Increased serum IgM antibodies directed against phosphatidyl inositol (Pi) in chronic fatigue syndrome (CFS) and major depression: Evidence that an IgM-mediated immune response against Pi is one factor underpinning the comorbidity between both CFS and depression

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Abstract

Major depression and chronic fatigue syndrome (CFS) are accompanied by signs of oxidative and nitrosative stress (O&NS) and an inflammatory response. Phosphatidyl inositol (Pi) is thought to play a role in depression. The aim of the present study is to examine whether depression and CFS are characterized by an IgM-mediated immune response directed against Pi. Toward this end, this study examines the serum IgM antibodies directed against Pi in 14 patients with major depression, 14 patients with CFS, 14 subjects with partial CFS, and in 11 normal controls. We found that the prevalence and mean value for the serum IgM levels directed against Pi were significantly greater in patients with major depression and CFS than in normal controls and patients with partial CFS. There were significant and positive correlations between serum IgM levels directed against Pi and two symptoms of the FibroFatigue Scale, i.e. fatigue and depression. The results show that an IgM-related immune response directed against Pi may occur in both depression and CFS and may play a role in the pathophysiology of the key symptom of CFS and major depression. It is suggested that the above disorders in Pi result from increased O&NS in both depression and CFS. Autoanti-Pi antibodies may have biological effects, for example, by changing inositol 1,4,5-triphosphate (IP3), phosphatidylinositol-4,5-bisphosphate (PIP2), diacylglycerol and phosphatidylinositol-3,4,5-triphosphate (PIP3) production, thus interfering with intracellular signalling processes. Future research in major depression and CFS should focus on the functional consequences of the immune responses directed against Pi.

ORIGINAL ARTICLE

INTRODUCTION

There is a strong degree of comorbidity between chronic fatigue syndrome (CFS) and major depression [1]. Also, at the symptomatic level there is a significant overlap between both disorders [1]. For example, fatigue is one of the key symptoms of major depression [2] and CFS [3]. Depressive symptoms frequently occur in CFS [1,3]. There are several pathophysiological mechanisms which may underpin the phenomenological and clinical overlap between both CFS and depression, such as the activation of the inflammatory response system (IRS) and increased oxidative & nitrosative stress (O&NS), which occur in both disorders.

There is now evidence that depression is accompanied by IRS activation with increased levels of proinflammatory cytokines, activation markers and acute phase proteins, and lowered levels of negative acute phase reactants, such as serum zinc [4]. In CFS, signs of IRS activation are observed, e.g. perturbations in proinflammatory cytokines, increased expression of activation markers, an increased serum alpha-2 protein fraction as obtained by means of electrophoresis, and lowered serum zinc [5–11]. We have shown that this inflammatory reaction is driven by an intracellular inflammation characterized by an increased production of the transcription factor nuclear factor kappa beta (NFκβ), which in turn induces increases in two other inflammatory and oxidative mediators, i.e. cyclo-oxygenase (COX-2) and inducible NO synthase (iNOS) [12, 13]. Indeed, the production of NFκβ, COX-2 and iNOS by peripheral blood lymphocytes is significantly greater in CFS than in normal volunteers, while there are significant and positive correlations between COX-2, iNOS and NFκβ [13].

Fatigue may also be induced by pro-inflammtory cytokines. Thus, in patients with hepatitis-C, treatment with interferon-alpha (IFNα) induces fatigue and major depression in a considerable number of patients [14]. Almost all IFNα-treated subjects develop fatigue one week after starting IFNα treatment. The degree of fatigue one week after starting treatment predicts the severity of the cognitive symptoms of depression [14]. The IFNα-induced depressive symptoms are significantly related to the increase in proinflammtory cytokines, which indicates a causal link between IFNα-induced IRS activation and the occurrence of depression [15, 16]. The above results suggest that IRS activation may underpin both fatigue and depression.

