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Brief report

Increased serum IgA and IgM against LPS of enterobacteria in chronic fatigue syndrome (CFS): Indication for the involvement of gram-negative enterobacteria in the etiology of CFS and for the presence of an increased gut–intestinal permeability

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Abstract

There is now evidence that chronic fatigue syndrome (CFS) is accompanied by immune disorders and by increased oxidative stress.

The present study has been designed in order to examine the serum concentrations of IgA and IgM to LPS of gram-negative enterobacteria, i.e. *Hafnia alvei*; *Pseudomonas aeruginosa*, *Morganella morganii*, *Proteus mirabilis*, *Pseudomonas putida*, *Citrobacter koseri*, and *Klebsiella pneumoniae* in CFS patients, patients with partial CFS and normal controls.

We found that the prevalences and median values for serum IgA against the LPS of enterobacteria are significantly greater in patients with CFS than in normal volunteers and patients with partial CFS. Serum IgA levels were significantly correlated to the severity of illness, as measured by the FibroFatigue scale and to symptoms, such as irritable bowel, muscular tension, fatigue, concentration difficulties, and failing memory.

The results show that enterobacteria are involved in the etiology of CFS and that an increased gut–intestinal permeability has caused an immune response to the LPS of gram-negative enterobacteria. It is suggested that all patients with CFS should be checked by means of the IgA panel used in the present study and accordingly should be treated for increased gut permeability.

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1. Introduction

There is now some evidence that chronic fatigue syndrome (CFS) is accompanied by immune disorders and by increased oxidative stress. Immune activation is suggested by an increased expression of T lymphocyte activation markers, such as CD26 and CD38 and alterations in cytokine production. Poor cellular immunity is suggested by lowered natural killer cell cytotoxicity, decreased mitogen-induced lymphocyte responses and

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<http://hcr3.isiknowledge.com/author.cgi?&link1=Browse&link2=Results&id=5139> (M. Maes).

defects in early T cell activation. Inflammatory reactions are indicated by decreased serum zinc levels and increased serum concentrations of the alpha2 globulin fraction (review: Maes et al., 2005, 2006).

Increased oxidative stress in CFS is suggested by increased levels of isoprostanes and oxidized low density lipoproteins (Kennedy et al., 2005), higher LDL thiobarbituric acid reactive substances (TBARS) and decreased anti-oxidative defences, such as lower serum zinc and dehydroepiandrosterone-sulphate (Vecchiet al., 2003; Maes et al., 2005, 2006).

The occurrence of CFS may not only be triggered by viral and bacterial infections, stressful life events and physical stress, type III–IV allergies for food and heavy metals, but also by an increased permeability of the gut barrier (Maes, 2005).

The present study has been carried out in order to examine whether CFS is accompanied by an increased permeability of the gut barrier whereby an immune response is mounted to endotoxins secreted by gram-negative enterobacteria.

2. Subjects and methods

2.1. Subjects

Forty subjects participated in the present study, 11 unrelated controls (staff or their family members), and 29 patients admitted to the M-Care4U Outpatient Clinics, Belgium. We made the diagnosis of CFS by means of the

Centers for Disease Control and Prevention (CDC) criteria (Fukuda et al., 1994). Patients with chronic fatigue but not fulfilling all diagnostic CFS criteria were classified as suffering from partial CFS. The severity of CFS was measured by means of the FibroFatigue scale, i.e. the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale (Zachrisson et al., 2002). The inclusion and exclusion criteria have been presented elsewhere (Maes et al., 2005, 2006). Patients and controls gave written informed consent after the study protocol was fully explained. The study has been approved by the local ethical committee.

3. Methods

Fasting blood was sampled during the morning hours for the determination of the IgM and IgA against the LPS of 7 different enterobacteria (see Table 1). The analyses were performed by means of an indirect ELISA method according to the methods outlined by the manufacturer (Gemabio, The Ultimate Biopharmaceuticals, France) and described previously (Geffard et al., 2002). 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. The biological interassay CV values are <10%.

