The gut-brain barrier in major depression: Intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression

Michael MAES [1], Marta KUBERA [2] and Jean-Claude LEUNIS [3]

1. MCare4U Outpatient Clinics, Belgium;
2. Department of Experimental Neuroendocrinology, Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland;
3. Laboratory Ategis, Waver, Belgium.

Correspondence to: Prof. Dr. M.Maes, M.D., Ph.D. Director: M-Care4U Outpatient Clinics, Olmenlaan 9, 2610 Antwerp, Belgium. TEL: +32-3-4809282 FAX: +32-3-2889185 www.michaelmaes.com E-MAIL: crc.mh@telenet.be

Submitted: 2008-01-06 Accepted: 2008-01-27 Published online: 2008-02-22

Key words: **major depression; chronic fatigue syndrome; inflammation; enterobacteria; leaky gut; gut permeability; cytokines; LPS; oxidative stress**

Neuroendocrinol Lett 2008; **29**(1):117–124 **PMID:** 18283240 NEL290108A12

Abstract

There is now evidence that major depression (MDD) is accompanied by an activation of the inflammatory response system (IRS) and that pro-inflammatory cytokines and lipopolysacharide (LPS) may induce depressive symptoms.

The aim of the present study was to examine whether an increased gastrointestinal permeability with an increased translocation of LPS from gram negative bacteria may play a role in the pathophysiology of MDD. Toward this end, the present study examines the serum concentrations of IgM and IgA against LPS of the gram-negative enterobacteria, Hafnia Alvei, Pseudomonas Aeruginosa, Morganella Morganii, Pseudomonas Putida, Citrobacter Koseri, and Klebsielle Pneumoniae in MDD patients and normal controls.

We found that the prevalences and median values for serum IgM and IgA against LPS of enterobacteria are significantly greater in patients with MDD than in normal volunteers. These differences are significant to the extent that a significant diagnostic performance is obtained, i.e. the area under the ROC curve is 90.1%. The symptom profiles of increased IgM and IgA levels are fatigue, autonomic and gastro-intestinal symptoms and a subjective feeling of infection. The results show that intestinal mucosal dysfunction characterized by an increased translocation of gram-negative bacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. It is suggested that the increased LPS translocation may mount an immune response and thus IRS activation in some patients with MDD and may induce specific "sickness behaviour" symptoms. It is suggested that patients with MDD should be checked for leaky gut by means of the IgM and IgA panel used in the present study and accordingly should be treated for leaky gut.

ORIGINAL ARTICLE

INTRODUCTION

There is now evidence that activation of the inflammatory response system (IRS) plays a role in the pathophysiology of major depression (MDD). This theory has been described as the "cytokine" or "monocyte-T lymphocyte hypothesis" of depression, although recently we described this newly discovered pathway as the "IRS activation" theory [1–4] and now as the "inflammatory and neurodegenerative (I&ND) hypothesis" of depression [5].

The IRS findings in MDD show: a) an increased production of pro-inflammatory cytokines, such as interleukin-6 (IL-6), IL-1β and tumor necrosis factor alpha (TNFα); b) an increased expression of T lymphocyte activation markers, such as HLA-DR$^+$ and CD25$^+$; c) the presence of an acute phase response, with increased serum levels of haptoglobin, decreased serum zinc levels and increased serum concentrations of the alpha2 globulin fraction obtained by electrophoresis; d) and signs of poor cellular immunity, such as lowered natural killer cell cytotoxity and decreased mitogen–induced lymphocyte responses; and e) an increased induction of indoleamine oxidase (IDO) with consequent tryptophan depletion and formation of TRYCATs (tryptophan catabolites along the IDO pathway) [6].

Activation of the IRS is found in animal models of MDD, such as the chronic mild stress and the olfactory bulbectomized rat models of depression [7–10]. Newly generated rat models of depression are based on induced inflammation, e.g. the LPS-induced model [11] and sustained administration of IL-6 by infecting healthy MRL +/+, C3H.SW and Balb/C mice with adenovirus vector carrying cDNA for murine IL-6 or mice infected with Ad5mIL6 adenovirus [12,13].

