Searching for Serum Antibodies to Neuronal Proteins in Patients With Myalgic Encephalopathy/Chronic Fatigue Syndrome

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ABSTRACT

Purpose: A role for the immune system in causing myalgic encephalopathy/chronic fatigue syndrome (ME/CFS) is long suspected, but few studies have looked for specific autoantibodies that might contribute to the symptoms. Our aim was to look for evidence of antibodies to neuronal proteins in patients with ME/CSF.

Methods: Sera samples from 50 patients and 50 healthy individuals were sent coded to the Neuroimmunology Laboratory in Oxford. Screening for antibody binding to neuronal tissue was performed on brain tissue and neuronal cultures. Specific serum antibodies were assessed by antigen-specific cell-based assays and radioimmunoassays. After antibody testing, the associations between seropositive status and clinical data were investigated.

Findings: Overall, 8 patients and 11 participants were found to have some serum immunoreactivity toward neuronal or neuromuscular junction proteins, but only 1 patient and 2 participants had specific serum antibodies. Nevertheless, seropositive status in patients with ME was associated with shorter duration since onset and a more severe disease.

Implications: The results indicate no overall increased frequency of antibodies to neuronal proteins in ME/CSF and no evidence of a specific antibody that might be causative or contribute to clinical features in patients. However, the association of seropositive status with shorter duration of disease and more severe symptoms suggests a possible role of antibodies at onset in some patients and should be the focus of future studies. (*Clin Ther.* 2019;41:836–847)

Key words: antibodies, chronic fatigue syndrome, LRP4, myalgic encephalopathy, neuronal surface antigens, NMDA receptor.

INTRODUCTION

Patients with myalgic encephalopathy/chronic fatigue syndrome (ME/CFS) often complain of headache, pain in joints or skin, dizziness, clumsiness, difficulties in short-time memory and concentration ability, as well as the well-known symptoms of postexertional malaise, extreme fatigue, sleep disturbances, muscle pain, and weakness. A variety of studies have suggested an immune basis for ME/CFS, including a response to intravenous immunoglobulins,[1,2] or the initial trials that used the anti-CD20 monoclonal antibody rituximab.[3,4] Unfortunately, the latter finding was not confirmed in a randomized Phase III trial.[5]

Nevertheless, over the past 2 decades there has been increased appreciation of the role of antibodies toward both the neuromuscular junction (NMJ; acetylcholine receptor [AChR], muscle specific kinase [MuSK], and leucine-rich protein 4 [LRP4]) and central nervous system (CNS) antigens such as the N-methyl-D-aspartate receptor (NMDAR), leucine-rich glioma inactivated 1 (LGI1), and contactin-associated protein-like 2 (CASPR2), in the pathogenesis of autoimmune forms of muscle dysfunction and encephalopathy, respectively.[6,7] In particular, antibodies to neuronal proteins have been described in an increasing

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proportion of patients with neuropsychiatric disorders.[8] These observations raise the possibility that a proportion of patients with ME/CFS may have one or more of these specific antibodies and could therefore respond to immunotherapies.

Here, we studied the sera from 50 patients and 50 participants. We started by using 2 generic approaches that are widely accepted and used in identifying the presence of neuronal antibodies: immunohistology on rodent brain sections and immunofluorescence on cultured fetal rodent neurons.[9–12] The sera were also tested for specific CNS and NMJ antibodies by assays in routine diagnostic use.

METHODS

Clinical Data

The sera were taken at the time that the patients were recruited for the recent randomized and placebo-controlled Phase III trial of rituximab (NCT02229942). Clinical features were based on the self-reported scores of different symptoms. The control sera were obtained with informed consent from 50 healthy individuals matched to sex and age. The sera were coded, and unblinding was done after acquisition of all data. The project was approved by the Regional Committees for Medical and Health Research Ethics in Norway (2014/365).

