Neuroendocrinology Letters Vol. 35 No. 6 2014
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

Evidence for the existence of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) with and without abdominal discomfort (irritable bowel) syndrome

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Submitted: 2014-09-12 Accepted: 2014-09-28 Published online: 2014-11-29

Key words: **chronic fatigue syndrome; inflammation; gut; depression; bacterial translocation; cytokines; oxidative stress; irritable bowel syndrome**

Neuroendocrinol Lett 2014; **35**(6):445–453 **PMID:** 25433843 NEL350614A01

Abstract

BACKGROUND: There is evidence that Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is accompanied by gastro-intestinal symptoms; and IgA and IgM responses directed against lipopolysaccharides (LPS) of commensal bacteria, indicating bacterial translocation.

METHODS: This study was carried out to examine gastro-intestinal symptoms in subjects with ME/CFS versus those with chronic fatigue (CF). The two groups were dissected by dichotomizing those fulfilling and not fulfilling Fukuda's critera. In these groups, we examined the association between gastro-intestinal symptoms and the IgA and IgM responses directed against commensal bacteria.

RESULTS: Using cluster analysis performed on gastro-intestinal symptoms we delineated that the cluster analysis-generated diagnosis of abdominal discomfort syndrome (ADS) was significantly higher in subjects with ME/CFS (59.6%) than in those with CF (17.7%). The diagnosis of ADS was strongly associated with the diagnosis of irritable bowel syndrome (IBS). There is evidence that ME/CFS consists of two subgroups, i.e. ME/CFS with and without ADS. Factor analysis showed four factors, i.e. 1) inflammation-hyperalgesia; 2) fatigue-malaise; 3) gastro-intestinal symptoms/ADS; and 4) neurocognitive symptoms. The IgA and IgM responses to LPS of commensal bacteria were significantly higher in ME/CFS patients with ADS than in those without ADS.

CONCLUSIONS: The findings show that ADS is a characteristic of a subset of patients with ME/CFS and that increased bacterial translocation (leaky gut) is associated with ADS symptoms. This study has defined a pathway phenotype, i.e bacterial translocation, that is related to ME/CFS and ADS/IBS and that may drive systemic inflammatory processes.

ORIGINAL ARTICLE

INTRODUCTION

There is evidence that Myalgic Encephalomyelitis / Chronic Fatigue Syndrome (ME/CFS) is accompanied by an immune-inflammatory response and increased oxidative and nitrosative stress (O&NS) (Maes 2009; Maes & Twisk 2010; Morris & Maes 2013; 2014). Chronic, low-grade inflammation is indicated by increased levels of pro-inflammatory cytokines, such as interleukin (IL-1)α, IL-1β, IL-6, IL-8 and tumor necrosis factor (TNF)α (Maes 2009; Maes & Twisk 2010; Fletcher *et al.* 2009; Lorusso *et al.* 2009; Brenu *et al.* 2011; Maes *et al.* 2012a; Morris & Maes 2013); and increased levels of acute phase proteins, such as C-reactive protein and the α2-protein fraction obtained during protein electrophoresis (Maes *et al.* 2005; Raison *et al.* 2009; Spence *et al.* 2008). Immune activation is indicated by elevated serum neopterin, indices of interferon (IFN)-induced pathways; and altered expression of activation markers (Fletcher *et al.* 2009; Lorusso *et al.* 2009; Maes *et al.* 2012a; Chao *et al.* 1990; Bellman-Weiler *et al.* 2008; Zhang *et al.* 2010; Morris & Maes 2013). The above pathways in ME/CFS are partially mediated by activated cellular signalling networks, including activation of nuclear factor-κB (NF-κB) (Maes *et al.* 2007b; 2007c).

In ME/CFS, signs of O&NS are associated with the severity of fatigue and post-exertional malaise (Vecchiet *et al.* 2003; Kennedy *et al.* 2005; Morris & Maes 2013; 2014). O&NS processes are indicated by elevated levels of reactive oxygen species, damage to lipids, proteins and DNA and increased IgM-mediated (auto)immune responses to various oxidative specific epitopes (OSEs) and nitrosylated (NO) proteins (Maes *et al.* 2006; 2007e; Morris *et al.* 2014), suggesting that these O&NS damaged epitopes have became immunogenic (Amara *et al.* 1994; Bodet *et al.* 2004; Morris *et al.* 2014).

