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ORIGINAL ARTICLE

Normalization of leaky gut in chronic fatigue syndrome (CFS) is accompanied by a clinical improvement: effects of age, duration of illness and the translocation of LPS from gram-negative bacteria

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Abstract

BACKGROUND: There is now evidence that an increased translocation of LPS from gram negative bacteria with subsequent gut-derived inflammation, i.e. induction of systemic inflammation and oxidative & nitrosative stress (IO&NS), is a new pathway in chronic fatigue syndrome (CFS).

METHODS: The present study examines the serum concentrations of IgA and IgM to LPS of gram-negative enterobacteria, i.e. Hafnia Alvei; Pseudomonas Aeruginosa, Morganella Morganii, Pseudomonas Putida, Citrobacter Koseri, and Klebsielle Pneumoniae in CFS patients both before and after intake of natural anti-inflammatory and anti-oxidative substances (NAIOSs), such as glutamine, N-acetyl cysteine and zinc, in conjunction with a leaky gut diet during 10-14 months. We measured the above immune variables as well as the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale in 41 patients with CFS before and 10-14 months after intake of NAIOSs.

RESULTS: Subchronic intake of those NAIOSs significantly attenuates the initially increased IgA and IgM responses to LPS of gram negative bacteria. Up to 24 patients showed a significant clinical improvement or remission 10-14 months after intake of NAIOSs. A good clinical response is significantly predicted by attenuated IgA and IgM responses to LPS, the younger age of the patients, and a shorter duration of illness (< 5 years).

DISCUSSION: The results show that normalization of the IgA and IgM responses to translocated LPS may predict clinical outcome in CFS. The results support the view that a weakened tight junction barrier with subsequent gut-derived inflammation is a novel pathway in CFS and that it is a new target for drug development in CFS. Meanwhile, CFS patients with leaky gut can be treated with specific NAIOSs and a leaky gut diet.

INTRODUCTION

There is now evidence that activation of inflammatory and oxidative & nitrosative stress (IO&NS) pathways play a role in chronic fatigue syndrome (CFS) [1-3]. The activated pathways include intracellular inflammation, systemic inflammation with increased levels of pro-inflammatory cytokines, increased O&NS, and damage to DNA, membrane lipids and functional proteins caused by O&NS [3]. There is now also evidence that leaky gut is a novel inflammatory pathway in CFS. Recently, we found that CFS is accompanied by increased serum levels of IgM and IgA directed against lipopolysaccharide (LPS) of gram-negative enterobacteria and that the IgM and IgA values are significantly correlated to specific symptoms of CFS, such as fatigue, autonomic and gastro-intestinal symptoms, and a subjective feeling of infection [1]. This shows that the symptoms of CFS have a genuine immune pathophysiology [1-3].

These results indicate that in CFS an IgM and IgA-mediated immune response is raised against the LPS of different gram negative enterobacteria. The latter indicates that CFS is accompanied by an increased gut permeability or leaky gut . This condition is also labelled intestinal mucosal dysfunction. This suggests that one of the critical functions of the gut wall has been jeopardized, ie. the integrity of the tight junction barrier, which separates intestinal microorganisms from the interstitium.

Disruptions of the permeability of the gut tight junction barrier may be caused by IO&NS pathways [4-6]. Pro-inflammatory cytokines, which are often increased in CFS, i.e. and interleukin-1β (IL-1β), IL-6, tumor necrosis factor-alpha (TNFα), and interferon-gamma (IFNγ) [3] may cause a loss of the protective barrier function by enlarging the spaces between the cells of the gut wall, which results in an increased permeability in the intestinal epithelial tight junction barrier [4-10]. The IL-1β-induced increase in permeability is partly mediated by the activation of nuclear factor kappa beta (NFκβ) [7]. The TNFα-induced increase in intestinal epithelial tight junction permeability is mediated by NFκβ p50/p65 binding through activation of myosin light chain kinase (MLCK) promoter which eventually leads to a MLCK-mediated opening of the intestinal tight junction barrier [8,9]. Not only pro-inflammatory cytokines, but also oxygen free radicals may cause intestinal barrier impairment, e.g. following ischaemia / reperfusion [11]. Natural anti-inflammatory and anti-oxidative substances (NAIOSs), such as N-acetyl-L-cysteine (NAC), glutamine and zinc, may improve the integrity of the gut barrier [11–17].

