture supernatants were screened weekly for reverse transcriptase activity [6].

Enteroviruses. Sera were screened for enterovirus-specific IgM with use of a monoclonal antibody–capture ELISA similar to that described previously [7]. To detect active infection, we prepared RNA from stool samples and performed a reverse transcriptase PCR to detect enteroviral sequences [8]. For four

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All study participants were volunteers who gave informed consent. Complete study protocols were approved by the Centers for Disease Control and Prevention Human Subjects Committees. Human experimentation guidelines of the U.S. Department of Health and Human Services were followed in the conduct of this study.

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cases and three controls, materials were insufficient for enteroviral RNA testing.

Arboviruses. Sera were screened with a hemagglutination-inhibition assay [9] against a panel of 10 arboviruses. Antibodies against Colorado tick fever virus and vesicular stomatitis virus were detected by means of a CF assay [10]. Titers of ≥1:10 were considered positive.

Herpesviruses. Titers of antibody to cytomegalovirus, human herpesvirus 6, and varicella zoster virus were determined by means of an ELISA against the whole virus. Optical density readings were converted into antibody units with use of known standards [11]. Cytomegalovirus and varicella zoster virus values were considered positive at ≥100 antibody units, and human herpesvirus 6 values were considered positive at ≥200 antibody units. Epstein-Barr virus (EBV) early antigen antibody titers were determined with a commercial ELISA (Gull Laboratories, Salt Lake City), and a titer of ≥1:10 was considered positive. Antibodies specific for EBV viral capsid antigen (VCA) and nuclear antigen were titrated by indirect immunofluorescence assay (IFA) [12]. A titer of ≥1:10 was considered positive. Type-specific herpes simplex virus serology was performed with a glycoprotein G–based western blot assay [13].

Respiratory viruses. Sera were tested for the presence of antibodies to adenovirus; parainfluenza viruses 1, 2, and 3; and respiratory syncytial virus. Testing was performed by ELISA with a 1:100 serum dilution [14]. Antibodies to coronavirus subtypes OC43 and SN229E were detected with a microneutralization assay [15].

Hepatitis viruses. Sera were screened for antibody to hepatitis B or hepatitis C by ELISA, with use of commercially available kits (Abbott Laboratories, Abbot Park, IL). ELISA-positive sera were subjected to confirmatory testing by western blotting (Abbott Laboratories).

Other viruses. Antibody to measles nucleoprotein [16], rubella (Rubestat G, Bio Whittaker, Walkersville, MD), and parvovirus B19 [17] was detected by ELISA. Measles neutralization titers were also determined [18].

Rickettsiaceae. Serological testing for antibodies to *Rickettsia typhi, Rickettsia rickettsii, Coxiella burnetii,* and *Ehrlichia chaffeensis* was performed with use of IFA against fixed whole organisms [19]. A titer of ≥1:32 was considered positive.

Bartonella (formerly Rochalimaea species). Sera were screened for antibodies to *Bartonella henselae, B. quintana,* and *B. elizabethae* by means of IFA with fixed whole organisms [20]. A titer of ≥1:32 was considered positive.

Borrelia burgdorferi. Sera were screened by ELISA for antibodies against flagellin. A positive result was confirmed by western blotting (Robbins et al., manuscript in preparation).

Candida albicans. Precipitating antibodies were detected by both latex agglutination and immunodiffusion. The result was considered positive if either the immunodiffusion assay was positive or the latex agglutination titer was ≥1:4. We also screened for the circulating *Candida* antigens mannan A and mannan B [21].

Chlamydia. Sera were screened by IFA at 1:32 dilution against *Chlamydia trachomatis,* serotype L2. This contains the lipopolysaccharide antigen specific for the genus *Chlamydia* and thus detects antibodies to all *Chlamydia* species [22].

Statistical Analysis

We analyzed our data using matched analysis procedures. We used a nonparametric test (Cochran-Mantel-Haenzel statistic [23] with modified ridit scores [24]) to assess differences in continuous variables between cases and controls. The logarithm in base 2 of the titers of antibody to EBV-VCA, EBV–early antigen, EBV–nuclear antigen, and coronavirus OC43 as well as the measles neutralization titer were used in the analysis, since they have a natural interpretation when titers are considered (each dilution represents 1 unit in the log base 2 scale).

In all statistical testing we carried out, a P value of ≤.05 was considered significant. Since the design of the study was exploratory and not hypothesis-testing, we did not correct for multiple comparisons [25]. Statistical analyses were performed with the statistical software *SAS* (SAS Institute, Cary, NC).

