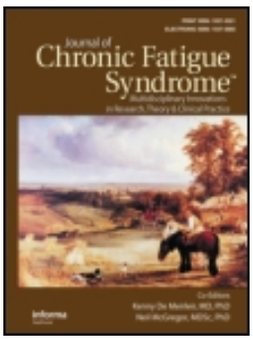

Journal of Chronic Fatigue Syndrome

ISSN: 1057-3321 (Print) 1547-0660 (Online) Journal homepage: http://www.tandfonline.com/loi/icfs20

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To cite this article: John W. Gow & Peter O. Behan (1996) Viruses and Chronic Fatigue Syndrome, Journal of Chronic Fatigue Syndrome, 2:1, 67-83, DOI: 10.1300/J092v02n01_06

To link to this article: http://dx.doi.org/10.1300/J092v02n01_06

Viruses and Chronic Fatigue Syndrome

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INTRODUCTION

Chronic fatigue syndrome (CFS) is a disorder in which the chief complaint is fatigue, made worse by exercise, together with myalgia and psychiatric and neurological symptoms. Night sweats, reversal of sleep pattern, atypical depression, and irritable bowel symptoms may also be present. The illness often follows an acute, viral-like illness and may occur sporadically or in epidemics (1,2). Routine clinical tests are normal. Involvement of the central nervous system is suggested by the neurological psychiatric symptoms and supported by recent evidence of hypothalamic dysfunction (3,4). Muscle may also be directly involved, as shown by prolonged jitter on single-fibre electromyography (5), abnormal metabolism on nuclear magnetic resonance testing (6), and, in a number of cases, atrophy of type-2 fibres and mitochondrial pleomorphism (7). Al-

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Journal of Chronic Fatigue Syndrome, Vol. 2(1) 1996

though clinical diagnostic criteria had been established as an aid to the differential diagnosis of CFS (8,9,10), more comprehensive guidelines have been published recently (11). These guidelines include recommendations for the clinical evaluation of fatigued persons, a revised case definition of CFS, and a strategy for subgrouping fatigued persons in formal investigations. Such guidelines are important since, although a large literature is available on fatigue syndromes prior to the Holmes (8) and Fukuda (11) definitions, the patient groups involved may not have met the strict modern criteria.

Several epidemics of fatigue disorders had been documented prior to the Holmes definition of CFS in 1988 (8), including those in Los Angeles (12), Akureyri (Iceland) in 1948 (13,14), the Royal Free Hospital in 1957 (1) and the Sick Children's Hospital, Great Ormond Street, London, in 1974 (15). Disorders which manifest themselves in epidemics suggest a shared exposure to a toxin or infectious agent, which has led clinicians and medical researchers to search for a common aetiological agent. Data which were collected at the time of the epidemics suggested that an infectious agent might have been involved with an incubation period of 8-10 days, followed by an acute febrile illness lasting for up to four weeks (1). After recovery from the initial illness, relapses followed which could persist for extended periods. Although no single causal agent has been isolated or identified, many viruses have been associated with the syndrome, including herpesviruses, retroviruses, influenza viruses, hepatitis-B, and the enteroviruses. The evidence for their involvement, which has been reviewed previously in two excellent articles (16,17), will now be considered.

HERPESVIRUSES

Epstein-Barr virus, cytomegalovirus, varicella zoster virus (1,7), and, more recently, herpesviruses 6 and 7 and herpes simplex virus (18) have all been associated with CFS.

Epstein-Barr Virus (EBV)

Several groups reported that an EBV infection in some patients may be followed by a chronic fatigue-like syndrome, the chronic

mononucleosis syndrome, which lasted for a year or more (19,20). Indeed, initial reports indicated that 20% of patients with CFS had significantly increased titres of EBV-specific early antigen (EA) antibodies, suggesting increased production of this virus (21). DNA hybridisation experiments using cloned EBV-specific constructs revealed the presence of EBV genomes in 9% (8 of 86) of muscle biopsies from patients with CFS (22). In the latter study, patients with EBV infection were negative for enteroviral sequences and those positive for enteroviral sequences were negative for EBV. A recent study by Natelson et al. (23) found that abnormal titres of EBV-DNA polymerase antibodies were found twice as often in patients with CFS as controls. Also, Carver et al. (24) have shown that 73% of U.S. Army Desert Storm reservists who met the study's diagnostic criteria for CFS were positive for either an acute or reactivated EBV infection. These data suggest that patients with CFS may have their symptoms increased and prolonged by secondary viral infections.