Immunohistology

The methods are as previously described by us and others.[9–12] Sprague Dawley rats were sacrificed with carbon dioxide, and their brains were removed, cut sagittally, and immediately immersed for 1 h in 4% paraformaldehyde (PFA). After fixation, brains were cryoprotected in 40% sucrose for 48 h, embedded in optimum citing temperature compound (Tissue-Tek) and snap-frozen in dry ice-cooled isopentane. Frozen brains were cut sagittally on a cryostat (12 μm; ThermoScientific Cryotome FSE). Brain sections were allowed to dry at room temperature and rinsed in cold tris-buffered saline (TBS) solution, and endogenous peroxidase was blocked with a solution of TBS-0.3% H_2O_2 for 15 min at room temperature. Slides were then washed with TBS (3 × 5 min) and blocked in normal goat serum (10% in TBS for 1 h) before incubation with patients' serum (dilution 1:100 in 3% normal goat serum−TBS) overnight at 4 °C. The day after slides were washed with TBS and incubated with secondary antibodies (Biotinylated Goat Anti-Human Immunoglobulin G [IgG] Antibody; Vector Laboratories, Peterborough, UK; dilution 1:1000 in TBS) for 2 h. After washing, a further incubation was performed with the avidin-biotin complex (dilution 1:100 in TBS, 1 hour; Vectastain Elite ABC Kit Standard). Sections were further washed with TBS, and the reaction was developed with the use of brown 3,3′-diaminobenzidine (ImmPACT DAB Peroxidase Substrate; Vector Laboratories) as per the manufacturer's instructions. Slides were left to dry in the fume hood before proceeding with dehydration procedure by immersion in progressive concentration of ethanol solutions, followed by p-Xylene.[12] Slides were mounted with DPX mountant for histology (Sigma−Aldrich, St. Louis, Mo). Sections were viewed with a light microscope (Nikon Eclipse E400; Nikon, Melville, NY), and images were taken with the Aperio ScanScope (Leica Biosystems, Wetzlar, Germany).

Hippocampal Neuronal Cultures

Hippocampal neuronal cultures were performed as previously described.[11,13] After 12 days in culture, live neurons were incubated with patients' sera (dilution 1:100) in neurobasal (NBS) medium supplemented with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (4.6 mg/mL) and 1% bovine serum albumin (BSA). Coverslips were then washed 3 times (NBS-HEPES) and fixed with PFA 3% in phosphate-buffered saline (10 min at room temperature). After 3 additional washes with phosphate-buffered saline, neurons were incubated with Alexa Fluor Goat anti-human IgG H&L 488 secondary antibody (dilution 1:1000 in complete NBS-1% BSA) before washing and mounting with 4′,6-diamidino-2-phenylindole (DAPI). Coverslips were visualized with a fluorescence microscope (Leica DM 2500; Leica Biosystems), and images were acquired with a confocal microscope (Zeiss LSM 710 TM; Zeiss, Welwyn Garden City, UK).

Binding of patients' IgG to neurons was scored subjectively on a scale of 0−4 (0 = none, 1 = weak but definitive, 2 = moderate, 3 = strong, 4 = very

strong). Positive sera were always repeated and checked for antibody specificity. The scores shown are the mean values.

Radioimmunoprecipitation Assays for AChR and MuSK Antibodies

Radioimmunoprecipitation assays were used to measure antibodies against AChR and MuSK, as used routinely.[14] Patients' sera were diluted 1:10 in 0.02M phosphate buffer-0.1% Triton X-100 (PTX) to a total volume of 50 μL and incubated with 50 μL of ^{125}I labelled AChR or MuSK overnight at 4 °C in Eppendorf 1.5-mL tubes. Sheep anti-human IgG (50 μL) was then added diluted 1:5 in PTX and incubated at room temperature until precipitation was visible (approximately 60 min). After addition of 500 μL of PTX to dilute the sample, the tubes were immediately centrifuged for 5 min at 13,000 *g* at room temperature. The supernatant was aspirated, and the pellets were washed twice with 500 μL of PTX each time. The radioactivity in the pellet was measured with a Wallac Wizard counter.

Cell-based Assays for Specific Antibodies

Samples clearly positive on both neurons and rodent brain tissue were screened for LGI1, CASPR2, glycine receptor, dipeptidyl-peptidase-like protein 6 DPPX, IgLON5, gamma-aminobutyric acid type A receptor, gamma-aminobutyric acid type B receptor, metabotropic glutamate receptor 5, and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor. LRP4 and NMDAR were selected as possible antigens for the screening of the whole cohort with the use of human embryonic kidney 293T cells after transfection with the plasmid of interest.[11,15]

Live cells were incubated with patients' serum (dilution 1:20) in Dulbecco's Modified Eagle Medium supplemented with HEPES and 1% BSA for 1 hour at room temperature. Coverslips were then washed and fixed in 4% PFA. After a further 3 washes, they were incubated with secondary antibodies (dilution 1:1000; Alexa Fluor 568 anti-human IgG H&L chain rises in goat for 1 hour at room temperature), then washed and mounted onto glass microscope slides (VWR) with DAPI. Because these secondaries can bind IgM as well as IgG, to further confirm the positivity and the relevance of the observed staining, confirming the presence of IgG antibodies, secondary antibodies against the human IgG Fc fragment were used.[15] In positive cases, serum dilutions were performed, when possible, to assess the antibody titer.