It is known that systemic LPS and administration of pro-inflammatory cytokines cause chronic central neuroinflammation. For example, systemic LPS results in rapid increases in brain TNFα levels, which may remain elevated for 10 months [14]. Moreover, brain microglia are activated to produce chronically elevated pro-inflammatory factors in the brain [14]. Central neuroinflammation and an increased production of pro-inflammatory cytokines, such as TNFα, IL-1β and IL-6, may induce a behaviour complex, i.e. sickness behaviour, characterized by the appearance of symptoms such as anorexia, psychomotor retardation, malaise, loss of interest, etc. [14]. This symptom complex is quite similar to the symptoms of MDD [15].

External (psychological) as well as internal (organic stressors) stressors, which induce IRS activation, are related to the appearance of depressive episodes. Examples are severe negative life events, some inflammatory and autoimmune illnesses, the postnatal period, etc. Maes et al. [16–18] were the first to show that – in humans – psychological stress induces IRS activation with increased production of pro-inflammatory cytokines, such as IFNγ and TNFα. In experimental animals increased IL-1β and IL-6 levels have been detected in the blood and various brain regions [19]. The IRS activation theory of MDD is fueled by the high comorbidity of MDD with inflammatory disorders such as multiple sclerosis (MS), coronary-heart disorder, HIV-infection, inflammatory bowel disease and rheumatoid arthritis [1,20]. For example, in MS, the depressive episodes are preceded by increased IFNγ production [21], suggesting that IRS activation may underpin depression in MS.

The aim of the present study is to examine another immune pathway which may underpin MDD, i.e. an immune response mounted against an increased translocation of LPS from gram-negative enterobacteria. Recently, we published that the median values for serum IgA and IgM against LPS of enterobacteria are significantly greater in patients with chronic fatigue syndrome (CFS) than in normal volunteers, suggesting that an increased translocation of LPS from enterobacteria is a new pathway underpinning CFS [22,23]. This condition can also be described as increased gut permeability or leaky gut and indicates intestinal mucosal dysfunction (IMD). Since there is a strong degree of comorbidity between MDD and CFS, and since fatigue is one of the key symptoms of major depression and gastrointestinal symptoms frequently occur in MDD [24,25], we hypothesized that also MDD might be accompanied by an increased translocation of LPS.

The specific aims of the present study are to examine whether MDD is accompanied by increased serum levels of IgM and IgA against the LPS of 6 enterobacteria, i.e. Hafnei Alvei, Pseudomanes Aeruginosa, Morganella Morganii, Pseudomanus Putida, Citrobacter Koseri, and Klebsiella Pneumoniae indicating an immune response directed to endotoxins secreted by gram-negative enterobacteria and which cannot be detected when the gut-intestinal lining is intact.

SUBJECTS AND METHODS SUBJECTS

Fifty-one subjects participated in the present study, 23 controls (staff or their family members), and 28 MDD patients admitted to the M-Care4U Outpatient Clinics, Belgium. The patients were classified according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition [26]. We have excluded:

a) subjects with life-time diagnosis of other psychiatric DSM-IV-TR disorders, such as anxiety disorders, schizophrenia, substance use disorders and organic mental disorders;
b) subjects with CFS as diagnosed by the CDC criteria [27]; 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.

The total sum of the FibroFatigue (FF) scale, i.e. the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale [28,29], was used in the MDD patients to compute the severity of specific symptoms, some of which have been shown to be correlated to the IgM and IgA levels against LPS in CFS [22]. 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. 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

Blood was taken during the morning hours for the determination of IgM and IgA against the LPS of 6 different enterobacteria, i.e. Hafnei Alvei, Pseudomanes Aeruginosa, Morganella Morganii, Pseud omanus Putida, Citrobacter Koseri and Klebsiella Pneumoniae. Serum IgM and IgA levels were measured by means of 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