Another factor which could explain the comorbidity between depression and CFS is increased O&NS [17–19]. Thus, major depression is accompanied by a) increased concentrations of 8-hydroxydeoxyguanosine in peripheral blood leukocytes [20], serum malondialdehyde (MDA) levels [21, 22], and blood and saliva peroxidase levels [23]; and b) decreased plasma levels of antioxidants, such as serum zinc, vitamin E and C, and glutathione peroxidase [22–25]. In addition, IFNα-induced inflammation is also accompanied by increased nitric oxide production and the latter appears to be involved in at least some forms of IFNα-induced depression [26]. Also CFS is accompanied by signs of O&NS, such as increased isoprostane levels and oxidized low density lipoproteins (LDL) [27], protein carbonyl levels [28], and LDL thiobarbituric acid reactive substances (TBARS) [29]. Animal models of stress-induced depression and CFS show that O&NS plays a key role in both conditions [30–32]. Moreover, the anti-oxidative defences may be decreased in CFS as indicated by lowered levels of antioxidants, such as serum zinc [9] and dehydroepiendrosterone-sulfate [33].

A third factor which may explain the comorbidity between depression and CFS and which is related to IRS activation and O&NS is autoimmunity. In inflammatory responses, lipid membranes and thus brain, muscle, and nerve cells can be damaged through the increased production of oxygen radicals [1]. During this process, chemical modifications may occur of lipids which change the natural structure of otherwise ubiquitous molecules to generate a variety of modified new epitopes (neoepitopes) which can change or abrogate the functions of the selfepitope and which can render these immunogenic [34]. The immunoglobulin-(Ig)-mediated (auto)immune response mounted against these neoepitopes can further change the biological activities of the self-epitope. This process probably plays a role in the pathophysiology of CFS because that disorder is accompanied by increased IgM levels directed against oleic, palmitic and myristic acid, MDA, azelaic acid, S-farnesyl-L-cysteine, and the N-oxide derivates, nitro-tyrosine, nitro-phenylalanine, nitro-arginine, nitro-tryptophan, and nitro-cysteinyl [35]. The above results suggest that CFS is characterized by an IgM-related immune response directed against disrupted lipid membrane components, by-products of lipid peroxidation, S-farnesyl-L-cysteine, and NO-modified amino-acids, which are normally not detected by the immune system but due to O&NS have become immunogenic [35].

Another selfantigen, which plays a role in the pathophysiology of depression, is phosphatidyl inositol (Pi). The lipid Pi makes up a significant component of cell membranes and is involved in many key functions of the cell. An autoimmune response directed against Pi has been observed in inflammatory disorders, such as multiple sclerosis and Guillain Barre syndrome [36, 37], two disorders which frequently are accompanied by fatigue. Depression is accompanied by lower inositol CSF levels and inositol may be useful in the treatment of major depression [38, 39].

The aim of the present study was to examine whether CFS and major depression are accompanied by an augmented IgM-related immune response directed against Pi and to examine whether the Ig-mediated immune response is related to the severity of specific symptoms common to major depression and CFS.

SUBJECTS AND METHODS

Subjects

Forty-two patients and 11 normal controls (staff or their family members) participated in the present study. The patients were admitted to the M-Care4U Outpatient Clinics, Belgium. The patients were classified according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV-TR) [40] and the Centers for Disease Control and Prevention (CDC) criteria [3]. The diagnostic CDC criteria for CFS are: a) the patient must have a severe chronic fatigue of six months or longer, while there is no other known medical condition which can explain the fatigue; and b) the patient must have four or more of the following symptoms: substantial impairment in short-term memory or concentration, sore throat, tender lymph nodes, muscle pain, multi-joint pain without swelling or redness, headache of a new type, pattern or severity, unrefreshing sleep, and post-exertional malaise lasting more than 24 hours. Patients presenting with criterion a) but who did not fulfill criterion b) were rated as partial CFS. Doing so, the patients were divided into 14 patients with major depression, 14 with CFS and 14 with partial CFS. The total sum of the FibroFatigue scale, i.e. the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale [40–43] was used in all patients to compute the severity of chronic fatigue. This scale measures 12 items reminiscent for CFS (and fibromyalgia): pain, muscular tension, fatigue, concentration difficulties, failing memory, irritability, sadness, sleep disturbances, autonomic disturbances, irritable bowel, headache, and subjective experience of infection.