Increased gut permeability is a driver of systemic inflammation [16]. For example, in abdominal postoperative patients the gut is an important source of systemic inflammation and decreases in intestinal permeability may attenuate the systemic inflammatory response [17]. The pathway which plays a decisive role in gut-derived inflammation is translocation of gram negative enterobacteria (bacterial translocation) or translocation of LPS from gram negative bacteria from the gut to the blood through the intestinal barrier failure. Thus, a leaky gut allows normally poorly invasive enterobacteria or the LPS from gram negative bacteria to exploit the enlarged spaces or lipid raft-mediated transcytotic pathways to cross the gut epithelium [1-4]. This causes systemic increases in LPS or infections in the peripheral blood. Depletion of NAIOSs, such as glutamine, may increase the risk towards cytokine-mediated bacterial translocation, while supplementation with glutamine may reduce bacterial translocation [18].

Bacterial or LPS translocation, in turn, may induce activation of NFκβ, the major upstream, intracellular mechanism which regulates the IO&NS pathways [19,20], such as cyclo-oxygenase (COX-2) and inducible NO synthase (iNOS) [19-21]. Previously, we have shown that CFS is accompanied by an increased production of NFκβ, iNOS and COX-2 by white blood cells, and other sings of activation of O&NS pathways [1-3,19,21-25]. As discussed above, pro-inflammatory cytokine-induced weakening of the tight junction barrier is mediated by NFκβ [7-9]. Consequently, NFκβ activation may be not only the cause, but also the consequence of the opening of the tight junction barrier and thus could perpetuate a vicious circle between NFκβ activation and weakening of the tight junction barrier.

Systemic activation of the IO&NS pathways by increased LPS translocation is accompanied by a central neuroinflammation and increased levels of pro-inflammatory cytokines and activation of microglia in the brain [26]. The latter two central pathways may remain activated for several months and are accompanied by a chronically activated production of pro-inflammatory cytokines, such as TNFα [26]. We have previously discussed that increased intracellular inflammation, induction of the O&NS pathways and the increased production of pro-inflammatory cytokines, such as IL-1β, IL-6, IFNγ, and TNFα, may induce many symptoms of CFS, such as fatigue, muscle pain, muscle tension, malaise, depressive feelings, and cognitive disorders [3]. Systemic LPS may provoke comparable symptoms in animal models [27,28].

By inference, the increased LPS translocation with subsequent gut-derived inflammation is a novel pathway which may explain the activated IO&NS pathways in CFS and the symptoms of CFS as well. It is also interesting to note that an increased gut permeability is induced by the established trigger factors of CFS, e.g. psychological stress [29,30], sustained strenuous exercise [31]; and inflammatory conditions, such as surgery, trauma and infections [32,33]. Moreover, the intestinal barrier may be compromised by other conditions which are known to be accompanied by chronic fatigue, but because of the CDC (Centers for Disease Control and Prevention) criterion no other medical condition may explain the chronic fatigue cannot be diagnosed as CFS

[34]. The symptoms and pathophysiology, however, of both CFS according to the CDC and secondary CFS due to other organic illnesses are quite similar [3]. These latter comprise – amongst others – the use of chemotherapeutic agents [35], prolonged use of antibiotics [36,37], radiation [38], AIDS/HIV [39], autoimmune disorders [40] and inflammatory bowel disorder [41].

In a case report, we have shown that normalization of the IgM and IgA response directed against LPS predicts a gradual remission to treatment with intravenous immunoglobins (IVIg), specific NAIOSs, e.g. glutamine, NAC and zinc, and a milk and gluten-free and low-carb diet, labelled the leaky gut diet [2]. This gradual normalization of the translocation of LPS during this treatment regime was also accompanied by a normalization of most IO&NS variables.

The present study has been carried out in order to examine the relationships between the translocation of LPS from gram negative bacteria and the clinical outcome in CFS.