Group mean differences were assessed by means of analysis of variance (ANOVA) or analysis of covariance (ANCOVA). The diagnostic performance was checked by means of ROC (receiver operating characteristics) analysis with computation of the area under the ROC curve, sensitivity, specificity and predictive value of a positive test result (PV+) and with kappa statistics. Data reduction was obtained by means of principal component (PC) analyses. In order to assess the "total LPS translocation load" we have employed three different indices: a) the first PC of the 12 Ig (IgM and IgA) values; b) the total sum of the 12 Ig (IgM and IgA) levels; and c) 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 first PCs of the 6 IgM and 6 IgA data; the peak IgM and peak IgA data; and the total sum of the 6 IgM and 6 IgA data. We employed Pearson's product moment correlation coefficients, Spearman's rank order correlations, and multiple regression analyses in order to examine the relationships between variables. The relationships between the IgM / IgA values and the symptoms of the FF scale were assessed by means of canonical correlation analyses and by means of stepwise (F-to-enter p=0.05) linear discriminant analysis (LDA) with the 12 FF scale items as the discriminatory variables and the dichotomized peak Ig (IgM and IgA) data as grouping variable. The significance was set at α=0.05 (two tailed).

RESULTS

There were no significant differences (F=1.6, df=4/49, p=0.2) in age between normal controls (mean ±SD = 40.0 ± 12.9 years) and MDD patients (44.3 ±11.2 years). There were no significant differences (χ^2 Yates = 0.1, df=1, p=0.7) in the female / male ratio between normal controls (16 / 7) and MDD patients (17 / 11). There were no significant correlations between age and any of the serum IgM or IgA levels directed against the LPS of the different enterobacteria and no significant differences in the serum IgM or IgA values between males and females. The FF score was not significantly different between men and women (F=0.06, df=1/26, p=0.8) and there was no significant correlation between age and the FF score (r=–0.33, p=0.08).

Table 1 shows the serum IgM values in the MDD patients and controls. ANOVAs showed that the IgM levels directed against the LPS from Pseudomonas Aeruginosa and Putida were significantly greater in MDD that in the normal controls. Table 1 shows that there were significant differences between the study groups in the 6 IgA levels against LPS except against Pseudomonas Aeruginosa. Covarying for age and sex did not change any of the above results. By means of Fisher's exact probability test we found a significantly (ψ = 0.50, p=0.0002) greater number of MDD patients (12 / 28) with abnormally increased IgM levels (i.e. anyone of the 6 IgM values > 2 Z values) than in controls (0 / 23). Also, the prevalences of MDD patients (11 / 28) with abnormally increased IgA levels (i.e. anyone of the 6 IgA > 2 Z values) were significantly (ψ = 0.35, p=0.01) higher than in normal controls (2 / 23).

Table 2 gives the peak serum Ig values (IgM or IgA, alone and together), the total sum of the Ig data (IgM or IgA, alone and together) and the first PC of the IgM and the IgA data. However, we were unable to find that one PC could reflect the 12 Ig (IgM and IgA) data: indeed, the first PC of the 12 Ig data explained only 38.2% of the variance, while the 6 IgM variables loaded highly on the first PC and the 6 IgA data on the second PC even

without performing a rotation. Thus, there is no common PC which can reflect the 12 Ig variables. The peak IgM and IgA values and the total sum of all 6 IgM and IgA values as well as the first PC subtracted from the 6 IgM and IgA values were significantly higher in MDD patients than in normal controls. The peak Ig (either IgM or IgA) values and the sum of the 12 Ig values were both significantly higher in MDD patients than in normal controls. Covarying for age and sex in ANCOVAs did not change any of these results. ROC analysis performed on the peak IgM or IgA values showed that the area under the ROC curve (AUC) was highly significant (AUC=90.8%). Accordingly, the diagnostic performance computed was highly significant; at a cut off value for the peak IgM or IgA values > 2.2 Z, the diagnostic performance was: sensitivity=67.9%, specificity=95.6%, and PV+=95.0% (κ=0.62, t=5.66, p=0.00002).