Results

Demographics. We recruited 26 patients with CFS (23 female and 3 male) and 52 matched controls. Two controls withdrew, leaving 2 cases with only 1 matched control. All participants were white (this finding is consistent with those of other studies). The median duration of illness for the cases was 5.3 years (range, 2.2–10.5 years), and the median age at onset of illness was 33.5 years (range, 16–49 years). Occupation, income, and education were comparable between cases and controls.

Agents infrequently detected in or absent from CFS cases. All 26 cases were seronegative for the following agents: hepatitis B and C; *R. typhi; R. rickettsii; B. henselae; B. burgdorferi;* the arboviruses St. Louis encephalitis virus, Powassan virus, Rio Bravo virus, yellow fever virus, eastern equine encephalitis virus, western equine encephalitis virus, Everglades virus, Cache Valley virus, Jamestown Canyon virus, LaCrosse virus, Colorado tick fever virus, and vesicular stomatitis virus; and the retroviruses HIV-1, HIV-2, and human T-lymphotrophic virus types I and II. In PBL cocultivation experiments designed to detect retroviral activity, no reverse transcriptase was detected at any of the four time points tested. Controls were also seronegative for these agents, with the exception of one control who had a 1:10 titer against St. Louis encephalitis virus.

We detected six (23.1%) or fewer CFS cases seropositive for a number of agents. These included *C. burnetii,* the causative agent for Q fever (3.9%); *E. chaffeensis* (3.9%); *Chlamydia* species (23.1%); herpes simplex virus type 2 (15.4%); *B. quintana* (3.9%); and *B. elizabethae* (19.4%). Only three (11.5%) of the cases had detectable circulating precipitating

Table 1. Comparison of antibody levels in CFS cases and controls.

Type of antibody detected	Cases (n = 26)	Controls (n = 50)	P value‡
Measles (nucleoprotein)*	.450 (96)	.440 (98)	.747
Respiratory syncytial virus*	.155 (92)	.150 (94)	.623
Parainfluenza virus 1*	.445 (100)	.415 (100)	.326
Parainfluenza virus 2*	.260 (100)	.290 (96)	.123
Parainfluenza virus 3*	.180 (100)	.190 (100)	.642
Adenovirus*	.400 (92)	.300 (78)	.112
Parvovirus B19 IgM*	.095 (92)	.090 (94)	.737
Parvovirus B19 IgG*	.930 (69)	.910 (74)	1.00
Cytomegalovirus†	1925 (62)	1185 (60)	.965
Human herpesvirus 6†	1460 (100)	1715 (100)	.808
Varicella zoster virus†	810 (100)	660 (100)	.330
Rubella†	2.46 (96)	2.34 (94)	.330
Herpes simplex virus 1	ND (50)	ND (50)	...

NOTE. Numbers in parentheses represent percentage positive for antibody; ND = not determined.

* Data expressed as median optical density.

† Data expressed as median antibody units.

‡ Cochran-Mantel-Haenzel statistic with modified ridit scores.

antibodies to *C. albicans,* a finding that may be indicative of systemic infection with *C. albicans,* and no case or control had detectable circulating mannan A or mannan B. Five cases (19.2%) and 11 controls (22%) had evidence of recent enteroviral infection, reflected by detectable enterovirus-specific IgM. Reverse transcriptase PCR analysis of stool specimens revealed the presence of enteroviral RNA in three cases (13.6%) and four controls (8.5%). There was no overlap between those who had enterovirus-specific IgM and those who had detectable viral RNA in their stool. There were no statistically significant differences between cases and controls for any of these agents.

Agents frequently detected in CFS cases. All other agents tested were detected in ≥25% of CFS cases, and antibody levels were compared between cases and controls. These agents included the herpesviruses cytomegalovirus, varicella zoster virus, human herpesvirus 6, EBV (early antigen, nuclear antigen, and VCA), and herpes simplex virus 1; the respiratory viruses adenovirus, respiratory syncytial virus, coronavirus, and parainfluenza viruses 1, 2, and 3; measles virus; parvovirus B19; and rubella (tables 1 and 2). The seropositivity rates in both cases and controls were ≥50%. There were no differences between the two groups in terms of seropositivity rates or virus-specific antibody levels, as measured by titer, by antibody units, or by optical density in an ELISA.