However, it has been suggested that EBV does not play a causative role in CFS (25). Swanink et al. (26) were unable to demonstrate a role for the reactivation of EBV, even in selected patients with high titres of antibody to viral capsid antigen (VCA) and early antigen (EA). Therefore, although reactivation of latent EBV appears possible in CFS, EBV serology is unlikely to be of diagnostic value (8,27).

Human Herpesvirus-6 (HHV-6)

Human herpesvirus 6 (HHV-6) has also been linked to CFS (28). In a large and carefully controlled study of 259 patients at outbreaks in Lake Tahoe and California/Nevada, Buchwald and co-workers (29) reported increased CD4/CD8 T-cell ratios and replication of HHV-6 in the peripheral blood lymphocytes of 70% of patients, as opposed to 20% of controls. The techniques used in this study included detection of virus by monoclonal antibodies to HHV-6 proteins and polymerase chain reaction (PCR) nucleic acid assays. These workers also reported that 78% of patients, compared to 21% of controls, had abnormal findings on magnetic resonance imaging studies of the central nervous system (CNS), suggesting there might be chronic, immunologically-mediated inflammation of the CNS.

One of the most common abnormalities in CFS patients is impairment of natural killer (NK) cell function (30,32). These cells, which are a non-T, non-B subset of lymphocytes, induce lysis of target cells such as neoplastic and virus-infected cells. Lusso et al. have recently shown that HHV-6 can directly target and kill NK cells and could thus suppress the anti-viral immunity of the host (33). However, no one has yet shown infection of NK cells in patients with CFS.

Although several other groups have described high HHV-6 seroprevalence or antibody titres of HHV-6 (34-38), the data are conflicting. Dale et al. (39) detected antibodies in 69% of patients with CFS compared with 12.5% of controls; however, Wakefield et al. (40) found no difference in seroprevalence between patients and controls.

Attempts have been made to further characterize the HHV-6 variant present in patients with CFS. Yalcin et al. (41), using PCR and hybridisation techniques, detected HHV-6 DNA in 7 of 13 CFS samples (53%) but in none of 13 healthy controls. Of those 7 patients, 4 were positive for HHV-6 variant A, and 3 were of variant B. The data from this study suggested that HHV-6 was actively replicating in patients with CFS. However, not all studies on the presence of HHV-6 have shown significant levels of active virus replication. In a PCR study carried out by Secchiero et al. (42) designed to detect viral DNA in serum or plasma as a marker of active infection, only 1 of 39 (2.6%, HHV-6 variant A) of CFS samples was positive compared with none of 37 healthy controls, 6 of 7 (85.7%) children with exanthem subitum, 3 of 13 (23.1%) bone marrow transplant recipients, and 4 of 18 (22.2%) HIV-infected patients.

Researchers have also reported on the incidence of human herpesvirus-7 (HHV-7) in patients with CFS. HHV-7 was isolated from the peripheral blood mononuclear cells of a patient with CFS by Berneman et al. (43). Subsequently, Secchiero et al. (44) isolated the virus from a patient with CFS and a healthy blood donor. Although a genetic polymorphism was detected between the two isolates, the significance of these findings with regard to CFS has yet to be fully investigated.

The above studies do, however, indicate that two human herpesviruses (EBV and HHV-6) may be actively replicating in some CFS patients. Whether this activation is an epiphenomenon, secondary to immune dysfunction or due to transactivation by another as yet

undetected virus, or whether the activation does contribute to the morbidity of the illness is not known. The evidence for a role for HHV-6 as an aetiologic or pathogenic cofactor is controversial. The correspondence by Reeves et al. (45), Alexander et al. (46), and Komaroff et al. (47) highlights the problems in this field.

RETROVIRUSES

Retroviruses are known to cause uncommon neurological disorders which have relapsing and remitting courses (48). DeFreitas and colleagues (49) attracted a great deal of interest when they published data to suggest that a human T lymphotropic virus type II (HTLV-II)-like retrovirus was present in the peripheral blood lymphocytes of patients with CFS and postulated a causal relationship. The idea of retroviral involvement in the syndrome is attractive because such a virus could disrupt the immune system and so allow other viruses to reactivate or persist. A retrovirus could also induce the chronic release of cytokines, which themselves have been shown to cause symptoms similar to those of CFS.