SUBJECTS AND METHODS

Subjects

Forty-one patients participated in the present study. They were consecutively admitted to the M-Care4U Outpatient Clinics, Belgium and selected on the basis of initial increases in the IgM and / or IgA responses to LPS [1,2]. The diagnosis CFS was made by means of the CDC criteria [34]. That is: i) 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 ii) 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. We employed the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale (FF scale) [42,43] in order to measure severity of illness. This scale measures 12 symptoms of CFS and fibromyalgia, that is pain, muscular tension, fatigue, concentration difficulties, failing memory, irritability, sadness, sleep disturbances, autonomic disturbances, irritable bowel, headache, and subjective experience of infection.

In the present study the following patients were excluded: those who had suffered from a life-time diagnosis of psychiatric DSM IV disorders, such as bipolar depression, anxiety disorders, schizophrenia, substance use disorders and organic mental disorders and patients with abnormal values for routine blood tests, such as alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), calcium, creatinine, electrolytes, and thyroid stimulating hormone (TSH). Also, patients with other medical illnesses, such as epilepsia, diabetes, inflammatory bowel disease, etc. were excluded. Patients gave written informed consent after the study was explained. The study has been approved by the local ethical committee. This is a non-interventional study. We did not intend to examine the effects of a specific treatment, but rather the IgM / IgA responses to LPS translocation in relation to clinical variables both in CFS patients without a treatment (baseline) and in the CFS patients who had been taking specific NAIOSs and had a leaky gut diet for 10-12 months (endpoint). All patients followed the leaky gut diet and took glutamine, zinc and NAC, in combination with other NAIOSs, which were given according to the immune and biochemical status of the patients, i.e. L-carnitine, coenzyme Q10, taurine and lipoic acid (in case of carnitine and/or coenzyme Q10 shortage); or curcumine and quercitine (in case of systemic or intracellular inflammation).

Methods

Fasting blood samples were taken during the morning hours for the assays of the serum IgM and IgA values against the LPS of 6 different enterobacteria, i.e. Hafnei Alvei, Pseudomanes Aeruginosa, Morganella Morganii, Pseudomanus Putida, Citrobacter Koseri and Klebsiella Pneumoniae. The analyses were performed as explained before [1,44,45]. In short we used an indirect ELISA method according to the methods outlined by the manufacturer (Gemacbio, The Ultimate Biopharmaceuticals, France). Each serum 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 were < 10%.

Statistics

Relationships between variables were assessed by means of Pearson's product moment correlation coefficients and multiple regression analyses. Group mean differences were checked by means of the analysis of variance (ANOVA) and by means of linear discriminant analysis. Repeated measurements analyses of variance (RM ANOVAs) were used to compare basal and endpoint measurements. When the interaction between time X groups was significant, analyses of simple effects were employed to examine the interaction pattern. The independence of classification systems was ascertained by means of analysis of contingence tables (χ^2-test). In order to assess the "total LPS translocation load" we have employed two different indices: a) the total sum of the 12 Ig (IgM and IgA) levels; and b) the peak Ig (IgM or IgA) levels, i.e. the highest of the 12 Ig values. The same indices were also employed to assess the IgM-versus the IgA-related translocation loads. Toward this end, we computed the peak IgM and peak IgA data; and

the total sum of the 6 IgM and 6 IgA data. The significance was set at α=0.05 (two tailed).

RESULTS

The mean age of the patients was 37.9 (±11.1) years. The male / female ratio was 7/34. The mean duration of illness was 7.9 (±6.6) years and the mean age at onset was 30.1 (±10.4) years.

Table 1 shows the measurements of the different IgM and IgA values both basal and at endpoint. RM design ANOVAs showed that the end point values of all 6 IgM values were significantly lower than the basal values. RM design ANOVAs showed that there were no significant differences in serum IgA to the 6 LPS values between basal and endpoint. RM design ANOVAs showed that the end point values of peak IgM and IgA were significantly lower than the basal peak IgM and IgA values, respectively. RM design ANOVA showed that the end point values of the peak Ig values, either IgA or IgM, were significantly lower than the basal peak Ig values.