We have excluded: a) subjects with life-time diagnosis of psychiatric DSM-IV-TR disorders, anxiety disorders, schizophrenia, substance use disorders and organic mental disorders; b) subjects with CFS who ever had major depressive episodes; and patients with major depression who also suffered from concurrent CFS; c) subjects with other medical illness, such as other inflammatory or autoimmune disorders; d) subjects who ever had been treated with anti-psychotic drugs or anticonvulsants and subjects who had been taking psychotropic drugs during the last year prior to the studies; e) subjects with abnormal values for routine blood tests, such as alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), calcium, creatinine, electrolytes, thyroid stimulating hormone (TSH), total protein, and iron or transferrin saturation; and f) subjects with acute inflammatory and allergic reactions for at least 1 month prior to the study. Patients and controls gave written informed consent after the study protocol was fully explained. The study has been approved by the local ethical committee.

Methods

The serum IgM values directed against Pi were analyzed by means of an enzyme-linked immunosorbent assay (ELISA) by a method described previously [43]. 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 as Z=f(OD) with Z=a OD + b. Thus, the Z value of the lowest standard can be negative. This curve allows to deduce the mean values of the duplicate measurements of the OD values. The biological interassay CV values are <10%.

Statistics

We employed Pearson's product moment correlation coefficients, Spearman's rank order correlations and (multiple) regression analysis to examine the correlations between variables. In order to examine differences among treatment means we used the analysis of variance (ANOVA) or covariance (ANCOVA). Post-hoc contrasts between multiple group means were ascertained by means of the Dunn test. The independence of classification systems was ascertained by means of analysis of contingence tables (χ^2-test) and Fisher's exact probability test. The significance was set at α=0.05 (two tailed).

RESULTS

There were no significant differences (F=0.3, df=3/49, p=0.8) in age between the study groups, i.e. normal controls (mean ± SD=41.4±8.7 years), partial CFS (41.4±10.9 years), CFS (44.6±12.2 years) and major depression (43.3±9.9 years). There were no significant differences (χ^2=0.5, df=3, p=0.9) in the male/female ratio between normal controls (3/8), partial CFS (4/10), CFS (4/10), and major depressive (6/8) patients. There were no significant correlations between age and the serum IgM levels directed against Pi (r=0.11, p=0.6) and no significant differences in the serum IgM values between males and females (F=0.5, df=1/45, p=0.5).

Figure 1 shows the serum IgM levels directed against Pi in the 4 study groups. By means of ANCOVA with age and gender as covariates we found that there were significant differences between the study groups (F=6.9, df=3/47, p=0.0009). By means of the Dunn test we found significantly higher values in CFS (t=4.14, p=0.0003) and major depressed (t=2.75, p=0.008) patients than in normal controls; patients with CFS had significantly higher values than those with partial CFS (t=3.43, p=0.002), whereas there was a trend towards higher values in major depressed than in partial CFS (t=1.95, p=0.054) patients. No significant differences were found in the serum IgM levels directed against Pi between normal controls and partial CFS patients (t=0.92, p=0.6) and between CFS and major depressed patients (t=1.48, p=0.14).

