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ORIGINAL ARTICLE

IgM-mediated autoimmune responses to oxidative specific epitopes, but not nitrosylated adducts, are significantly decreased in pregnancy: association with bacterial translocation, perinatal and lifetime major depression and the tryptophan catabolite (TRYCAT) pathway

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Abstract Immunoglubulin (Ig)M responses directed to oxidative specific epitopes (OSEs) and nitric oxide (NO)-adducts are significantly associated with major depression and physiosomatic symptoms. End of term serum IgM responses to OSEs and NO-adducts were assayed in pregnant women with ($n = 24$) and without prenatal depression ($n = 25$) as well as in 24 nonpregnant women. Associations of IgM/IgA responses to Gram-negative gut commensal bacteria (leaky gut index) and IgA/IgM responses to tryptophan catabolites (TRYCATs) were analyzed. IgM responses to OSEs, but not NO-adducts, were significantly reduced at the end of term. There were no significant associations between IgM responses to OSEs and perinatal depression, whilst IgM responses to NO-adducts, especially NO-cysteinyl, were significantly associated with a lifetime major depression. IgM responses to OSEs and NO-cysteinyl were significantly associated with IgA/IgM responses to Gram-negative bacteria, especially *Morganella morganii*, *Klebsiella pneumoniae* and *Citrobacter koseri*. IgM responses to NO-adducts and OSEs, especially malondialdehyde and myristic acid, and C-reactive protein (CRP) were inversely associated with TRYCAT pathway activity, whilst a lifetime depression and *Pseudomonas putida* were positively associated. The attenuation of natural IgM-mediated responses to OSEs at the end of term may indicate lowered activity of this part of the compensatory (anti-)inflammatory reflex system and may be partly explained by lowered bacterial translocation. Increased IgM responses to NO-cysteinyl is a biomarker of lifetime depression and may be induced by bacterial translocation. Natural IgM-mediated autoimmune responses, increased nitrosylation and higher CRP levels may have negative regulatory effects on the TRYCAT pathway.

Keywords Depression · Inflammation · Immune · Kynurenine · Cytokines · Compensatory (anti-)inflammatory reflex system (CIRS)

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Introduction

Clinical depression is accompanied by increased peripheral immune-inflammation coupled to increased levels of oxidative

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and nitrosative stress (IO&NS), as indicated by raised levels of acute phase proteins, such as C-reactive protein (CRP), pro-inflammatory cytokines, oxidative stress biomarkers and signs of hypernitrosylation (Anderson and Maes 2014). The immune picture in depression shows increased levels of M1 macrophage cytokines, including interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α as well as Th1 cytokines, including interferon (IFN)-γ (Eyre et al. 2013; Maes et al. 2012a, b; Miller et al. 2009). Increased levels of oxidative stress in depression is indicated by damage to cell membranes, lipids, proteins and DNA (Anderson and Maes 2016; Czarny et al. 2016). Increased levels of malondialdehyde (MDA), a toxic product of polyunsaturated lipids degradation following damage by reactive oxygen species (Pryor and Stanley 1975), is one of the most frequently observed biomarkers in unipolar and bipolar depression (Del Rio et al. 2005; Pryor and Stanley 1975).

Major depression, and especially when chronic, is accompanied by increased immunoglobulin (Ig)M-mediated autoimmune responses against oxidative specific epitopes (OSEs), including MDA, azelaic acid (Maes et al. 2013a, b; Maes et al. 2013a, b; Zheng et al. 2013), phosphatidyl inositol (Pi), and the anchorage molecules, myristic acid, palmitic acid and farnesyl-cysteine (Maes et al. 2011a, b; Maes et al. 2012a, b), indicating oxidative damage to membranes and a consequent IgM-mediated natural or regulatory autoimmune response. This IgM response may aid in the clearance of oxidatively damaged, apoptotic or dying cells expressing OSE markers, including MDA (Maes et al. 2011a, b; Maes et al. 2013a, b; Maes et al. 2013a, b; Maes et al. 2011a, b; Maes et al. 2012a, b; Tsuboi et al. 2013; Zheng et al. 2013). These natural IgM antibodies not only recognize neoantigens, including OSEs, but also foreign antigens, including invading pathogens (Kanchanatawan et al. 2017; Schwartz-Albiez et al. 2009). As such, natural IgM antibodies target OSEs thereby attenuating proinflammatory responses and comprise a first-line defence against pathogens.