The mean basal FF score was 38.8 (±7.5) and the endpoint FF score was 14.0 (±11.1). The variability in the basal FF data was 19.5% and in the endpoint FF data was 79.4%. We have divided the groups into patients who showed a good clinical response and those who did not. Towards this end, we have made the regression of the endpoint FF values on the basal FF values. Both values were strongly correlated (R2=23.8%, r=0.49, p=0.001). The residualized values were consequently dichotomized to yield two groups, labelled as responders (greater reduction in the FF score) versus non-responders. Table 1 shows the measurements of the FF values both basal and endpoint in the responders and the non-responders. RM design ANOVA showed significant differences in the FF values between responders and non-responders (F=18.4, df=1/39, p=0.0002); significant effects of treatment (F=105, df=1/39, p<10E-4) and a significant interaction between groups X time (F=12.3, df=1/39, p=0.001). Analyses of simple effects showed that at baseline, there were no significant differences between the FF values between responders and non-responders (F=0.47, df=1/78, p=0.5) and that at endpoint there were significant differences between both groups (F=49.5, df=1/78, p<10E-4). Overall, 26 (that is 63.5%) of the patients showed a good clinical response, whereas 15 (that is 36.5%) showed less clinical improvement, although still statistically significant.

Table 2 shows the FF measurements in groups divided according to duration of illness (longer versus shorter than 5 years). The FF score was significantly higher in patients with a longer duration of illness as compared with those with a shorter duration of illness (F=7.5, df=1/39, p=0.009); the interaction time x groups was significant (F=17.4, df=1/39, p=0.0003). Simple effects showed that in baseline conditions, there were no significant differences between the FF values in both groups (F=0.4, df=1/78, p=0.5), but at endpoint, patients who suffered from CFS for more than 5 years showed significantly higher FF values than those who did not (F=16.9, df=1/78, p=0.0002).

Table 2 shows the measurements of the basal and endpoint FF values in patients dichotomized according to their age (<38.0 years versus > 38 years). RM design ANOVA did not show significant differences in the FF values between older and younger subjects (F=1.5, df=1/39, p=0.2) but there was a significant interaction

Table 1. The measurements of serum IgM and IgA levels against LPS of Hafnia Alvei, Pseudomonas Aeruginosa, Morganella Morganii, Pseudomonas Putida, Citrobacter Koseri and Klebsiella Pneumoniae in basal conditions and at endpoint in 41 patients with chronic fatigue syndrome.

Variables		basal	endpoint	F value*	p value (df=1/39)
Hafnei Alvei	IgM	1.82 (2.26)	0.61 (2.03)	9.0	0.005
	IgA	0.71 (2.60)	0.53 (1.72)	0.2	0.7
Pseudomonas Aeruginosa	IgM	2.38 (1.90)	0.77 (1.60)	21.9	0.01
	IgA	0.80 (2.12)	0.97 (1.93)	0.4	0.6
Morganella Morganii	IgM	2.51 (2.40)	1.24 (1.82)	9.7	0.004
	IgA	0.76 (2.80)	0.62 (1.80)	0.5	0.5
Pseudomonas Putida	IgM	2.77 (2.78)	1.27 (2.19)	12.7	0.001
	IgA	1.68 (4.40)	0.96 (2.41)	3.3	0.07
Citrobacter Koseri	IgM	2.12 (2.11)	0.90 (1.99)	13.4	0.001
	IgA	1.05 (2.95)	0.49 (1.63)	1.37	0.2
Klebsiella Pneumoniae	IgM	2.24 (4.23)	1.10 (1.84)	6.0	0.02
	IgA	1.73 (3.96)	0.55 (1.56)	3.6	0.06
Peak IgM	IgM	4.22 (3.31)	2.06 (2.31)	19.6	0.0002
Peak IgA	IgA	4.07 (4.55)	2.15 (2.51)	13.7	0.0009
Peak	IgM or IgA	6.83 (3.67)	3.20 (2.55)	57.2	<10E-5

All results are shown as mean (±SD).
*All results of RM design ANOVAs.