Recently, it has been shown that individuals with ME/CFS as compared with those with chronic fatigue (CF), i.e. patients not fulfilling with ME/CFS criteria, and controls, have increased IgA and IgM responses to lipopolysaccharides (LPS) of gram negative gut bacteria (Maes *et al.* 2007b; 2007c). In those patients, the increased IgA/IgM responses to LPS are significantly associated with increased plasma IL-1, TNFα, and serum neopterin levels and increased autoimmune responses to serotonin (Maes *et al.* 2012b; 2013). Patients with ME/CFS also show specific alterations of intestinal microbiota (Fremont *et al.* 2013) and treatment with probiotics significantly decreases anxiety symptoms in those patients, suggesting that the gut-brain interface is mediated by "microbes that reside or pass through the intestinal tract" (Rao *et al.* 2009). In an animal model of ME/CFS (the forced swim test) "per-oral administration of Lactobacillus acidophilus (LAB) and LAB loaded alginate beads" significantly attenuates fatigue-like behaviors (immobility and post-swim fatigue time) is association with inflammation (TNFα levels) and O&NS in the brain (Singh *et al.* 2012).

All in all, the results suggest that increased bacterial translocation following leaky gut (increased gut permeability) and gut dysbiosis may play a role in the immune pathophysiology of ME/CFS (Maes *et al.* 2007a; 2007d; Rao *et al.* 2009; Lakhan & Kirchgessner 2010; Fremont *et al.* 2013). Normally, immunocytes are not primed against the LPS of gram-negative commensals, because the tight junction barrier geographically and functionally separates the immune system from the gut bacteria. When the tight junction barrier is hyperpermeable, however, gut bacteria may translocate into the lamina propria and the mesenteric lymph nodes (MLNs) (Berg & Garlington 1979; Wiest & Garcia-Tsao 2005). Consequently, the immunocytes are activated by LPS through binding with the Toll-like receptor-4 (TLR4) complex (Wiest & Garcia-Tsao 2005), which in turn, activates cellular signalling networks, including NF-κB, mediating pro-inflammatory and O&NS gene expression (Tsukamoto *et al.* 2010). Disruption of the tight junction barrier is accompanied by increased serum LPS, and inflammatory and O&NS responses, processes that are reversible following treatment of gut permeability (Zhou *et al.* 2003; Quan *et al.* 2004; Schietroma *et al.* 2006; Forsyth *et al.* 2009).

There is a strong comorbidity between ME/CFS and irritable bowel syndrome (IBS) (Simsek 2011; Sperber & Dekel 2010; Hamilton *et al.* 2009; Riedl *et al.* 2008; Wojczynski *et al.* 2007; Schur *et al.* 2007; Moss-Morris *et al.* 2006; Whitehead *et al.* 2002). Low-grade mucosal inflammation, alterations in intestinal and colonic microflora, including small intestinal bacterial overgrowth (SIBO), are potential pathogenic mechanisms in IBS (Simsek 2011; Ford & Talley 2011). Previously, we reported that in ME/CFS there is an association between increased IgA/IgM responses directed against LPS and gastro-intestinal symptoms (Maes *et al.* 2007d) as measured by item 10 of the Fibromyalgia and Chronic Fatigue Rating Scale (Zachrisson *et al.* 2002).

Based on the abovementioned findings, it may be hypothesized that both ME/CFS and the gastro-intestinal symptoms in ME/CFS are associated with increased bacterial translocation. The aims of the present study were therefore to examine the prevalence of gastro-intestinal symptoms in participants with ME/CFS and CF and their association with increased IgM/IgA responses to commensal bacteria.

SUBJECTS AND METHODS

Subjects

The participants were 34 outpatients with CF and 94 patients with ME/CFS admitted to the Maes Clinics (Belgium). We used the Centres for Disease Control and Prevention (CDC) criteria to make the diagnosis of ME/CFS (Fukuda *et al.* 1994). Individuals who presented with symptoms of chronic fatigue for more than 6 months but did not fulfil the CDC diagnostic criteria were diagnosed as chronic fatigue (CF). The diagnosis

of IBS was based upon the Rome II Diagnostic Criteria for Functional Gastro-intestinal Disorders (Thompson *et al.* 2000). We excluded patients with a life-time and actual diagnosis of other psychiatric axis-1 disorders (except somatization disorders). Axis-1 disorders were diagnosed with the DSM-IV-R and included bipolar disorder, depression, substance abuse, psychotic and organic mental disorders. We also omitted patients who were treated with antidepressants, anti-psychotic drugs, mood stabilizers, antibiotics, antivirals, glucocorticoids, statins, beta-blockers, and dietary supplements, e.g. omega-3 polyunsaturated fatty acids, coenzyme Q10, etc. Patients with other clinically significant medical illnesses were excluded, e.g. inflammatory bowel disease, diabetes type 1, chronic obstructive pulmonary disease, rheumatoid arthritis, lupus erythematosus, epilepsia, etc. Finally we excluded those who showed infectious or allergic reactions the last two months prior to this study. Patients gave written informed consent after the study protocol was fully explained. The study was approved by the local ethical committee.