DeFreitas examined 30 patients with chronic fatigue and immune dysfunction syndrome (CFIDS) and 13 "exposure" and 10 "non-exposure" controls (exposure defined as having had sexual or casual contact with a patient). PCR amplification was carried out using primers designed to amplify three regions: HTLV-I *gag*, HTLV-II *gag* and HTLV-II *tax*. More than 75% of patient samples were positive for HTLV-II *gag*-like sequences compared to 34% of exposure controls and 0% of non-exposure controls. Western immunoblotting was used to screen sera for the presence of HTLV antibodies, and more than 50% of the patients demonstrated antibodies to at least two viral gene products. None of the non-exposure controls were positive in this test although over 30% of exposure controls were. *In situ* hybridisation was also used to demonstrate the expression of active HTLV-II-like *gag* genes.

Several laboratories have now tried to reproduce these findings. We examined DNA from blood samples of 30 patients with CFS and 30 controls together with skeletal muscle biopsies from 15 patients and 15 controls for the presence of retrovirus (50). The PCR primers used included two sets of HTLV-II *gag* primers (in-

cluding the sequences by DeFreitas), two *pol* primers which amplified both HTLV-I and HTLV-II, HTLV-II *env* primers and *tax* primers common for both HTLV-I and HTLV-II. This primer combination would be expected to amplify not only the specific viruses but also any related ones. Apart from endogenous sequences in both patients and controls, all PCR experiments were negative.

We also examined 20 serum samples from CFS patients and 20 controls for the presence of antibodies to foamy or spumavirus. Western immunoblotting experiments and indirect immunofluorescence (IFA) assays were negative for all patient and control samples.

Other laboratories in the USA (51,52,53,54) and Japan (55) have now reported negative findings similar to our own. It appears unlikely, therefore, that an HTLV-II-like retrovirus is present in CFS although Martin et al. (56) have reported the isolation of two 1.5 kb PCR fragments of a novel CMV-related virus using HTLV *tax* primers.

ENTEROVIRUSES

In the United Kingdom much of the research involved with detection of viruses implicated in the pathogenesis of CFS has focused on the enteroviruses. This was due to earlier reports which suggested that symptoms of fatigue during outbreaks were similar to a mild form of poliomyelitis. For example, after the epidemic in Akureyri, a widespread outbreak of poliomyelitis (type 1 virus) occurred in the rest of Iceland, but interestingly none of the patients who had the fatigue syndrome was affected. Serologic studies showed that the patients were immune to poliomyelitis, having already been exposed to a similar agent (13,14). In addition, an American airman at the US base in Reykjavik who developed poliomyelitis in the 1955 epidemic, after returning home to Pittsfield, Massachusetts, experienced a small outbreak of fatigue syndrome (57,58).

Enteroviral Persistence

Enteroviruses cause a variety of diseases in man, affecting nervous tissue, skeletal muscle, the heart, and possibly the pancreas. In most cases the infection is acute and self-limited, but occasionally it

becomes chronic as in culture-negative meningoencephalitis (59) and in the progression of myocarditis to congestive cardiomyopathy (60).

Several studies have shown that specific point mutations in the 5′ non-translated region of poliovirus affect neurovirulence (61-63); however, the mutations which predispose to the development of a chronic infection are still unknown.

There are significant differences between the effects of acute and chronic, or persistent, viral infection. There may be no evidence of cell damage, and no recognizable change in the vital functions of the cell. Nonetheless, there may be a major change in the differentiated, or luxury, function of the cell with the production of disease. For instance, neonatal mice with neuronal infection by lymphocytic choriomeningitis virus (LCMV) show decreased neuronal production of neurotransmitters. In neuroblastoma cells chronically infected with LCMV there is no cytopathic effect (cpe) and cloning efficiency is normal; however, there is a complete cessation of the normal production of acetylcholine (64).

The idea that a persistent viral infection can cause such defects in neurotransmitter production without evidence of cell damage is important. We have recently detected (post-mortem) enterovirus in the hypothalamus of a patient who suffered from CFS (65), and this finding may further implicate both virus and hypothalamic dysfunction in the pathogenesis of the disorder. The functional activity of hypothalamic 5-hydroxytryptamine receptors has been shown to be upregulated in CFS patients (3), and impairment of the hypothalamic-pituitary-adrenocortical axis has been reported (4).