Antibody binding to the expressed antigen was observed with a fluorescence microscope (Leica DM 2500; Leica Biosystems). A subjective visual scoring system was adopted to assess the presence and the intensity of the antibody binding to the cells of each coverslip (adapted from Ricken et al[10]). The scoring system was the following: 0 = no labelling; 1 = weak, considered as a low positive; 2 = moderate; 3 = moderate-strong; 4 = strong.[15,16]

RESULTS

Immunohistology on Brain Sections and Binding to Hippocampal Neurons

The 100 samples tested were 50 baseline serum samples before intervention from patients with ME/CFS who were participating in the RituxME trial and 50 healthy control samples. The identity of the samples was unknown during testing and reporting of the results. Figure 1A shows the typical staining of an NMDAR antibody-positive serum, from a patient with autoimmune encephalitis, with selective binding to the hippocampal region (detailed in insert). A healthy control serum binds only weakly to the hippocampus or cerebellum (Figure 1B and details at higher magnification). By contrast, 2 examples of ME/CFS sera show binding to the hippocampus (Figure 1C) and to the cerebellar molecular layer (Figure 1D). Although there was some nuclear staining detected with both sera, the binding to the molecular layers found in both Figures 1C and 1D is typical of antibody binding to neuronal surface proteins.

Binding to live cultured neurons was a good test of the potential pathogenicity of the antibodies, because it detected only those antibodies that could bind in vivo, but was only performed on 60 samples, including the 11 positive samples by immunohistochemistry (IHC). Two sera, both positive by IHC, bound clearly in a typical punctuate pattern (Figure 2), as shown in many previous studies of cell-surface antibodies[9,10] and supporting the specificity of the IHC binding shown above. Two other sera bound less strongly on IHC and to neurons, but 11 bound on IHC or to neurons but not both.

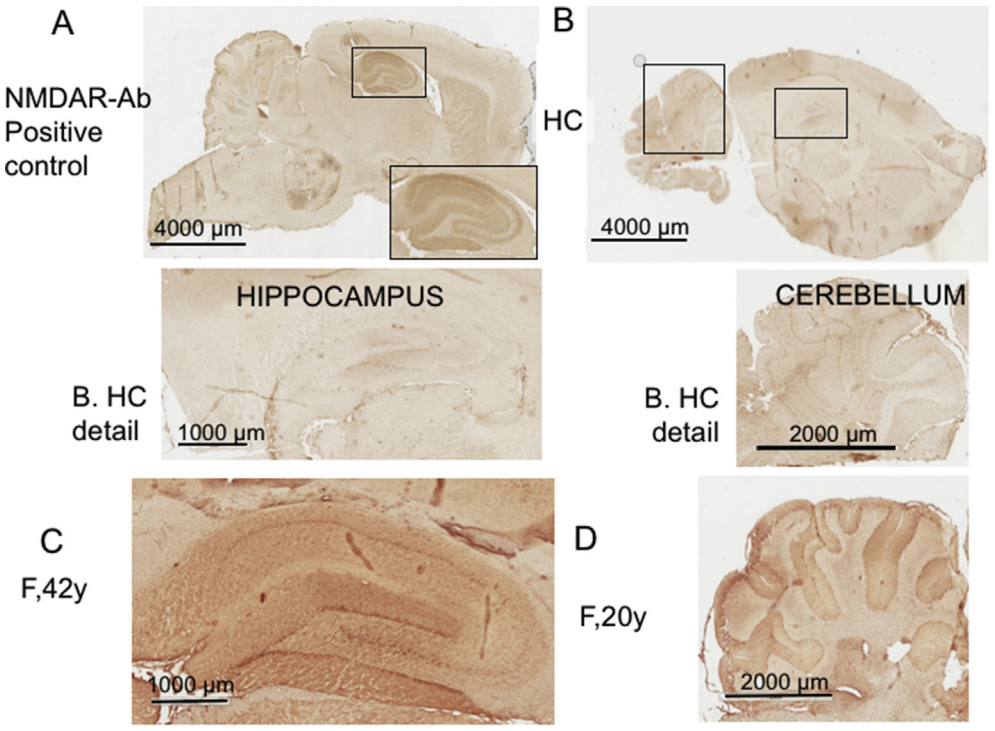

Figure 1. (Top panel) Examples of a positive and a negative serum control staining performed with the avidin-biotin peroxidase technique (brown color). (A) An N-methyl-D-aspartate receptor (NMDAR) antibody-positive serum indicated selective binding to the hippocampus, displayed at a higher magnification in the bottom right panel. (B) A healthy serum control (HC) showed no binding in the same area. (C) Details from the hippocampus and the cerebellum (see black squares) from the same HC showing no clear binding. (D) A serum sample from a patient with myalgic encephalopathy/chronic fatigue syndrome (ME/CFS) showed hippocampal binding, mainly to the dentate gyrus; another ME/CFS serum showing mainly binding to the molecular layer and Purkinje cells of the cerebellum. Ab = antibody, F = female.