Table 2. Measurements of the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale (FF scale) both at baseline and at endpoint according to different dichotomies.

criterion	FF basal	FF endpoint	n
responders	38.2 (7.8)	7.7 (5.7)	26
non responders	39.9 (7.3)	24.9 (9.7)	15
duration of illness < 5 years	37.8 (7.3)	7.3 (4.8)	17
duration of illness > 5 years	39.5 (7.8)	18.7 (11.9)	24
age < 38 years	39.2 (7.5)	10.6 (8.2)	21
age > 38 years	38.4 (7.8)	17.6 (12.7)	20

All results are shown as mean (±SD).
See text for results of statistical analyses.

between age groups X time (F=6.9, df=1/39, p=0.01). Analyses of simple effects showed that at baseline there were no significant differences between the FF values between older and younger patients (F=0.07, df=1/78, p=0.8) and that at endpoint older subject had higher FF values than younger ones (F=5.9, df=1/78, p=0.02).

We have also computed the differences between the responders and the non-responders as defined above. Responders tended to be younger that non-responders (F=3.3, df=1/39, p=0.07). The duration of illness was significantly (F=20.2, df=1/39, p=0.0001) higher in non-responders (mean=12.9 ±5.9 years) than in responders (mean=4.9 ±5.2 years). There were no significant differences between responders and non-responders in gender distribution, age at onset, baseline IgM or IgA values, and the endpoint IgM values. Non-responders (3.29 ±2.33 SD) had significantly (F=5.4, df=1/39, p=0.02) higher peak IgA values at endpoint than responders (1.50 ±2.41 SD). Also the residual sum of the 6 IgA values (obtained by the regression from endpoint IgA on baseline IgA values) was significantly (F=5.2, df=1/39, p=0.02) higher in non-responders (2.34 ±6.34 SD) than in responders (-1.35 ±3.98 SD). We found that some of the endpoint IgM or IgA values against LPS were significantly higher in non-responders than in responders, i.e. IgM against Pseudomonas Aeruginosa (F=5.2, df=1/39, p=0.02); Pseudomanas Putida (F=4.7, df=1/39, p=0.03) and Hafnia Alvei (F=4.1, df=1/39, p=0.047); and IgA against Citrobacter Koseri (F=4.3, df=1/39, p=0.04); and Klebsiella Pneumoniae (F=4.5, df=1/39, p=0.04). By means of linear discriminant analysis a significant discrimination of non-responders from responders was obtained by means of two significant discriminatory variables, i.e. IgM against Pseudomonas Aeruginosa and IgA against Citrobacter Koseri (F=6.7, df=2/38, p=0.003).

We have also examined the correlations between the residualized FF values (endpoind FF value with the baseline FF values covaried out) and various predictors. We found significant correlations between the residualized FF values and age (r=0.32, p=0.03), duration of illness (r=0.61, p<10E-4) and the residualized IgA values (r=0.34, p=0.02), but not with the age at onset (r=-0.05, p=0.7). We found that 46.7% of the variance in the residualized FF values could be explained by the regression on age (F=24.4, df=1/37, p=0.00008), age of onset (F=16.2, df=1/37, p=0.0005) and the residualized sum of the IgA values (F=5.8, df=1/37, p=0.01). Age at onset was negatively loaded, the other explanatory variables were positively loaded.

By means of regression analyses with the various endpoint FF symptoms as dependent variables and the basal FF symptom values, duration of illness and the residualized IgA or IgM values as explanatory variables we found that 58.9% of the variance in endpoint values of the FF item aches and pain was explained by the regression on basal FF values of aches and pain (F=23.8, p=0.00009), duration of illness (F=16.3, p=0.0004) and the residualized IgA values (F=5.1, p=0.02). 60.2% of the variance in endpoint muscle tension was explained by the regression on baseline muscle tension (F=19.4, p=0.0002), duration of illness (F=11.2, p=0.002) and the residualized IgA values (F=10.1, p=0.003). 41.0% of the variance in memory disturbances could be explained by the regression on duration of illness (F=17.3, p=0.0003) and the residualized IgA values (F=7.0, p=0.01). 52.9% of the variance in gastro-intestinal symptoms was explained by the regression on the baseline symptom values (F=10.7, p=0.002), duration of illness (F=10.5, p=0.003) and the residualized IgA values (F=11.2, p=0.002).