Evidence for Enterovirus

Cases which occurred in the UK from 1980 to 1983 implicated Coxsackie virus because of the cardiac involvement in some cases (66). Studies revealed that 82% of patients, as opposed to 10% of control subjects, had increased titres of neutralising antibodies to Coxsackie B virus antibodies (67). In a further study, the structural protein antigen VP1 was found in more than 50% of patients, compared to 0% of controls. Four months later, 89% of the patient group were still positive (68). These early data are difficult to interpret, however, because they are based on IgG rather than IgM

antibodies. Later studies identified no difference in specific anticoxsackie antibody titres between patients and controls (69,70).

More recently, molecular hybridisation studies have suggested enteroviral involvement. The presence of enteroviral sequences in muscle from patients with CFS was demonstrated by Archard et al. (71). Twenty-four percent of patient skeletal muscle biopsies harboured enteroviral sequences, as compared to 0% of control biopsies. Moreover, it was postulated that the true figure might be higher, since virus may be missed due to the focal nature of persistent virus in muscle and to the small amount of biopsy tissue available (72).

Cunningham et al. (73) then reported 4 of 8 (50%) patients' muscle biopsies were positive for enteroviral sequences, and Bowles et al. (74) found 25 of 96 (26%) of patients' muscle samples positive, compared to 41 of 158 (25.9%) samples from patients with inflammatory myopathies.

Our initial study using more sensitive PCR technology involved 60 patients who had undergone extensive investigation to exclude other conditions, and 41 controls from the same catchment area, none of whom had any evidence of fatigue or neuromuscular disorders. Serological tests showed no difference between the patient and control groups (25% and 24.4% significant titres respectively). Of the 60 patients with CFS, 32 (53%) were positive, and of the controls, 6 of 41 (15%) were positive for enteroviral RNA sequences (70). PCR results from leukocyte samples from patients with CFS and controls showed that 16% of both patients and controls were positive for enteroviral sequences. These results suggested that enteroviral infection of muscle was present in a significantly high number of patients. Clements et al., also using PCR technology, suggested that enterovirus was present in 42% of CFS samples (75).

Our later study, however, has presented a different picture. A search for enteroviral nucleic acid sequences was carried out on 121 muscle biopsies from patients with CFS and 101 control samples from patients with other neuromuscular diseases. Thirty-two (26.4%) of the patient biopsies were positive on PCR assay, compared to 20 (19.8%) from the other group of patients, a difference which was not significant (76) although the figures correspond well with those of Bowles et al. (see above, 74). DNA sequence analysis of PCR products, however, did confirm that positive samples were not due

to contamination from the CBV3 positive control used and were several different enteroviruses. Also Swanink et al. (77) have shown no significant differences between 76 patient and 76 matched control samples by serological and PCR testing.

The results are in accord with the hypothesis that enterovirus may be found in various types of muscle damage (78) and strengthen the hypothesis that muscle is affected in patients with CFS. The results do not exclude a role for enterovirus in initiating the disorder, but they do suggest that the illness is not dependent on one specific serotype of enterovirus. More importantly, the pathogenesis of this syndrome does not seem to be dependent on persistent enteroviral infection.

Enterovirus Replication

In an acute, lytic infection by coxsackie viruses, a negative strand of viral RNA forms the template for the production of infectious virus and the ratio of negative to positive (genomic) strands is approximately 1:100. It has been suggested that in persistent infections the ratio may be dramatically altered to 1:1 (79). Klingel et al. (80) demonstrated that ongoing enterovirus-induced myocarditis was based on persistent infection and that the persistently infected tissue had similar amounts of plus and minus strand RNA. Virus persistence was associated with restricted viral RNA and capsid protein synthesis.

With regard to CFS, Cunningham et al. (81) demonstrated that RNA samples from muscle biopsies from patients with CFS contained similar amounts of plus and minus viral RNA strands. This is an important result since it would indicate that virus is indeed persistent and therefore may be responsible for the morbidity of CFS rather than being an epiphenomenon.

When we carried out a similar experiment on RNA from muscle samples of patients, the slot blot hybridisation results suggested that there was more plus strand genomic viral RNA present than negative strand template RNA. There are problems associated with slot blot analysis, including the small tissue/RNA samples. No size separation of viral RNA from genomic RNA occurs in the slot blot analysis, and also cross hybridisation of viral probes to host endogenous genes may take place. Thus, a PCR technique was devised to

determine the relative amounts of enteroviral RNA present in the tissue (82). Using this semi-nested PCR technique we found that in all the enterovirus positive cases examined there was a higher level of positive strand RNA present than negative strand RNA (83), suggesting that the replication of the virus in muscle was normal, at least at the level of transcription. The results were also in keeping with the idea that any virus present was not replication defective and therefore argued against persistence. Indeed, the term 'enteroviral persistence' should not be used in connection with CFS, as no one to date has demonstrated unequivocally the presence of enterovirus nucleic acid sequences in serial samples.