Binding of Serum CNS Antibodies by Cell-based Assays

Human embryonic kidney cells transfected with the NR1 and NR2b subunits of the NMDA receptor were used to test for these antibodies. Only 1 patient had any reactivity, and this serum was not positive in any of the other tests. The 2 sera that had bound strongly to hippocampal neurons and on immunohistology were tested for all available CNS antigens (Table I), but neither serum was positive.

Binding of Serum Antibodies to NMJ Antigens

Finally, all sera were negative by radioimmunoprecipitation assays for AChR and MuSK antibodies. However, LRP4 antibodies, tested by a live cell-based assay, were positive in 2 sera. These sera had not been positive in any of the other tests.

Results of All Antibody Tests

After acquisition of the antibody results, the samples were decoded. The heat-maps in Figure 3 show the results of the 50 patients and 50 participants, and Table I summarizes the sex and ages of the participants, and the number of positive results for each test. Clearly, no appreciable differences were found between patients with ME/CFS and participants, and no evidence for an antibody specific

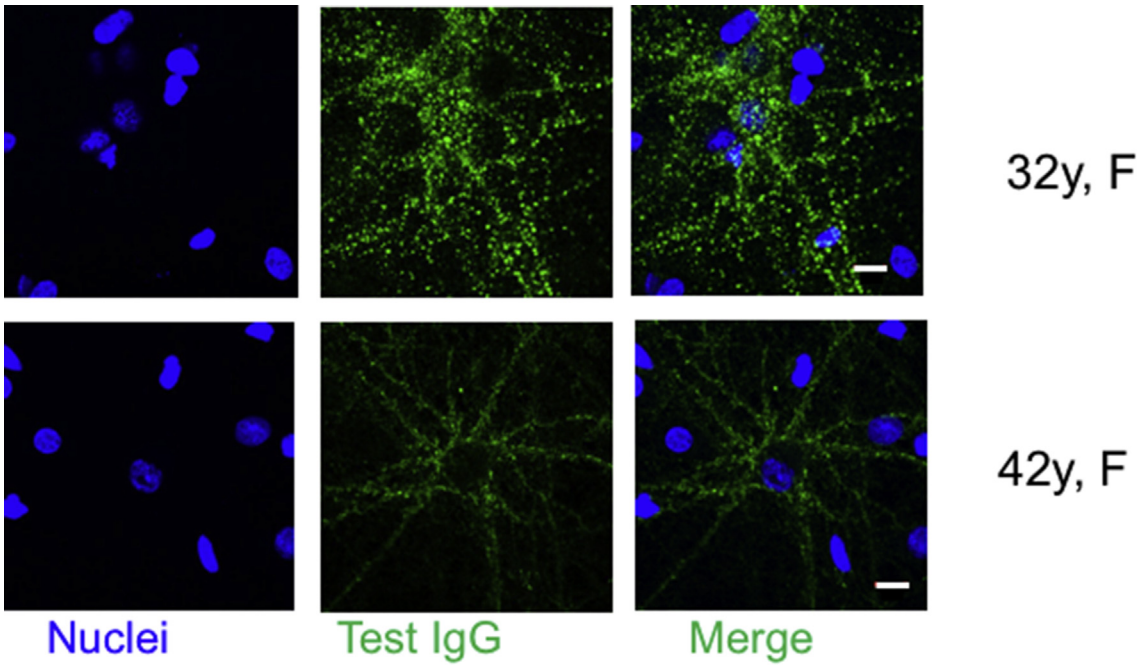

Figure 2. Microphotographs of serum binding to cultured live hippocampal neurons. Serum antibodies (green) bind to the surface of the hippocampal neuropil in both a participant (top) and a patient with myalgic encephalopathy (ME) (bottom). Magnification × 60; scale bar, 10 μm. F = female; IgG = immunoglobulin G.

to patients with ME/CFS; indeed more positive results were found in the participants (n = 11) than in the patients (n = 8).