Clinical depression is accompanied by enhanced levels of inducible NO synthase (iNOS), NO and NO metabolites and increased nitrosation and nitrosylation of proteins (Gałecki et al. 2012; Maes et al. 2013a, b, 2011a, b; Morris et al. 2016a, b). Increased autoimmune responses to NO-adducts have been observed in depression, and especially when chronic, indicating hypernitrosylation (Maes et al. 2013b; 2011a). Nitrosylation may have a regulatory role; for example, it may aid in DNA repair mechanisms, whilst hypernitrosylation may lead to deleterious and neurodegenerative effects (Morris et al. 2016a, b). In addition, some IgM-mediated autoimmune responses to NO-adducts are detrimental and neurotoxic, including increased IgM responses to NO-cysteinyl (Morris et al. 2016a, b; Moylan et al. 2014). No studies have examined these IgM responses to OSEs and NO-adducts during pregnancy or in prenatal depression.

Th1 cytokines, especially IFN-γ, M1 macrophage cytokines and oxidative stress activate indoleamine 2,3-dioxygenase, which drives tryptophan to the production of tryptophan catabolites (TRYCATs), including quinolinic, picolinic and xanthurenic acid, kunurenine and 3-OH-kynurenine (Anderson et al. 2014a, b; Martin-Subero et al. 2016; Morris et al. 2016a, b). Research projects are being performed to clarify as to whether TRYCAT pathway activation is a characteristic of depression per se or physio-somatic (formerly psycho-somatic) that often accompany clinical depression (Anderson et al. 2014a, b). Recently, we found no significant differences in TRYCAT levels in association with perinatal depression, although increased TRYCAT levels were associated with physio-somatic symptoms at the end of term (Maes et al. 2008). To date, no previous studies have investigated associations between TRYCAT pathway activation and IgM-mediated immune responses to OSEs and nitrosylation in perinatal depression.

Increased gut permeability followed by increased bacterial translocation (leaky gut) is another condition that may be associated with clinical depression, especially when chronic (Maes et al. 2008). This condition is indicated by increased serum IgM / IgA responses directed against antigens and LPS of Gram-negative bacteria, including *Hafnia alvei, Pseudomonas aeruginosa, Morganella morganii, Pseudomonas putida, Citrobacter koseri* and *Klebsiella pneumoniae* (Roomruangwong et al. 2016). Bacterial translocation may drive gut-derived inflammation and oxidative stress as well as IgM-mediated autoimmune responses to OSEs and NO-adducts (Anderson et al. 2014a, b; Maes et al. 2013a, b). However, no studies have investigated possible associations between bacterial translocation and IgM-mediated autoimmune responses in the perinatal period.

The aims of the current study are: 1) to assess whether prenatal depression is accompanied by alterations in serum IgM responses to OSEs and NO-adducts and whether there are any changes in these autoimmune responses during pregnancy; and 2) delineate the associations between these IgM-mediated autoimmune responses and IgM/IgA responses to gut commensal bacteria and TRYCAT pathway activation.

Subjects and methods

Participants

Seventy-three participants were recruited, comprised of 24 non-pregnant women and 49 pregnant women who attended the Antenatal Clinic of the King Chulalongkorn Memorial Hospital, Bangkok, Thailand. Inclusion criteria for pregnant women were: a) having planned to give birth and attend postpartum follow ups at the Department of Gynecology, King Chulalongkorn Memorial Hospital; b) 18 years of age or older; and c) being able to read and write Thai language. Exclusion criteria for pregnant women were: a) axis 1 disorders other than bipolar disorder and major depressive disorder, including autism

and schizophrenia; b) positive VDRL or HIV serology; and c) medical or obstetric condition(s) that impede ability to complete the questionnaires. Exclusion criteria for normal controls were current and lifetime diagnoses of any axis 1 disorders. Exclusion criteria for non-pregnant and pregnant women were medical disorders, including immune and autoimmune disorders, hypertension and diabetes. The study was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (IRB No.321/57, COA No. 615/2014). Written informed consent was obtained from all participants.