Discussion

We have screened a well-characterized group of patients with CFS for evidence of infection with a wide range of agents and have compared percentages of infection with age-, race-, and sex-matched controls. We found no evidence of a common infectious agent in the patients studied. There were no differences in prevalence of current enteroviral infection between cases and controls, as determined by levels of circulating enterovirus-specific IgM, nor in the frequency of detectable enterovirus in stool samples. Thus, in the group of patients from Atlanta, enteroviral infection was not more common in CFS cases. We were unable to detect evidence of infection with any known human retrovirus, and our inability to detect reverse transcriptase activity in cocultures suggests that there is no common, unknown retrovirus in these patients. This is consistent with other studies that have been unable to detect retroviruses in CFS [26, 27]. We found no evidence of infection with *B. burgdorferi,* the causative agent of Lyme disease. Infection with *B. burgdorferi* can lead to a CFS-like illness, and in areas where Lyme disease is endemic, it can contribute to CFS-related estimates [28]. In this group of patients from Atlanta, there was no evidence that Lyme disease is a factor in CFS.

Other agents that had <25% seroprevalence in these patients included arboviruses, hepatitis B and C, Rickettsiaceae, *Bartonella* species, *Chlamydia* species, and precipitating antibodies to *C. albicans.* We also looked for circulating *Candida* antigens, since these are frequently found in severely immunodeficient patients. None of our patients with CFS had detectable mannan A or mannan B, which suggests that these patients were not immunodeficient. The lack of precipitating antibodies to *C. albicans* argues against the occurrence of a chronic candidal infection in patients with CFS.

Herpesviruses—in particular, EBV and human herpesvirus 6—have been associated with CFS. Although elevated titers to EBV-VCA and EBV early antigen were initially described as occurring in patients with CFS [29, 30], subsequent studies made it clear that these were not specific to CFS [31–33]. In this study, we found no differences in titers to EBV-VCA or EBV nuclear antigen between cases and controls. However, the titer of early antigen was slightly elevated in the cases compared with that in controls, although the difference was not statistically significant.

In conclusion, we were unable to find evidence of a single infectious agent associated with CFS in this patient population, nor did we find evidence of elevated antibody titers consistent

Table 2. Comparison of antibody titers in CFS cases and controls.

Type of antibody detected*	Cases (n = 26)	Controls (n = 50)	P value†
EBV early antigen	57.5 (96)	35.2 (87)	.095
EBV nuclear antigen	26.4 (92)	21.1 (86)	.747
EBV viral capsid	89.0 (100)	83.6 (96)	.847
Measles (neutralization)	82.9 (92)	144.5 (98)	.101
Coronavirus OC43	75.8 (100)	64.5 (100)	.086

NOTE. Numbers in parentheses represent percentage positive for antibody; EBV = Epstein-Barr virus.

* Data expressed as reciprocal of geometric mean titer (log base 2).

† Cochran-Mantel-Haenzel statistic with modified ridit scores.

either with reactivation of a latent virus or with generalized immune activation.