By means of Spearman's rank order correlation coefficients we found a significant positive correlation between the peak IgM-IgA data and the total sum on the FF scale (r=0.49, p=0.007). Two items of the FF scale were significantly related to the peak IgM-IgA data, i.e fatigue (r=0.54, p=0.003) and irritable bowel (r=0.56, p=0.002). Table 3 shows the results of a canonical correlation analysis with peak IgM and peak IgA values as explanatory variables and the 12 items of the FF scale as dependent variables. We found that the symptom profiles of increased IgM and IgA were fatigue, autonomic disturbances, irritable bowel and a subjective feeling of infection. In order to further examine the relationships between serum IgM and IgA levels and the severity of the FF scale, we carried out stepwise LDA with the 12 FF scale symptoms as the explanatory variables and the dichotomized peak IgM (higher versus lower than 2 Z values) and peak IgA (higher versus lower 2 Z values) as groups. We found that sadness (loading=0.71), irritable bowel (loading=0.58) and muscular tension (loading=0.40) were significant discriminatory variables for the dichotomized peak IgM values (F=18.5, df=1/26, p=0.0004). The dichotomized peak IgA groups were best discriminated (F=5.7, df=1/26, p=0.02) using fatigue (loading = 0.86) and irritable bowel (loading = 0.77) as discriminatory variables. Finally, the dichotomized peak IgM or IgA values were best predicted by fatigue (loading = 0.87), irritable bowel (loading = 0.75) and a subjective feeling of infection (loading = 0.65) (F=18.2, df=1/26, p=0.0004).

DISCUSSION

The findings of the present study show that MDD is accompanied by increased serum levels of IgM and IgA directed against LPS of gram-negative enterobacteria and that the IgM-IgA values are related to symptoms reminiscent of MDD and CFS, e.g. fatigue, autonomic and gastro-intestinal symptoms, and a subjective feeling of infection.

The results of our study show that there is an IgM and IgA-related immune response raised to the LPS of enterobacteria in MDD. Increments in serum IgM levels can be seen in immune activation and mucosal immunity. B1 lymphocytes are a significant source of natural serum IgM and they constitute a first line of defence against systemic viral and bacterial infections [30]. Moreover, B1 cells migrate to the intestinal lamina propria to differentiate into IgA-producing serum cells, which in turn play a role in mucosal immunity [30].

The increased serum IgM and IgA levels against LPS in MDD indicate that MDD is accompanied by an increased gut permeability and that there is an immune response directed against LPS of enterobacteria. Indeed, the intestinal epithelial barrier has critical functions, such as a) the formation of a barrier, which separates the luminal contents from the interstitium, and which protects against micro-organisms including gram negative bacteria, larger toxic and antigenic molecules; b) the transportation of fluids, electrolytes and nutrients across the intestinal wall; and c) the secretion of IgA to bind to bacteria thus preventing their attachment to epithelial cells.

The function of the intestinal barrier may be compromised by IRS activation. The latter may cause a loss of the protective barrier function [31–33], which in turn causes enlarged spaces between the cells of the gut wall [31–33]. In this respect, the important inflammatory mediators which induce "leaky gut", IFNγ and IL-6, are both significantly increased in MDD [34,35]. These disruptions of the intestinal epithelium allow normally poorly invasive enterobacteria to exploit lipid raft-mediated transcytotic pathways or the enlarged spaces to cross the gut wall [31–33]. Thus, IRS activation – through an increased production of IFNγ and IL-6 – is an essential factor in the loss of the epithelial barrier function [31,33]. The former may induce an increased translocation of LPS and thus cause increased serum concentrations of LPS which, in turn, may trigger an IgM or IgA-mediated immune response to LPS [22,23].

Systemic increases in LPS not only cause a systemic inflammation, but also a central neuroinflammation; increased brain tumor necrosis factor-α (TNFα) activities, which may remain elevated for 10 months; and activation of brain microglia with a chronically elevated production of pro-inflammatory mediators [14]. It is well-known that an increased production of pro-inflammatory cytokines, such as IL-1, IL-6 and TNFα, either peripheral or central, and brain neuroinflammation may induce the sickness behaviour complex [14]. Also systemic LPS may provoke sickness behaviour [36,37]. As explained previously, symptoms of sickness behaviour, such as anorexia, soporific effects, disturbances of locomotor activity and exploration, and anhedonia bear a strong similarity with those of MDD [15].

Increased LPS translocation may play a role in the O&NS, which occurs in MDD. Indeed, MDD is ac-

Table 1. Measurements of serum IgM levels against the LPS of Hafnia Alvei, Pseudomonas Aeruginosa, Morganella Morganii, Pseudomonas Putida, Citrobacter Koseri and Klebsiella Pneumoniae in normal controls and MDD patients.