4. Statistics

Relationships between variables were assessed by means of Pearson's product moment correlation

Table 1

Age and gender distribution and the measurements of serum IgM and IgA levels against the LPS of *Hafnia alvei*, *Pseudomonas aeruginosa*, *Morganella morganii*, *Proteus mirabilis*, *Pseudomonas putida*, *Citrobacter koseri* and *Klebsiella pneumoniae* in normal controls, patients with partial CFS and patients with CFS

Variables		Normal controls	Partial CFS	CFS	F or χ	p value (df=2/37)
Age sex		41.5 (10.4) 3/8	41.4 (11.0) 4/10	44.5 (10.4) 5/10	F=0.4 $\chi=0.13$	0.7 0.9 (df=2)
<i>Hafnia alvei</i>	IgM	-0.46 (1.12)	-0.19 (1.24)	0.20 (1.32)	F=0.9	0.6
	IgA	-0.84 (0.61)	-0.40 (0.55)	0.43 (1.59)***	F=4.7	0.01
<i>Pseudomonas aeruginosa</i>	IgM	-0.45 (1.23)	-0.10 (0.96)	0.75 (1.53)	F=3.1	0.054
	IgA	-0.02 (1.20)	0.45 (1.69)	2.66 (3.33)***	F=5.0	0.01
<i>Morganella morganii</i>	IgM	-0.25 (0.96)	-0.03 (1.07)	0.76 (1.80)	F=1.6	0.2
	IgA	-0.78 (0.51)	-0.08 (1.13)	2.03 (3.65)***	F=5.2	0.01
<i>Proteus mirabilis</i>	IgM	-1.18 (1.52)	0.22 (1.27)*	0.90 (1.49)**	F=6.9	0.003
	IgA	-0.94 (0.82)	0.19 (1.20)	2.95 (4.33)***	F=6.9	0.003
<i>Pseudomonas putida</i>	IgM	-0.19 (0.92)	0.50 (1.60)	1.44 (1.68)**	F=3.9	0.02
	IgA	-0.45 (0.58)	0.31 (1.21)	3.82 (4.53)***	F=8.5	0.001
<i>Citrobacter koseri</i>	IgM	-0.14 (1.09)	0.39 (1.82)	0.94 (2.10)	F=1.2	0.3
	IgA	-0.44 (0.54)	0.04 (1.11)	3.54 (4.11)***	F=9.5	0.0007
<i>Klebsiella pneumoniae</i>	IgM	-0.69 (0.74)	0.71 (1.76)	1.58 (3.15)**	F=3.3	0.047
	IgA	-0.92 (1.99)	0.28 (1.10)	1.89 (2.61)**	F=6.3	0.004

All results are shown as mean (\pm SD).

*Significantly different from normal controls at $p < 0.01$; **significantly different from normal controls at $p < 0.05$; ***significantly different from normal controls and partial CFS at $p < 0.05$ (all results of Dunn tests).

coefficients, canonical correlation analysis and Spearman's rank order correlations. Group mean differences were examined by means of analysis of variance (ANOVA) or covariance (ANCOVA) and by means of linear discriminant analysis. 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 contingency tables (χ^2 -test) and Fisher's exact probability test.

5. Results

Table 1 shows that there were no significant differences in age or in sex distribution between the study groups. At the $p=0.01$ level, no significant relationships were found between age or gender and the serum IgM and IgA levels. Patients with CFS had significantly greater scores on the FibroFatigue scale than patients with partial CFS (50.9 ± 5.5 versus 32.6 ± 5.3 , $F=84.7$, $df=1/24$, $p < 10^{-5}$).

Table 1 shows that the serum IgM levels against LPS of *Proteus mirabilis* were significantly greater in patients with partial CFS and CFS than in the normal controls. The IgM levels against LPS of *Pseudomonas putida* and *Klebsiella pneumoniae* were significantly higher in CFS patients than in normal controls. We found a significantly greater number of CFS patients (40%) with abnormally increased IgM levels (i.e. anyone of the 7 IgM values >3 Z values) ($\psi=0.47$, $p=0.02$) as compared to partial CFS patients (16.7%) and controls (0%). Serum IgA levels to LPS of all enterobacteria were significantly higher in CFS patients versus normal controls and patients with partial CFS. The prevalence of CFS patients with abnormally increased IgA levels (i.e. anyone of the 7 IgA >3 Z values) was significantly higher in CFS patients (66.7%) than in normal controls (0%, $\psi=-0.68$, $p=0.0006$) and in partial CFS patients (7.1%, $\psi=-0.61$, $p=0.001$).