Measures

Clinical assessments

Pregnant women were evaluated by a senior gynecologist and psychiatrist at 2 time points, namely end of term (T1) and 4–6 weeks after delivery (T2). All non-pregnant women and pregnant women were assessed using a structured interview, including the Edinburgh Postnatal Depression Scale (EPDS), Thai validated translation (Pitanupong et al. 2007; Vacharaporn et al. 2003) and Spielberger's State-Trait Anxiety Inventory (STAI), state version in a Thai validated translation (Spielberger and Vagg 1984; Thapinta 1991) Pregnant women with an EPDS score ≥ 11 were diagnosed as suffering from depression, either antenatal (T1) or postpartum (T2) (Pitanupong et al. 2007; Vacharaporn et al. 2003). The STAI had been used to assess anxiety among pregnant women (Abedian et al. 2015; Bayrampour et al. 2015). In non-pregnant women and pregnant women at T1 we used the Mini International Neuropsychiatric Interview (M.I.N.I) - Thai version (Kittirattanapaiboon and Khamwongpin 2005) – to assess the diagnosis of "a life time history of mood disorders" (namely major depressive disorder, bipolar disorder or dysthymia) and "a life time history of depression" (namely major depressive disorder or dysthymia). At T1, all participants completed a questionnaire to obtain socio-demographic, medical and obstetric information. Body height (in m) was assessed with a manual height measure instrument and body weight in kilograms (kg) with a digital weighing scale. Body mass index (BMI) was calculated using the formula: BMI = body weight (kg) / height $(m)^2$. The senior psychiatrist also assessed premenstrual syndrome (PMS), namely a recurrent pattern of physio-somatic symptoms (body aches, tender breasts, fatigue, bloating) and mood symptoms (depression and anxiety symptoms, tension and irritability, decreased concentration), appearing after ovulation in the luteal phase and resolving with menstruation.

Assays of IgM responses directed to OSEs

At 8.00 a.m., after an overnight fast, blood was sampled in non-pregnant and pregnant woment (at T1) for the assay of

IgM-mediated autoimmune responses directed against OSEs and NO-adducts, IgA/IgM responses directed against antigens/LPS of Gram-negative bacteria and TRYCATs, and serum CRP. An enzyme-linked immunosorbent assay (ELISA) was used to measure IgM levels directed against conjugated azelaic acid, MDA, phosphatidylinositol (Pi) and three conjugated anchorage molecules, namely palmitic acid, myristic acid and farnesyl cysteine (Boullerne et al. 1996; Geffard et al. 2003, 2002). Azelaic acid, myristic acid, palmitic acid and farnesyl cysteine, were linked to free fatty acid BSA. Ten mg of each hapten was dissolved in 2 ml of methanol containing 40 μl of triethylamine (TEA). For each coupling, 20 mg of free fatty acid BSA was dissolved in 2 ml of water containing 40 μl TEA. The carboxylic group of each hapten was activated by ethylchloroformate (ECF) diluted in DMSO. After 10 min, BSA solutions were slowly mixed with the activated hapten solutions. After the coupling, each conjugate was purified by dialysis against phosphate buffer with 10^3 M CaCl2. Phospatidylinositol (Pi) was linked to BSA, as described for conjugated TRYCATs (see below). MDA was linked to BSA using the following protocol: 5.280 ml of MDA was mixed with 720 μl of 4 N HCL in 20 ml of water. After 10 min stirring at 37 °C, this solution was mixed in 20 ml of water containing 200 μl of BSA. The pH was adjusted at 7 during 2 h at 37 °C. Then, the mixture was purified by dialysis with stirring water. Polystyrene 96-well plates (NUNC) were coated with 200 μl solution containing the conjugate at 10 μg/ml for Pi, azelaic, palmitic and myristic acids and at 80 μg/mL for MDA in 0.05 M carbonate buffer at pH 9.6. Well plates were incubated at 4 °C for 16 h under agitation. Then, a 200 μl blocking solution (PBS, 2.5 g/l BSA) was added for 1 h and placed at 37 °C. Following three washes with PBS, plates were filled with 100 μl of sera diluted at 1:1000 in the blocking buffer A (PBS, 2 g/l BSA) and incubated at 37 °C for 1 h 45. After three washes with PBS-0.05% Tween 20, plates were incubated at 37 °C for 1 h with peroxidase-labeled anti-human IgM secondary antibodies diluted respectively at 1: 15,000, in the blocking buffer (PBS, 2 g/l BSA). They were then washed three times with PBS 0.05% Tween 20, and incubated with the detection solution for 10 min in the dark. Chromogen detection solution was used for the peroxidase assay at 8% in 0.1 M acetate and 0.01 M phosphate buffer (pH 5.0) containing 0.01% H_2O_2. The reaction was stopped with 25 μl 2-N HCl. Optical densities (OD) were measured at 492 nm using a multiscan spectrophotometer. All assays were carried out in duplicate.