Viruses and Mitochondrial Damage

There are two schools of thought on the involvement of muscle in CFS: the first suggests that muscle function is normal and it is the perception of fatigue which is disturbed in the patients; the second argues that muscle function is directly affected. We have shown that mitochondrial morphology in muscle from some patients may be abnormal, with pleomorphism and proliferation of cristae (7,76,84). Recently, Kuratsune et al. (85) produced data to illustrate that patients with CFS had a deficiency of serum carnitine. Carnitine has an important role in energy production and modulation of intramitochondrial coenzyme A (CoA)/acyl-CoA ratio in skeletal muscle, so that a deficiency could directly affect skeletal muscle and therefore result in fatigue. It has also been shown that persistent viral infection can cause a specific stimulation of the mitochondrial gene, encoding for subunit 1 of cytochrome oxidase (86). Thus it may be possible to link the presence of virus to the fatigue.

CONCLUSIONS

It is clear that although an infectious agent is a prime candidate in the aetiology of CFS, as yet no specific agent has been identified. Despite numerous attempts at viral culture (and success in isolated cases), no conclusive data have been obtained.

It may be that no one virus is the aetiological agent in this disor-

der. It is possible that any of a variety of viruses can trigger an abnormal immune response; e.g., Gauntt et al. (87), have demonstrated molecular mimicry whereby anti-$CBV3_m$ neutralizing monoclonal antibodies can participate in a sustained chronic disorder in the absence of continued virus replication. Also, Hepatitis-B vaccination (rather than a viral infection per se) has been associated with CFS (88,89). In a comprehensive study on 375 patients with CFS, Hilgers and Frank (90) suggested that CFS is associated with, or the beginning of, manifest autoimmune disease. However, when Manian (91) measured titres of antibodies to EBV, HHV-6, HSV-I, HSV-II, and coxsackieviruses B1 and B4, the data suggested that in the majority of cases of CFS, elevation of antibody titres is not due to a nonspecific polyclonal immune response.

Other agents have been postulated to play a role in CFS, including organophosphate compounds, heavy metals, and neurotoxins. Organophosphates and heavy metals are stored in adipose tissue, from which their slow release may give rise to CFS-like symptoms (92). It has been shown also that acute viral infection can alter the tissue distribution of these agents as well as other toxins (93) and may therefore trigger CFS-like symptoms.

Viruses can also affect cell-membrane ion channels. It has been postulated that chronic fatigue syndrome may be caused by viral injury to muscle cell ion channels (94). HIV-I gp120 viral coat proteins can alter calcium flux through voltage-dependent calcium ion channels (95). Picornavirus VP1 and HIV TM contain sequences which mimic insect charybdotoxin and induce changes in sodium and potassium monovalent cation channels (96). Data from preliminary experiments suggested that there may be increased expression of the sarcoplasmic reticulum Ca^{2+} ATPase RNA and the dihydropyridine receptor RNA in skeletal muscle of patients with CFS compared with healthy controls (97).

The question of whether active virus is the aetiological agent in the disorder or whether reactivation of latent or persistent virus is a secondary event has to be considered. If reactivation of latent virus is a secondary event, does that virus then contribute to the ongoing pathogenesis of the chronic disorder?

A picture is slowly emerging to indicate that the host immune system is subtly disrupted with abnormal NK cell function and

increases in CD8+ cells. This may allow the reactivation of common latent viruses, such as EBV and HHV-6, and also inhibit the immune system from clearing endemic viruses, such as the enteroviruses, with normal efficiency. It is also conceivable that cytokine activation, produced by an overactive immune response to a persistent virus or virus which has been eliminated, gives rise to the symptoms.

As more and more clinical and scientific studies are being carried out on CFS, we can look forward to a clearer understanding of the role of virus in the aetiology and pathogenesis of this intriguing disorder.

ACKNOWLEDGMENTS

The authors are grateful to the Barclay Trust, the McFeat Bequest, and the Institute of Neurological Sciences Trust for their support in the course of our research into chronic fatigue syndrome. The authors thank K. Simpson for help in preparing the manuscript.

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