DISCUSSION

This study shows that normalization of the IgA and IgM responses to translocated LPS may predict the clinical outcome in CFS. A younger age at onset, a shorter duration of illness and a younger age of the patient are significantly predict a better outcome in CFS.

The first major finding of this study is that the intake of specific NAIOSs may attenuate the initially increased IgM and IgA responses to LPS, which indicates that those NAIOSs may reduce gut-derived inflammation and by inference may tighten the opened tight junction barrier. However, the normalization of the IgM values was more pronounced than that of the IgA values. This may be explained since increases in serum IgA indicate the more chronic pathogenic conditions [1]. Different

pathways may be involved in this improvement of gut permeability.

a) NAIOSs, such as glutamine [12], NAC [13], and zinc [14,15], have been shown to have a significant efficacity in the treatment of increased gut permeability. In a recent review article it was discussed that in laboratory and clinical settings, glutamine can attenuate gut permeability and enhance the protection of the gut epithelial barrier through its ability to induce the cellular protective stress response in the gut. Thus, glutamine may attenuate gut injuries and may attenuate the subsequent gut-derived systemic inflammatory responses [16]. Foitzik et al. [46], using an animal model of acute necrotizing pancreatitis, examined the effects of total parental nutrition (TPN) or TPN combined with glutamine. They found that glutamine significantly increased transmucosal resistance, and decreased the mannitol flux through the epithelium and the prevalence of pancreatic infections. Ann et al. [47] showed that glutamine might effectively reduce nonsteroid anti-inflammatory drugs (NSAID)-induced gut damage and bacterial translocation in the rat. Thus, diclofenac causes an increase in gut damage, enteric bacterial numbers and bacterial translocation, whereas glutamine may reduce the above changes induced by diclofenac. In another study, glutamine administration was compared to placebo in patients undergoing abdominal surgery [17]. It was found that in the glutamine treated group, there were significant reductions in gut permeability, serum endotoxin concentrations, serum malondialdehyde and WBC counts [17]. Similar findings were reported by Zhou et al. [48]: enteral glutamine supplementation improved gut permeability and decreased plasma endotoxin concentrations in thermally injured patients. In Caco-2 cells, TNFα induces a translocation of E. Coli when there is a simultaneous depletion of glutamine [18]. Addition of glutamine significantly inhibits the bacterial translocation [18]. This indicates that in inflammatory conditions, the availability of glutamine is essential for the preservation of a functional barrier to microorganisms [18]. The above results show that glutamine reduces the permeability of the colon; the opening of the tight junction barrier; and bacterial translocation by stabilizing the intestinal mucosal barrier; and that glutamine attenuates gut-derived inflammation. These effects of glutamine may be obtained through the augmentation of small bowel villus morphology; the maintenance of intestinal functions; intestinal permeability and immune function; and prevention of clinical infection related to bacterial translocation [49].

Dietary supplementation with zinc improves methotrexate-damaged rat intestine and in particular stimulates gut repair [50,51]. In Crohn's disease, zinc supplementation tightens "leaky gut". Thus, the lactulose / mannitol ratio was significantly higher before zinc supplementation than after, whereas during follow-up, most of the patients had normal intestinal permeability and did not relapse. This indicates that zinc can resolve permeability alterations and improves intestinal barrier function, which in turn contributes to a reduction of relapses in Crohn's disease [14,52]. Zinc-carnosine at concentrations that are found in the gut lumen stabilises the gut mucosa. Thus, in volunteers, indomethacin caused a threefold increase in gut permeability, whereas no significant increase in permeability was seen when zinc carnosine was co-administered [53]. NAC pretreatment results in improved barrier integrity and less pronounced reticuloendothelial system activation, indicating that NAC ameliorates gut-derived inflammation through an increased gut barrier function [11].