Using a cut-off point for the anti-IgM antibody titers directed against Pi of >2 Z values, we found a significantly greater number of CFS (7/14, ψ=0.55, p=0.007) and major depressed (5/14, ψ=0.44, p=0.03) patients with abnormally increased IgM antibodies than in the

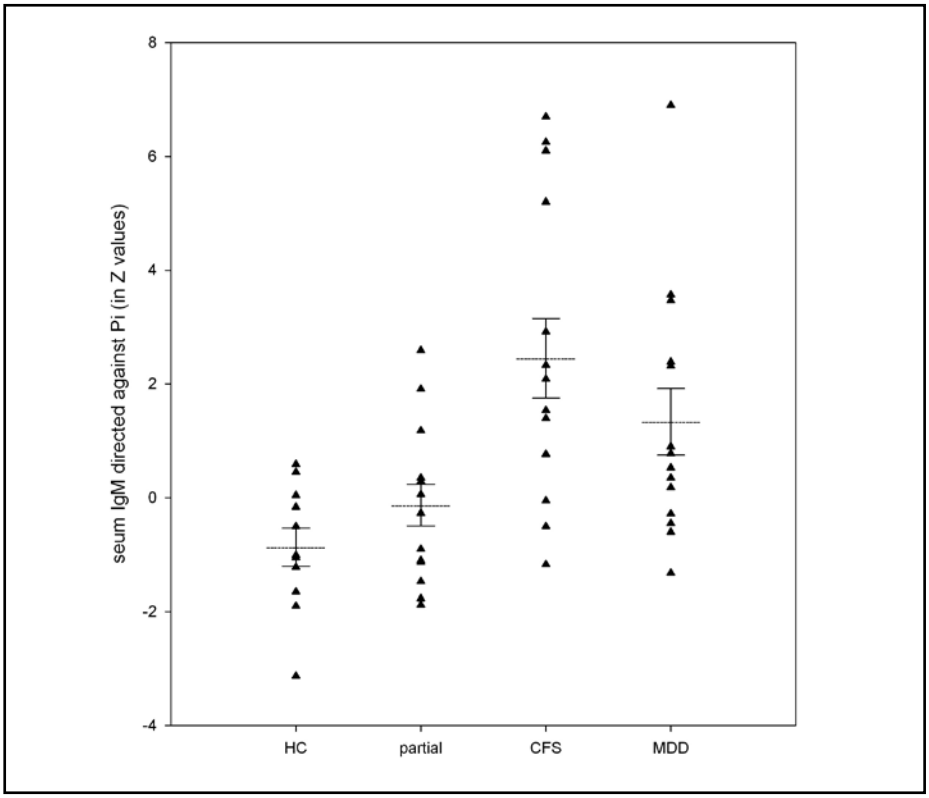

Figure 1. Scatter diagram of the serum IgM titers directed against phosphatidyl inositol in healthy volunteers (HC), partial chronic fatigue syndrome (CFS), CFS and major depression (MDD). Shown are the mean and SEM values.

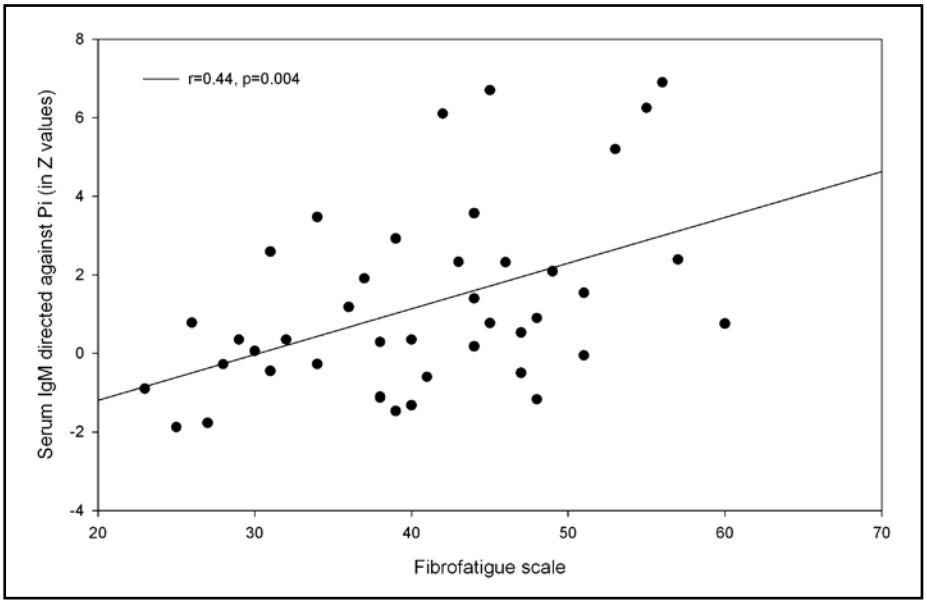

Figure 2. Relationship between the total score in the FibroFatigue Scale and serum IgM titers directed against phosphatidyl inositol in 42 patients with partial chronic fatigue syndrome (CFS), CFS and major depression.