Variables		normal controls	MDD	p
Hafnei Alvei	IgM	–0.41 (0.98)	0.33 (2.03)	0.1
	IgA	–0.85 (0.71)	0.32 (2.13)	0.01
Pseudomonas Aeruginosa	IgM	– 0.28 (1.00)	0.68 (1.83)	0.02
	IgA	–0.40 (1.14)	0.27 (1.83)	0.13
Morganella Morganii	IgM	–0.10 (0.91)	0.98 (2.51)	0.052
	IgA	–0.88 (0.58)	1.00 (2.73)	0.002
Pseudomonas Putida	IgM	–0.15 (0.85)	1.19 (2.40)	0.01
	IgA	–0.56 (0.94)	0.26 (1.44)	0.02
Citrobacter Koseri	IgM	–0.18 (1.05)	0.61 (1.81)	0.07
	IgA	–0.60 (0.62)	1.09 (3.16)	0.01
Klebsiella Pneumoniae	IgM	–0.46 (0.76)	0.07 (1.67)	0.17
	IgA	–0.93 (1.39)	0.99 (1.90)	0.0004

All results are shown as mean (±SD). All results of ANOVAs (df=1/49)

Table 2. Measurements of peak IgM or IgA values, total sum of the 6 IgM or 6 IgA data, the first principal component (PC) of the 6 IgM and 6 IgA data, as well as the peak, total sum and the first PC of the 12 Ig (IgM and IgA) data.

Variables	normal controls	MDD	F value	p
Peak IgM	–0.48 (0.83)	2.09 (2.67)	7.7	0.007
Sum of the 6 IgM	–1.57 (4.48)	3.87 (10.63)	5.2	0.02
First PC of the 6 IgM data	2.39 (1.09)	3.68 (2.56)	5.1	0.03
Peak IgA	0.13 (1.12)	2.59 (3.09)	13.2	0.0009
Sum of the 6 IgA	–4.22 (3.81)	3.90 (10.79)	11.8	0.002
First PC of the 6 IgA data	–0.95 (085)	0.78 (2.32)	11.5	0.002
Peak Ig (IgM and IgA) data	0.13 (1.12)	2.59 (3.09)	27.5	0.00003
Sum of the 12 Ig (IgM and IgA) data	–5.79 (6.41)	7.77 (13.89)	18.6	0.0002

All results are shown as mean (±SD). All results of ANOVAs (df=1/49)

Table 3. Results of canonical correlation analysis with the regression of the first principal component (PC) of the IgM and the first PC of the IgA data directed against the LPS of Hafnia Alvei, Pseudomonas Aeruginosa, Morganella Morganii, Pseudomonas Putida, Citrobacter Koseri, and Klebsiella Pneumoniae on the one hand and the symptoms of the FibroFatigue scale, on the other.

IgM	**0.78**
IgA	**0.54**
Aches & pain	0.24
Muscular tension	0.10
Fatigue	**0.36**
Concentration difficulties	0.21
Failing memory	0.28
Irritability	0.28
Sadness	0.24
Sleep disturbances	–0.13
Autonomic disturbances	**0.59**
Irritable bowel	**0.86**
Headache	0.18
Subjective experience of infection	**0.47**
Canonical correlation coefficient	r=0.67

Shown are the significant loadings (> 0.35) of the canonical regressions of the IgM / IgA data on the different symptoms of the FibroFatigue scale.

companied by O&NS as indicated by increased levels of malondialdehyde (MDA), a byproduct of polyunsaturated fatty acid peroxidation and arachidonic acid, 8-hydroxy-2-deoxyguanosine, indicating damage to DNA by oxygen radicals [38–40]; increased catalase and MDA levels [41,42], increased peroxidase and catalase activities in blood and saliva [43]; and increased IgM levels directed against nitro-BSA (bovine serum albumin), indicating increased nitrosative stress [44]. It is well known that LPS causes induction of nuclear factor kappa beta (NFκβ), the major upstream, intracellular mechanism which regulates inflammatory and O&NS mediators, such as cyclo-oxygenase (COX-2) and inducible NO synthase (iNOS) [45,46].