b) The other NAIOSs employed in this study, e.g. a) carnitine, coenzyme Q10, and lipoic acid; and curcumine and quercetine, may normalize the activation of the IO&NS pathways, e.g. through inhibition of oxygen radical formation; protecting tissues and mitochondria from O&NS damage; inhibiting the production of NFκβ, iNOS and COX-2 by white blood cells; and decreasing the production of pro-inflammatory cytokines [54-56]. For example, curcumine significantly reduces the production of TNFα in colon mucosa cells, and can attenuate the production of COX-2 and iNOS immunosignals and nitrite production as well [57]. Curcumine is also a specific inhibitor of NFκβ [58]. In intimal cells, propionyl-carnitine has been shown to be an inhibitor of NFκβ [59]. In MonoMac6 cells (a human monocytic cell line) stimulated with TNFα, lipoic acid significantly suppresses the activity of NFκβ [60]. Lipoic acid and NAC reduce the oxidative stress associated with zinc deficiency and the subsequent triggering of NFκβ-activation in neuronal cells [61]. Quercetin inhibits the activation of NFκβ and iNOS protein and mRNA expression and inhibits NO production in a dose-dependent manner [62].

In our case report [2], the patient had been treated with NAIOSs in conjunction with IVIg. In this patient, IVIg was used primarily as an immunomodulator because IVIg shows an efficacy in treating inflammatory and autoimmune responses, the patient suffered from. Indeed, IVIg may attenuate cytokine-induced NFκβ production; the production of pro-inflammatory cytokines; T-cell activation; and LPS-stimulated cytokine production; IVIg may favour phagocytosis and neutralize infectious agents; and IVIg contains antiidiotypic antibodies against human autoantibodies [63-68]. Importantly, IVIg decreases bacterial translocation beyond the mesenteric lymph nodes and decreases the number of translocated bacteria thus preventing bacterial translocation spread [69]. However, none of the patients in the present study has been treated with IVIg. Since the patients took only NAIOSs, we may conclude that NAIOSs in conjunction with the leaky gut diet may tighten the weakened tight junction barrier in CFS.

The second major finding of this study is that the abovementioned normalization of the IgA and IgM val-

ues in CFS predicts a better clinical outcome. These results support the view that an increased translocation of LPS of gram-negative bacteria is a novel pathway in CFS. Indeed, previously, we reported that the IgM and IgA levels against LPS are significantly increased in patients with CFS as compared with normal controls [1]. In the abovementioned case report, we found that a gradual normalization of the translocation of LPS was accompanied by a gradual clinical improvement and eventually remission of CFS [2]. In the present study, we found that the normalization in serum IgA and IgM directed against LPS predicts the clinical outcome in patients with CFS. This indicates that a normalization of the immune responses to LPS and thus of leaky gut is accompanied by an improvement in the severity of CFS and in some patients to a clinical remission. As explained, the NAIOSs which had been taken by the patients are known to restore the openings of the tight junction barrier which likewise is followed by an attenuation of gut-derived activation of the IO&NS pathways and thus a decrease in the symptoms of CFS. Phrased differently, NAIOSs may have a clinical utility in CFS because they attenuate the IO&NS pathways and restore the gut barrier as well.

The third major finding of this study is that a longer duration of illness and older age are factors that increase the probability towards a worse clinical outcome in CFS. This may be explained since a longer duration of illness may have allowed the IO&NS pathways to be activated for longer times, thus resulting in more damage caused by O&NS, such as lipid peroxidation, damage to DNA and proteins, and consequently a higher probability of autoimmune responses, which frequently occur in severe CFS (Maes et al., in preparation). Also, older age is a predisposing factor toward increased IO&NS responses. Thus, increasing age is accompanied by increased inflammatory [70] and oxidative processes [71].

In conclusion, in this study we report that the normalization of the increased LPS translocation during treatment with specific NAIOSs and the leaky gut diet is accompanied by a clinical improvement or remission of CFS. The results of the present support the view that leaky gut is a novel pathway in CFS. At this point, this condition may be treated by some NAIOSs and the leaky gut diet. However, treatment with these NAIOSs may take a long time (around 1 year) and is rather expensive. Therefore, future drug development in CFS should target the weakening of the tight junction barrier and the subsequent gut-derived inflammation.

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