normal controls (0/11) (all results of Fisher's exact probability tests). No significant differences were established either between normal controls and partial CFS patients or between CFS and major depressed patients. Combining the CFS and major depressed patients, we found a significantly greater number of CFS and major depressed (12/28) patients with abnormally increased IgM antibodies than in normal controls (0/11, ψ=0.42, p=0.008) and partial CFS patients (1/14; ψ=0.36, p=0.02). Using a more conservative cut-off point, i.e. anti-IgM values >3 Z values, we found a significantly greater number of CFS (4/14) and major depressed (3/14) patients (total: 7/28) with abnormally increased IgM antibodies, than in controls and partial CFS patients together (0/25; ψ=0.37, p=0.008).

ANOVA showed that the total score on the FibroFatigue scale was significantly greater (F=17.7, df=2/41, p=0.00003) in CFS patients (mean ±SD score: 48.0 ±5.6,

t=5.92, p=0.00001) and major depressed patients (41.5 ±8.8; t=3.52, p=0.001) than in those with partial CFS (32.6 ±5.3). The Fibrofatigue score was significantly greater in CFS patients than in those with major depression (t=2.59, p=0.01). Figure 2 shows the correlation between the total score on the FibroFatigue scale and the serum IgM levels directed against Pi (r=0.44, p=0.004). There were also significant and positive correlations between the serum IgM levels directed against Pi and two symptoms only of the FF scale, i.e. fatigue (r=0.42, p=0.006) and depression (r=0.46, p=0.003). Automatic multiple regression analysis (with an F-to-enter of p=0.05) showed that 26.5% of the variance in the serum IgM levels directed against Pi was explained (F=7.0, df=2/39, p=0.003) by the regression on fatigue (F=6.8, p=0.01) and depression (F=4.1, p=0.04).

DISCUSSION

The results of the present study show that major depression and CFS are accompanied by an increased IgM-mediated immune response directed against Pi and that the increased IgM values are strongly related to two symptoms of the FibroFatigue Scale, i.e. depression and fatigue.

Thus, in major depression and CFS there is an immune response directed against autoepitopes (Pi-epitopes) that are normally hidden from the immune system. The increased IgM antibodies directed against Pi indicate that in major depression and CFS the natural lipid membrane structures have been modified to generate a modified Pi lipid structure with immunogenic determinants. Previously, it has been found that in major depression and CFS there are increased antibody titers to epitopes of oxidized LDL and increased levels of MDA, a byproduct of lipid peroxidation [21,22,27,29] and against lipids, such as oleic, palmitic and myristic acid [35]. By inference, O&NS may have changed otherwise inactive Pi-autoepitopes to Pi-antigens which have acquired immunogenicity and thus may serve as a trigger to impair or bypass immunological tolerance. Thus, one hypothesis is that the Pi autoepitopes may be recognized since O&NS has damaged or disrupted the Pi lipid membrane structure resulting in the formation of neoantigens and consequently in a mounted IgM response against the Pi neoepitopes.