The severity of the FibroFatigue scale was significantly related to the first principal component (PC) of the IgA ($r=0.55$, $p=0.002$) data, which explained 71.7% of its variance. There were significant correlations between this first PC and muscular tension ($r=0.38$, $p=0.04$), fatigue ($r=0.41$, $p=0.03$), concentration difficulties ($r=0.39$, $p=0.03$), failing memory ($r=0.36$, $p=0.048$), and irritable bowel ($r=0.69$, $p=0.0001$).

6. Discussion

The findings of the present study show that CFS is accompanied by increased serum levels of IgA and, to a

lesser extent, IgM against the LPS of gram-negative enterobacteria.

Increments in serum IgM levels can be seen in mucosal immunity and immune activation, e.g. in the B1 lymphocytes. The latter are a significant source of natural serum IgM, thereby serving as a first line of defence against systemic bacterial and viral infections (Thurnheer et al., 2003). B1 cells can migrate to the intestinal lamina propria and differentiate into IgA-producing serum cells, playing a role in mucosal immunity (Thurnheer et al., 2003). The results of our study suggest that there is a chronic immune response raised to the LPS of enterobacteria.

Many conditions can cause the mucosal barrier to become more permeable, whereby enlarged spaces between the cells of the gut wall cause a loss of the protective barrier. This may induce an increased bacterial translocation and thus increased serum endotoxin concentrations which, in turn, may trigger an immune response (Wu et al., 2004). Thus, the increased serum IgA and IgM levels against the LPS of gram-negative enterobacteria in CFS indicate the presence of an increased gut permeability and an immune response mounted against LPS of the enterobacteria. The relationship established between irritable bowel and the serum IgA to enterobacteria suggests that irritable bowel in CFS reflects in part disorders in gut–intestinal permeability rather than psychological stress as most psychiatrists tend to confirm.

Interestingly, the intestinal barrier may be compromised by factors which are known to trigger CFS, e.g. psychological stress (Meddings and Swain, 2000); sustained strenuous exercise (Davis et al., 2005); food allergies (Andre et al., 1987), surgery and trauma (Pape et al., 1994), but also inflammation. The latter through an increased production of interferon-gamma and interleukin-6 is an essential factor in the loss of the epithelial barrier function (Yang et al., 2003). Normally poorly invasive enterobacteria may, in situations of inflammatory stress, exploit lipid raft-mediated transcytotic pathways to cross the intestinal epithelium, and these effects may precede cytokine-induced disruption of tight junctions (Clark and Diehl, 2002).

The increased gut permeability may also explain the occurrence of autoimmunity in many patients with CFS, such as against neurofilaments, gangliosides, and serotonin (Maes, 2005; Maes et al., in preparation). Thus, enterobacteria may have caused the autoimmunity in CFS, for example, by acting as superantigens for T lymphocytes or by a mechanism called molecular mimicry (Levin et al., 2002). Indeed, those enterobacteria have antigenic sites very similar to those of neuronal tissue and its lipid structures. These antigens in turn

will go into various tissues and trigger inflammation and once autoantibodies are formed the inflammation may become chronic. Thus, various trigger factors, such as viral and bacterial infections, psychological stress, physical exhaustion, food allergies, increased gut permeability or other sources of inflammation, e.g. injury, may induce immune activation, oxidative stress and inflammation and thus the symptoms of chronic fatigue (Maes, 2005). Inflammation may in turn induce an increased gastro-intestinal permeability, which may aggravate the inflammation in a preexisting fatigue syndrome or cause autoimmunity (Maes, 2005).

The results of the present study show that patients with CFS and other forms of chronic fatigue with a known etiology should be checked for the presence of increased gut permeability by the measurements of IgA/IgM against the LPS of gram-negative bacteria. In addition, we suggest that patients with CFS who suffer from an increased gut permeability should be treated with specific antioxidants.

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