Based on the above, we may conclude that an increased translocation of LPS is another pathway which may explain the inflammatory pathophysiology of MDD and which underpins the link between IMD and MDD. For example, external stressors (psychological stress) may compromise the intestinal barrier [47,48] as well as inducing the cytokine network causing increased IFNγ and IL-6 production [16], which in turn may further endanger the mucosal gut barrier.

Increased LPS translocation offers also an explanation for the increased incidence of MDD during alcohol dependence. Thus, alcoholism is known to induce an increased translocation of LPS [49] and is accompanied by activation of the IRS with an increased catabolism of tryptophan [50,51]. Internal (organic) stressors, such as infections (AIDS/HIV) [52], autoimmune disorders [53] and inflammatory bowel disease [54] – which all show some degree of comorbidity with depression – may endanger gut mucosal function and may increase gut barrier permeability. Thus, the LPS pathway may either primarily (an increased translocation of LPS inducing inflammation) or secondarily (a primary inflammation may induce LPS translocation through increased IL-6 and IFNγ production) be involved in the inflammatory pathophysiology of MDD. Consequently, the intertwined systemic inflammation and the IgM and IgA-mediated immune response following LPS translocation may further aggravate the depressogenic effects of IRS activation.

The symptom profiles of increased IgM and IgA levels to LPS of enterobacteria in MDD are fatigue, sadness, autonomic and gastro-intestinal symptoms, muscular tension, and a subjective feeling of infection. These findings corroborate our previous report that, in CFS, there are significant positive correlations between the IgA responses to LPS of enterobacteria and symptoms of the FibroFatigue scale, such as muscular tension, fatigue, concentration difficulties, failing memory, autonomic disturbances, irritable bowel and the subjective experience of infection [22]. These correlations probably reflect causal relationships between IRS activation and O&NS inducing the abovementioned symptoms occurring in CFS and MDD. As discussed above, LPS and pro-inflammatory cytokines are depressogenic and induce sickness behaviour, e.g. fatigue, anorexia, weight loss, sleep disorders, psychomoror retardation, etc. Also, O&NS is implicated in the production of musceloskeletal pain, muscle fatigue resistance, reduced responses to aerobic exercise and reduced maximal exercise time [55–59]. The significant correlation between gastro-intestinal symptoms and the Ig-mediated immune response against LPS in MDD and in CFS indicates that the gastro-intestinal symptoms in some patients with MDD (and in CFS) reflect – in part – disorders in gut-intestinal permeability and cannot be considered as a symptom of mental stress as most psychiatrists tend to confirm. The significant positive correlation between the IgM and IgA-mediated immune response against LPS and the subjective experience of infection in MDD indicates that the latter is an index of the inflammation from which the patients are suffering. The canonical correlation analysis which was carried out showed also a significant correlation between the Ig-mediated immune response against LPS and autonomic disturbances, such as gastroparesis, neuropathy, vascular neuropathy, and dysautonomia. This may be explained since LPS as well as IRS activation and O&NS have multivarious and profound effects on the autonomous nervous system [60–66].

Increased gut permeability may be another factor explaining the occurrence of autoimmunity in MDD. There are many reports that MDD is accompanied by autoimmune responses, such as against phopholipids [67]. Indeed, enterobacteria may act as superantigens for T lymphocytes or may induce autoimmunity through a mechanism called molecular mimicry [68,69]. This may be explained since enterobacteria have antigenic sites which are very similar to those of the lipid structures of neuronal tissue. These antigens will go into various tissues and trigger inflammation and once autoantibodies are formed the inflammation may become more chronic. Thus, systemic LPS caused by an increased translocation not only induces peripheral inflammation and O&NS, but also induces a central neuroinflammation and eventually an autoimmune responses directed against neuronal tissues.

The results of the present study show that patients with MDD should be checked for the presence of leaky gut by the measurements of IgM and IgA against the LPS of gram-negative bacteria. The results of the present study suggest that MDD patients who have a leaky gut should be treated with specific antioxidants with an efficacity for leaky-gut and a leaky gut diet [23].

Acknowledgments

The research reported was supported by a NARSAD Distinguished researcher award to M.Maes and by M-CARE4U and CRC-MH, Antwerp, Belgium. The secretarial assistance of Indra Corten is greatly appreciated.

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