Clinical Data of Individual Patients With ME/CFS

Despite these disappointing results it was of interest to see if there were any distinguishing features in those patients who did have evidence of some neuroimmunoreactivity. Figure 4 shows the averaged self-reported scores for fatigue, pain, cognitive, sleep, and autonomic problems. Table II summarizes the more severe (grade ≥4 on a self-reported scale from 0 to 10) clinical features of the 50 patients with ME and their antibody test results at the time of serum sampling. Positivity for CNS immunoreactivity did not clearly relate to specific clinical features, preceding infectious cause, or treatment responses. Overall, there was, however, shorter duration since onset in the 8 patients with ME/CFS (mean [SD]: 3.5 [1.6] years) with evidence of neuronal antibodies compared with patients without antibodies (5.8 [2.4] years; n = 42; $P = 0.013$, t test), and patients with antibodies having more severe disease (3 of 8) compared with patients without antibodies (3 of 42; $P = 0.044$, Fisher's exact test). In addition, 1 of the only 2 patients without cognitive involvement had LRP4 antibodies, which are associated with NMJ dysfunction.

DISCUSSION

The cause of ME/CFS is unclear, although it is likely to be a heterogeneous disorder that covers different causes, pathologic processes, and is often accompanied by neuropsychiatric features. One aspect that has not been so systematically investigated is the possibility of antibodies to neuronal proteins. Here, we report the results of sera from a large cohort of patients with ME/CFS tested systematically for the presence of antibodies to brain tissue and neuromuscular antigens with the use of well-established methodological approaches.[10] The results were not, overall, different from participants,

Table I. Summary of results in patients with ME/CFS and participants.

Variable	Age, mean (SD), years		Positive for binding on immunohistochemistry (n = 100)	Positive for binding to live neurons (n = 60)	Positive NMDAR Ab (n = 100)	Positive LRP4 Ab (n = 100)
	Women (n = 80)	Men (n = 20)				
Patients with ME/CFS	38 (11)	37 (14)	5	3/40	0	1
Healthy participants	32 (5)	40 (10)	6	5/20	1	1

Two sera binding on both immunohistochemistry and live neurons were routinely tested for antibodies to known central nervous system antigens (NMDAR, leucine-rich glioma inactivated 1, contactin-associated protein-like 2, glycine receptor, dipeptidyl aminopeptidase-like protein 6, IgLON5, gamma-aminobutyric acid type A receptor, gamma-aminobutyric acid type B receptor, metabotropic glutamate receptor 5, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) but were negative. All sera were negative for neuromuscular junction antigens (acetylcholine receptor and muscle specific kinase) except the two (1 patient, 1 participant) with LRP4 Abs.
Ab = antibody; LRP4 = leucine-rich protein 4; ME/CFS = myalgic encephalopathy/chronic fatigue syndrome; NMDAR = N-methyl-D-aspartate receptor.

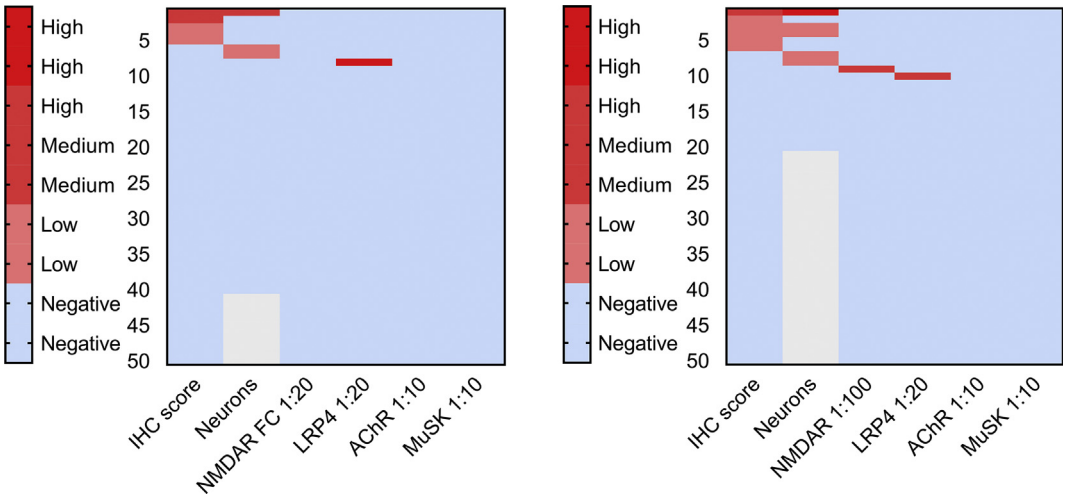

Figure 3. Heatmaps of the 50 myalgic encephalopathy/chronic fatigue syndrome (ME/CFS) sera and the 50 healthy control (HC) sera samples, illustrating the variability of positive results (mostly low to moderate binding binding) seen on immunohistology, live neurons, or cell-based assays for specific antibodies (N-methyl-D-aspartate receptor [NMDAR], leucine-rich protein 4 [LRP4]), and the large number of negative serum results for all antigens. AChR = acetylcholine receptor; IHC = immunohistochemistry; MuSK = muscle specific kinase.