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References

1. Fukuda K, Straus SE, Hickie I, et al. The chronic fatigue syndrome: a comprehensive approach to its definition and study. Ann Intern Med **1994**;121:953–9.
2. Mawle AC, Reyes M, Schmid DS. Is chronic fatigue syndrome an infectious disease? Infectious Agents and Disease **1994**;2:333–41.
3. Gunn WJ, Connell DB, Randall B. Epidemiology of chronic fatigue syndrome: the Centers for Disease Control Study. Ciba Foundation Symposium **1993**;173:83–93.
4. Holmes GP, Kaplan JE, Gantz NM, et al. Chronic fatigue syndrome: a working case definition. Ann Intern Med **1988**;108:387–9.
5. Brodine SK, Kaime EM, Roberts C, Turnickey RP, Lal RB. Simultaneous confirmation and differentiation of human T-lymphotropic virus types I and II infection by modified Western blot containing recombinant envelope glycoproteins. Transfusion **1993**;33:925–9.
6. Kitamuro K, Rudolph DL, Goldsmith C, Folks TM, Lal RB. Isolation, characterization, and transmission of human T-lymphotrophic virus types I and II in culture. Curr Microbiol **1993**;27:355–60.
7. Helfand RF, Khan AS, Pallansch MA, et al. Echovirus 30 infection and aseptic meningitis in parents of children attending a child care center. J Infect Dis **1994**;169:1133–7.
8. Gow JW, Behan WMH, Clements GB, Woodall C, Riding M, Behan PO. Enteroviral RNA sequences detected by polymerase chain reaction in muscle of patients with postviral fatigue syndrome. BMJ **1991**; 302:692–6.
9. Clarke DH, Casals J. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. Am J Trop Med Hyg **1958**;7:561–73.
10. Casey HL. Adaptation of LECF microtechnique in standardized diagnostic complement fixation method and adaptation to micro test. Public Health Monograph 74, Public Health Service Publication 1228, part 2. Washington, DC: U.S. Government Printing Office, 1965.
11. Holmberg SD, Stewart JA, Gerber AR, et al. Prior herpes simplex type 2 infection as a risk factor for HIV infection. JAMA **1988**;259:1048–50.
12. Henle G, Henle W. Immunofluorescence in cells derived from Burkitt's lymphoma. J Bacteriol **1966**;91:1248–56.
13. Sanchez-Martinez D, Schmid DS, Whittington W, et al. Evaluation of a test based on baculovirus-expressed glycoprotein G for detection of herpes simplex virus type–specific antibodies. J Infect Dis **1991**; 164:1196–9.
14. Blomberg J, Nilsson I, Andersson M. Viral antibody screening system that uses a standardized single dilution immunoglobulin G enzyme immunoassay with multiple antigens. J Clin Microbiol **1983**;17:1081–91.
15. Hierholzer JC, Halonen PE, Bingham PG, Coombs RA, Stone YO. Antigen detection in human respiratory coronavirus infections by monoclonal time resolved fluoroimmunoassay. Clinical and Diagnostic Virology **1994**;2:165–79.
16. Hummel KB, Erdman DD, Heath J, Bellini WJ. Baculovirus expression of the nucleoprotein gene of measles virus and utility of the recombinant protein in diagnostic enzyme immunoassays. J Clin Microbiol **1992**;30:2874–80.
17. Erdman DD, Usher MJ, Tsou C, et al. Human parvovirus B19–specific IgG, IgA, and IgM antibodies and DNA in serum specimens from patients with erythema infectiosum. J Med Virol **1991**;35:110–5.
18. Erdman DD, Anderson LJ, Adams DR, Stewart JA, Markowitz LE, Bellini WJ. Evaluation of monoclonal antibody–based capture enzyme immunoassays for detection of specific antibodies to measles virus. J Clin Microbiol **1991**;29:1466–71.
19. Newhouse VF, Shepard CC, Redus MD, Tzianabos T, McDade JE. A comparison of the complement fixation, indirect immunofluorescent antibody, and microagglutination tests for the serological diagnosis of rickettsial diseases. Am J Trop Med Hyg **1979**;28:387–95.
20. Regnery RL, Olson JG, Perkins BA, Bibb W. Serological response to *Rochalimaea henselae* antigen in suspected cat-scratch disease. Lancet **1992**;339:1443–5.
21. Kaufman L, Reiss E. Serodiagnosis of fungal diseases. In: Rose NR, de Macario EC, Fahey JL, Friedman H, Penn GM, eds. Manual of clinical laboratory immunology. 4th ed. Washington, DC: American Society for Microbiology **1992**:511–5.
22. Saikku P, Paavonen J. Single antigen fluorescence test for Chlamydia antibodies. J Clin Microbiol **1978**;8:119–22.
23. Landis RJ, Heyman ER, Koch GG. Average partial association in three-way contingency tables: a review and discussion of alternative tests. International Statistics Review **1978**;46:237–54.
24. van Elteren PH. On the combination of independent two-sample tests of Wilcoxon. Bulletin of the International Statistical Institute **1960**; 37:351–61.
25. Rothman KJ. No adjustments are needed for multiple comparisons. Epidemiology **1990**;1:43–6.
26. Gow JW, Simpson K, Schliephake A, et al. Search for retrovirus in the chronic fatigue syndrome. J Clin Pathol **1992**;45:1058–61.
27. Heneine W, Woods TC, Sinha SD, et al. Lack of evidence for infection with known human and animal retroviruses in patients with chronic fatigue syndrome. Clin Infect Dis **1994**;18(suppl 1):S121–5.
28. Wokke JHJ, de Koning J, Stanek G, Jennekens FGI. Chronic muscle weakness caused by *Borrelia burgdorferi* meningoradiculitis. Lancet **1988**;1:146–50.
29. Jones JJ, Ray CG, Minnich LL, Hicks MJ, Kibler R, Lucas DO. Evidence for active Epstein-Barr virus infection in patients with persistent unexplained illness: elevated anti-early antigen antibodies. Ann Intern Med **1985**;102:1–6.
30. Straus SE, Tosato G, Armstrong G, et al. Persisting illness and fatigue in adults with evidence of Epstein-Barr virus infection. Ann Intern Med **1985**;102:7–16.
31. Buchwald D, Sullivan JL, Komaroff AL. Frequency of chronic active Epstein-Barr virus infection. JAMA **1987**;257:2303–7.
32. Holmes GP, Kaplan JE, Stewart JA, Hunt B, Pinsky PF, Schonberger LB. A cluster of patients with a chronic mononucleosis-like syndrome: is Epstein-Barr virus the cause? JAMA **1987**;257:2297–302.
33. Matthews DA, Lane TJ, Manu P. Antibodies to Epstein-Barr virus in patients with chronic fatigue. South Med J **1991**;84:832–40.