Assays of IgM responses directed against NO adducts

In order to mimic nitrosylation processes, several conjugates, namely NO-tryptophan (NOW), NO-arginine and NO-cysteinyl, were synthesized by linking haptens to bovine serum albumin (BSA, Sigma-Aldrich) using glutaraldehyde (G) (Boullerne et al.

1996; Geffard et al. 2003, 2002). The synthesis of these conjugates have been described previously (Boullerne et al. 1995). Each hapten conjugate was nitrosylated using sodium nitrite (NaNO$_2$) dissolved in 2 ml of each conjugate, in 0.5 M HCl at 37 °C for 2 h, while shaking in the dark. Conjugates were then dialyzed at 4 °C for 24 h against a Phosphate Buffered Saline (PBS: 10^{-2} M NaH$_2$PO$_4$, 12H$_2$O; 0.15 M NaCl; pH 7.4) solution. S-nitrosothiol bond formation was determined by spectrophotometry. The S-nitrosothiol compounds possess two absorbance maxima, at 336 and 550 nm, respectively: e_{336} nm = 900 M^{-1} cm^{-1} for the conjugates, e_{550}nm = 4000 M^{-1} cm^{-1} for BSA. Absorbance was evaluated in order to determine NO concentrations linked to the compounds.

The assays for the titers of IgM have been extensively described elsewhere (Boullerne et al. 2002; Geffard et al. 2002). Briefly, polystyrene 96-well plates (NUNC) were coated with 200 μl solution containing the conjugates at 10 μg/ml in 0.05 M carbonate buffer at pH 9.6. Well plates were incubated at 4 °C for 16 h under agitation. Then, a 200 μl of blocking solution (PBS, 2.5 g/l BSA) was added for 1 h and placed at 37 °C. Following three washes with PBS, plates were filled up with 100 μl of sera diluted at 1:1000 in the blocking buffer A (PBS, 0.05% Tween 20, 10% Glycerol, 2.5 g/l BSA, 1 g/l BSA-G) and incubated at 37 °C for 2 h. After three washes with PBS-0.05% Tween 20, plates were incubated at 37 °C for 1 h with peroxidase-labeled anti-human IgM secondary antibodies diluted respectively at 1: 15,000, in the blocking buffer (PBS, 0.05% Tween 20, 2.5 g/l BSA). They were then washed three times with PBS-0.05% Tween 20, and incubated with the detection solution for 10 min in the dark. Chromogen detection solution was used for the peroxidase assay at 8% in 0.1 M acetate and 0.01 M phosphate buffer (pH 5.0) containing 0.01% H$_2$O$_2$. The reaction was stopped with 25 μl 2-N HCl. Optical densities (OD) were measured at 492 nm using a multiscan spectrophotometer. All assays were carried out in duplicate. The intra-assay CV values were <6%.

CRP and TRYCAT pathway assays

hs-CRP was measured using the high sensitivity CRP Vario assay (Abbott Laboratories, Abbott Park, Illinois) on Architect cSystems. Intra-assay CV values were <4.0%. The synthesis of TRYCAT conjugates was performed as explained previously (Duleu et al. 2010; Kanchanatawan et al. 2017). The 6 TRYCATs were dissolved in 200 μL dimethylsulfoxide (DMSO) (Acros). Bovine serum albumin (BSA) (ID Bio) was dissolved in 3 mL 2-morpholino-ethanesulfonic acid monohydrate (MES Acros) buffer 10^{-1} M at pH = 6.3 (Acros). The TRYCATs were then mixed with the BSA solution and supplemented with 15 mg N-hydroxysuccinimide (Sigma) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (Acros) as coupling agents. The conjugates were synthesized by linking 3-