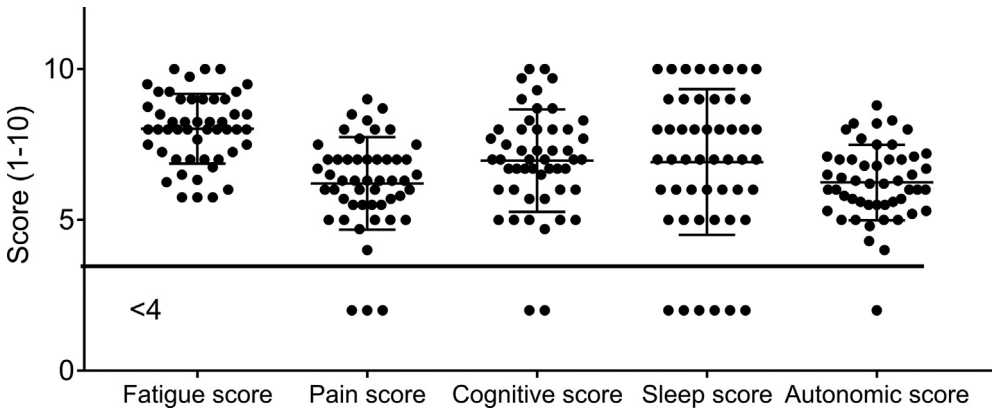

Figure 4. Self-reported scores of the patients with myalgic encephalopathy/chronic fatigue syndrome showing the wide range of neurologic features.

and there was no strong evidence of a relationship between disease phenotype and the existence of antibody reactivity, although associations were found between positive antibodies and shorter duration of disease or disease severity.

Several previous studies in patients with ME/CFS found the presence of autoantibody reactivity directed against intracellular[17–19] or membrane[19,20] components and a variety of neurotransmitters and neurotransmitter receptors.[19–21] Antibodies to the muscarinic M1 AChR were associated with feeling of muscle weakness.[21] These receptors are mainly expressed in the CNS, although there is evidence for modulatory effects on neuromuscular transmission. Therefore, we investigated the presence of antibodies against the nicotinic AChR, expressed at the NMJ, and against other proteins expressed at the same site and associated with muscle fatigue. No patients or participants had antibodies against nicotinic AChR or MuSK, whereas both a patient and a participant were positive for antibodies to LRP4, which are found in a small proportion of patients with myasthenia gravis, sometimes negative for AChR and MuSK antibodies, as they were here. The LRP4 antibody-positive patient was indeed the only one of 2 with no evidence of CNS involvement, consistent with a peripheral basis for their weakness and fatigue. In other respects, the clinical data found no obvious relationship between the serum immunoreactivity and the clinical features, the preceding infections, or the responses to rituximab. Overall, the negative results of the present study are consistent with the lack of beneficial results from the randomized Phase III trial that evaluated rituximab versus placebo for patients with ME/CFS.[5]

NMDAR antibodies are commonly associated with a form of autoimmune encephalitis, frequent in young children and adults.[22] Some of the features found in patients with severe ME/CFS, such as autonomic and movement disorders, could represent the presence of a previous autoimmune disorder that, by the time of testing, had left little vestige of the original disorder. It is known that some patients with this condition have spontaneous remissions with falling levels of antibodies. However, the only positive NMDAR antibody in the present study was found in an apparently healthy individual.

Two patients had antibodies that bound strongly to the neuropil of the hippocampus on immunohistology and also to neurons in culture. This is now a classic manner to show the potential pathogenicity of antibodies to new, undefined, synaptic proteins.[9,10] The full identification of the antigen requires immunoprecipitation from the cultures by the patients' serum IgG antibodies and may in the future provide evidence of a specific CNS antigen in this condition. However, 1 of the 2 strong positive sera was from a healthy individual. Nevertheless, it is clear that the existence of a serum CNS antibody by itself may not be sufficient to cause disease, which

Table II. Predominant self-reported clinical features and antibody results in 50 patients with myalgic encephalopathy/chronic fatigue syndrome.