We assessed the severity of ME/CFS symptoms using the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale (FF scale) (Zachrisson *et al.* 2002). The FF scale measures 12 symptoms reminiscent of ME/CFS and fibromyalgia on a scale with defined scale steps from 0 to 6. A score of 0 indicates absent / transient symptoms, while 6 indicates severely interfering or persisting symptoms. The total sum of the 12 items was employed as a measure of the severity of ME/CFS symptomatology. The 12 FF symptoms are: FF1: muscle pain; FF2: muscular tension; FF3: fatigue; FF4: concentration difficulties; FF5: failing memory; FF6: irritability; FF7: sadness; FF8: sleep disturbances; FF9: autonomic disturbances; FF10: irritable bowel; FF11: headache; and FF12: a subjective experience of infection. We assessed whether 10 gastro-intestinal (GI) symptoms (listed below as GI1-GI10) were present (for at least 12 weeks in the preceding 12 months) or not present. The first nine items are based on the symptoms of the Rome II criteria, i.e.: GI1: abdominal discomfort/pain which is relieved with defecation; GI2: onset associated with more than 3 bowel movements per day; GI3: onset associated with less than 3 bowel movements per week; GI4: onset associated with a change in form of stool; GI5: abnormal straining; GI6: abnormal urgency; GI7: feeling of incomplete bowel movement; GI8: passing mucus during bowel movement; GI9: bloating; and GI10: abdominal pain or cramps.

Laboratory Methods

Fasting plasma was sampled between 8.30 a.m. and 11.30 a.m. for the assay of IgM and IgA responses directed against the LPS of Hafnei Alvei, Pseudomonas Aeruginosa, Morganella Morganii, Pseudomonas Putida, Citrobacter Koseri, and Klebsiella Pneumoniae. The assays were performed with an indirect ELISA according to manufacturer methods (GEMAC-IDRPHT, New Innovative Therapies, St Jean d'Illac, France). In short, each plasma sample was measured in duplicate and tested simultaneously with three standard solutions. The optical densities (OD) of the three standards are expressed as Z values and from this the reference linear curve is calculated. The Z ratio equals: the optical density (OD) of the patients serum minus the OD of control serum assayed at the same time and in the same microplate divided by the standard deviation of the ODs of the control samples. This Z value represents the position of the patients serum versus the mean of "normal" serum samples. Doing so, a Z value > indicates an antibody titer that is higher than the mean of 95.4% of the healthy controls sera. The analytical interassay CV values were <6%. The peak IgM and peak IgA responses to the 6 IgA and IgM measurements were used as an overall measure of IgM and IgA responses to LPS.

Statistics

Correlations between the variables were determined by using Pearson's product-moment correlation coefficients and regression analyses. Differences between group means are assessed with analyses of variance (ANOVA). When significant, we used the Dunn-Scheffe test with Bonferroni corrected p-values to assess multiple comparisons among treatment means. Associations between classification systems were assessed with the analyses of contingence Tables (χ^2-test). The significance was set at α=0.05 (two tailed). To examine the dimensions in the data set we employed factor analysis (principal component method) followed by varimax rotation. The latter was employed as an aid for the interpretation of the data structure. The number of factors was determined by means of Kaiser's criterion (only factors with an eigenvalue >1 are retained). Loadings ≥0.400 were used for interpretation of the factors. Cluster analysis, i.e. Forgy's centroid method, was used to assess the relevance of classification systems and to generate new classifications in the data set (Derde & Massart 1982; Massart & Kaufman 1983; Maes *et al.* 1990a; 1990c; 1998). Consequently, we used supervised learning methods, i.e. ANOVAs, χ^2-tests, and LDA (Linear Discriminant Analysis) to examine the differences between the cluster-analysis-generated categories. The classification ability of the LDA classification rule was checked with the Jackknife method. The generalizability of the diagnostic classifications generated by cluster analysis was checked against external criteria (Aldenderfer *et al.* 1986; Maes *et al.* 1990b), i.e. the IgM and IgA mediated immune responses against LPS.

RESULTS

Characteristics of ME/CFS and GI symptoms

Table 1 shows age, gender, peak IgA and IgM responses to LPS, total FF score, the 10 GI items, and the prevalence of IBS in individuals with CF and ME/CFS. There