Increased autoantibody titers to Pi have been detected in other inflammatory disorders, such as Guillain-Barre syndrome and multiple sclerosis (MS). The former is an acute inflammatory polyneuropathy related to autoimmunity [36]. Also, Guillain-Barre patients develop anti-Pi antibodies of the IgM family. This production appears to be related to acute inflammation since treatment with gamma-globulin intravenously (IgIV) decreases the levels of anti-Pi autoantibodies 1 day after starting the treatment [36]. Antibodies directed against Pi have also been detected in remitting-relapsing multiple sclerosis (MS) [44,45]. Also in MS, the circulating IgM antibody titers appear to be related to the presence of inflammation since IgM antibodies appear during relapses and decrease during remissions [37]. Since CFS appears to be an inflammatory disorder with autoimmune responses against O&NS-damaged neoepitopes, it has been proposed [46] that the clinical efficacy of IgIV treatment in CFS [47,48] may be explained by its normalization of the inflammatory and autoimmune responses. The results of our study also suggest that treatment with IgIV could have some clinical efficacy in major depression.

As described in the Introduction, Pi is an important lipid, both as a key membrane constituent and as a participant in essential metabolic processes. Pi is converted to one of the key intermediates in intracellular signaling, i.e. phosphatidylinositol-4,5-bisphosphate (PIP2). The latter is the precursor of three very important second-messenger molecules, i.e. inositol-1,4,5-triphosphate (IP3), diacylglycerol and phosphatidylinositol-3,4,5-triphosphate (PIP3). These substances modulate intracellular calcium levels, regulate cell survival, growth, polarization and proliferation, activate phosphorylization of cellular proteins, function as lipid messengers at the plasma membrane to the effector in the nucleus, and activate protein kinase C (PKC) [49]. Also, the neurotransmitter serotonin requires Pi for proper functioning [50]. Thus, it may be hypothesized that the above Pi-related functions may become disturbed by the damage caused by O&NS to the lipid membrane structures and by the consequent autoimmune response. Therefore we may hypothesize that damage to Pi by O&NS, inflammation and autoimmune responses may cause dysfunctions in proper cell functioning.

Interestingly, inositol has been found to be decreased in the CSF of depressed patients [37], while altered PKC-mediated phosphorylation is involved in bipolar disorder [51]. Decreased serotonin 5-HT2A receptor-stimulated phosphoinositide signaling may occur in melancholic depression [50] and serotonergic disturbances not only play a role in major depression [52] but also in CFS [53].

Another major finding of this study is that the mounted IgM response to the Pi neoepitopes is significantly correlated to the key symptoms of both CFS and major depression, i.e. fatigue and depressive feelings. These results extent those of Vecchiet et al. [29] who found that – in CFS – increased O&NS and decreased antioxidant defences are related to the extent of fatigue. Our results are also in agreement with our previous report that in CFS there are significant and positive correlations between the serum IgM levels directed against fatty acids, MDA and azelaic acid and the severity of illness (as measured by the FibroFatigue scale) and symptoms, such as aches and pain, muscular tension and fatigue [35]. The results extent those of another report [54], which found depressive symptoms to be correlated to lipid peroxydation. Previous findings showed that IFNα-induced IRS activation and O&NS induce fatigue and depression [14,26]. Thus, it may be hypothesized that O&NS and IRS activation and the IgM-mediated autoimmune

response directed against Pi induces symptoms, such as fatigue and depression. Also, the comorbidity and clinical overlap between CFS and major depression may be explained by a common immune pathophysiology, such as O&NS, IRS activation and autoimmunity. Differences in other pathophysiological factors may further differentiate both syndromes into two different diagnostic classes, e.g. defects in the interactions between proinflammatory cytokines and the turnover of tryptophan-serotonin, which appears to be a hallmark for major depression [55]; and increased gut permeability (intestinal mucosal dysfunction), which frequently occurs in CFS [56].

In summary, the present results add to the view that major depression and CFS are disorders characterized by IRS activation and O&NS, phenomena which cause damage to lipids, such as Pi, which, in turn, a) may become immunogenic and cause an IgM-related autoimmune response to neoepitopes; and b) may cause disturbed functional activity in Pi and consequently in a number of cell functions. It is hypothesized that O&NS and consequent autoimmune responses directed against important cell lipid components, such as PI, are pathophysiological factors in the symptoms, such as fatigue and depression, and, therefore, may underpin the comorbidity between both CFS and major depression.

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