Sex	Age, y	Severity	Duration, y	Fatigue score*	Pain score*	Cognitive score*	Sleep score*	Autonomic score*	ICH/live neurons†	NMDAR Ab‡	LRP4 Ab‡	Positive antibody summary
M	41	Severe	5	9.00	7.0	5.0	8	6.3	1/0	0	0	ICH only
F	42	Severe	2	9.50	7.0	6.7	10	7.0	1/3	0	0	ICH and neurons
F	41	Moderate	5	7.50	6.3	6.7	7	5.7	1/0	0	0	ICH only
F	20	M-M	2	8.00	5.5	5.0	7	5.8	1/0	0	0	ICH only
F	46	M-M	2	5.75	4.0	6.0	<4	6.2	2/0	0	0	ICH only
F	46	Severe	5	9.00	6.3	7.7	9	8.3	0/1	0	0	Neurons only
F	42	M-S	5	8.50	<4	7.3	7	4.3	0/1	0	0	Neurons only
M	31	Moderate	2	5.75	7.0	<4	6	5.7	0/0	0	4	LRP4 Ab only
M	38	Mild	10	8.00	5.5	8.3	8	5.6	0/0	0	0	None
F	48	Mild	5	8.00	5.0	6.0	<4	5.0	0/0	0	0	None
F	29	Mild	5	7.25	6.0	5.0	5	7.0	0/ND	0	0	None
F	55	M-M	5	7.25	6.0	6.5	7	6.0	0/0	0	0	None
F	51	M-M	5	8.25	6.0	8.0	9	6.0	0/0	0	0	None
F	56	M-M	5	8.00	7.0	8.0	8	7.1	0/0	0	0	None
F	30	M-M	2	8.25	6.3	6.7	6	4.8	0/0	0	0	None
F	47	M-M	5	6.25	<4	5.0	5	5.2	0/0	0	0	None
F	33	M-M	10	6.00	5.0	5.0	6	5.0	0/0	0	0	None
F	43	M-M	10	8.50	<4	7.7	7	5.5	0/0	0	0	None
F	45	M-M	5	7.00	5.8	5.7	5	5.5	0/0	0	0	None
M	23	M-M	2	7.00	7.0	<4	<4	6.0	0/0	0	0	None
M	65	M-M	5	6.33	7.0	8.0	<4	<4	0/0	0	0	None
F	35	M-M	5	7.00	6.3	6.0	<4	4.0	0/0	0	0	None
M	39	M-M	10	7.00	6.3	7.0	5	6.3	0/0	0	0	None
F	39	M-M	5	6.75	5.0	7.0	10	8.0	0/0	0	0	None
F	31	M-M	5	8.25	5.5	6.7	10	6.8	0/ND	0	0	None
F	37	Moderate	5	10.00	5.0	6.7	10	5.3	0/ND	0	0	None
M	19	Moderate	5	6.50	5.7	7.5	9	5.0	0/ND	0	0	None
M	38	Moderate	5	8.00	6.5	4.7	7	5.0	0/ND	0	0	None
F	28	Moderate	10	7.67	5.5	7.0	6	8.0	0/ND	0	0	None
F	51	Moderate	5	7.50	8.0	8.7	8	6.0	0/0	0	0	None

(*continued on next page*)

Table II. (*Continued*)

Sex	Age, y	Severity	Duration, y	Fatigue score*	Pain score*	Cognitive score*	Sleep score*	Autonomic score*	ICH/live neurons†	NMDAR Ab‡	LRP4 Ab‡	Positive antibody summary
F	58	Moderate	10	8.00	8.0	7.0	10	8.2	0/0	0	0	None
F	46	Moderate	5	8.00	7.5	8.3	9	7.2	0/0	0	0	None
F	34	Moderate	5	8.00	9.0	6.0	5	7.5	0/0	0	0	None
F	57	Moderate	5	5.75	5.0	5.7	7	5.6	0/0	0	0	None
F	36	Moderate	5	8.00	6.0	7.0	6	7.0	0/0	0	0	None
F	22	Moderate	5	9.00	7.0	9.0	8	7.1	0/0	0	0	None
F	42	Moderate	2	8.00	6.7	6.7	5	5.3	0/0	0	0	None
F	30	M-S	10	8.50	7.0	8.0	8	6.5	0/0	0	0	None
M	21	M-S	5	8.75	6.3	7.3	6	7.7	0/0	0	0	None
F	38	M-S	10	8.25	5.7	7.0	10	6.2	0/0	0	0	None
M	53	M-S	5	10.00	8.7	10.0	9	8.2	0/0	0	0	None
F	47	M-S	5	9.25	8.3	9.7	8	7.5	0/0	0	0	None
F	20	M-S	5	9.75	7.5	9.7	<4	6.4	0/0	0	0	None
F	45	M-S	10	8.25	6.0	7.3	10	6.8	0/ND	0	0	None
F	21	M-S	5	9.25	7.7	10.0	10	8.8	0/ND	0	0	None
F	21	M-S	5	9.00	4.7	8.7	7	5.5	0/ND	0	0	None
F	21	M-S	5	9.00	8.0	8.0	9	6.7	0/ND	0	0	None
M	27	Severe	5	9.50	8.5	6.7	8	6.5	0/0	0	0	None
F	34	Severe	2	10.00	7.0	9.3	8	7.0	0/0	0	0	None
F	22	Severe	5	9.25	6.5	7.3	6	6.0	0/0	0	0	None

The clinical scores were based on the average scores of different features self-reported (scale from 1 to 10) by the patients. Only values of 4 or more were included in the mean values for different symptoms that are reported here.

Ab = antibody; F = female; ICH = immunohistochemistry; LRP4 = leucine-rich protein 4; M = male; M-M = mild to moderate; M-S = moderate to severe; ND = note done; NDMDAR = N-methyl-D-aspartate receptor.

* 1 = yes; 0 = no.

† Scores of binding on scale 0 to 3.

‡ Scores of binding on scale 0 to 4.

may require break down of the blood–brain barrier or specific recruitment of the antibody-secreting B cells into the CNS to be pathogenic.

Another possibility is a role for circulating cytokines that could influence CNS function. Peripheral inflammation is associated with the increase of blood proinflammatory cytokines that, through different mechanisms, can reach the brain and trigger a sickness behavior that share several of the ME/CSF symptoms. Cytokines activate microglia, the resident macrophages of the brain, and astrocytes, triggering their production of chemokines and cytokines and therefore a local inflammatory response, potentially resulting in neuronal damage and blood-brain barrier disruption. In addition, mast cells, found in the area postrema, the choroid plexus, and the parenchyma of the thalamic hypothalamic region,[23] are an important source of inflammatory mediators[24,25] such as interleukin-6, interleukin-1β, tumor necrosis factor-α, and histamine[26] and can cause glia activation. The role of mast cells could be relevant in ME/CFS, as suggested by the clinical overlap between ME/CFS and mast cell activation disorder. Despite the lack of strong evidence for an increase of these mediators in these patients,[27] cytokine levels might correlate with symptoms severity,[28] and infections and depression[29] can also trigger stress and inflammation,[30,31] which have also been implicated in ME/CSF.[25,32]

Several other mechanisms have been implicated in the pathogenesis of ME/CFS, including dysfunction of the neuroendocrine system. Fatigue has been suggested to be the consequence of abnormalities in energy metabolic processes.[33] Patients with ME/CSF have also been reported to have a higher risk of metabolic syndrome and diabetes,[34] and, as such, fatigue associates with diabetes.[35,36] Diabetes can also cause impairment of cognition, neurotransmission, and synaptic plasticity, a condition known as diabetic encephalopathy.[37] Moreover, metabolic dysfunctions are linked to increased oxidative stress, exerting a proinflammatory activity.

CONCLUSIONS

Some evidence was found for antibodies binding to neuronal antigens but none that was specific for ME/CFS, and in general the binding of the antibodies, although above our threshold for positivity, was not strong and did not reach this threshold when sera were diluted. The presence of similar antibodies in patients and participants could reflect that low levels of antibodies, as found here, require other factors to be causative. Further studies are needed to explore more fully the potential pathogenic role of any serum antibodies that are identified in the patients.

CONFLICTS OF INTEREST

Dr. Giannoccaro has nothing to declare; Dr. Cossins discloses nothing. Ms. Sørland reports grants from The Kavli Trust and The Norwegian Regional Health Trusts, during the conduct of the study. Haukeland University Hospital has a patent on B-cell depletion therapy in chronic fatigue syndrome pending, in which Dr. Fluge is mentioned as an inventor. Prof. Vincent reports grants from GlaxoSmithKline outside the submitted work; in addition, Oxford University and Prof. Vincent have a patent for muscle specific kinase antibodies for diagnosis of myasthenia with royalties paid from Athena Diagnostics, and a patent for leucine-rich glioma inactivated 1 and contactin-associated protein-like 2 antibodies for autoimmune encephalitis with royalties paid from Euroimmun AG. The authors have indicated that they have no other conflicts of interest regarding the content of this article.

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Dr. Giannoccaro was responsible for investigation, analysis, and interpretation of data and for drafting and revising the work; Dr. Cossins was responsible for investigation and revising the work; Ms. Sørland and Dr. Fluge were responsible for providing serum samples from patients and participants and for clinical data collection; Prof. Vincent was responsible for conceptualization, analysis, and interpretation of data and for drafting and revising the work. All authors provided critical revisions of the publication for intellectual content and approved the final version for submission.

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