OH-kynurenine (3HK) (Sigma), kynurenic acid (KA) (Acros), quinolinic acid (QA) (Acros), anthranilic acid (AA) (Acros), xanthurenic acid (XA) (Acros) and picolinic acid (PA) (Acros) to 20 mg BSA. The coupling reaction proceeded at 37 °C for 1 h in the dark. The coupling was stopped by adding 100 mg hydroxylamine (Sigma-Aldrich) per conjugate. Protein conjugates were dialyzed with 10^{-1} M NaCl solution, pH = 6 for 72 h and the bath solution was changed at least four times per day. The conjugated TRYCATs and BSA concentrations were evaluated by spectrophotometry. The coupling ratio of each conjugate was determined by measuring the concentration of TRYCATs and BSA at 310–330 nm and 280 nm, respectively. ELISA tests were used to determine plasma titers of serum immunoglobulin (Ig) M and IgA. Towards this end, polystyrene 96-well plates (NUNC) were coated with 200 μL solution containing 10–50 μg/mL TRYCAT conjugates in 0.05 M carbonate buffer (pH = 9.6). Well plates were incubated under agitation at 4 °C for 16 h. Then, 200 μL blocking buffer A (Phosphate Buffered Saline, PBS, 2.5 g/L BSA, pH = 7) was applied and all samples were incubated at 37 °C for 1 h. Well plates were washed with PBS solution and filled up with 100 μL serum diluted 1:130 in blocking buffer and incubated at 37 °C for 1 h and 45 min. Well plates were washed 3 times with PBS, 0.05% Tween 20, incubated with peroxidase-labeled goat anti-human IgA (SouthernBiotech) antibodies at 37 °C for 1 h. The goat anti-human IgM antibody was diluted at 1:5.000 and the IgA antibody was diluted at 1:10,000 in blocking buffer (PBS, 2.5 g/L BSA). Plates were then washed three times with PBS, 0.05% Tween 20. Fifty μL of 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate (SouthernBiotech) was added and incubated for 10 min in the dark. The reaction was stopped using 50 :l of TMB stop solution (SouthernBiotech). Optical densities (ODs) were measured at 450 nm using Varioskan Flash (Thermo Scientific). All assays were carried out in duplicate. The analytical intra-assays CV values were <7%. The OD scores were expressed as z scores.

Measurements of IgA/IgM responses to gram-negative bacteria

Antigens derived from the six commensal bacteria were assayed after sonication, namely *C. koseri, H. alvei, K. pneumoniae, M. morganii, P. aeruginosa,* and *P. putida.* Protein concentration was assayed by the Bradford method. Briefly, polystyrene 96-well plates (NUNC) were coated with 200 μl solution containing bacterial components at 4 μg/ml in 0.05 M carbonate buffer at pH 9.6. Well plates were incubated at 4 °C for 16 h under agitation. Then, we added 200 μl blocking solution (PBS, Tween 20 0.05%, 5 g/l BSA) for 1 h and placed at 37 °C. Following two washes with PBS, plates were filled up with 100 μl of sera diluted at 1:1000 in the blocking buffer A (PBS, 0.05% Tween 20, 2.5 g/l BSA) and incubated at 37 °C for 105 min. After three washes with PBS-0.05% Tween 20, plates were incubated at

37 °C for 1 h with peroxidase-labeled anti-human IgM or IgA secondary antibodies diluted respectively at 1: 15,000 and 1: 10,000 in the blocking buffer (PBS, 0.05% Tween 20, 2.5 g/l BSA). Afterwards, plates were washed three times with PBS-0.05%Tween 20, and incubated with the detection solution for 10 min in the dark. Chromogen detection solution (Tetramethylbenzedine) was used for the peroxidase assay at 16.6 ml per liter in 0.11 M sodium acetate trihydrate buffer (pH 5.5) containing 0.01% H_2O_2. The reaction was stopped with 25 µl 2-N HCl. After addition of stop solution (H_2SO_4 or HCl), the obtained, proportional absorbance in the tested sample (compared to established concentration of respective antibodies), was measured at 450 nm with one alpha of correction at 660 nm.

Statistical analysis

We employed analyses of contingency tables (X^2-test) to assess associations between sets of categorical variables and analyses of variance (ANOVAs) to check differences in continuous variables among treatment means. To check post-hoc differences between group means we used protected least significant difference (LSD). Pearson's product moment correlation analyses were employed to assess the univariate correlations between two continuous variables. We used multivariate general linear model (GLM) analyses to assess the multivariate effects of primary explanatory variables on a set of dependent variables. For example, we used the IgM responses to OSEs or NO-adducts as dependent variables and diagnosis as primary explanatory variable while adjusting for background variables, such as age. We also used the IgM responses to OSEs or NO-adducts as dependent variables and IgA/IgM responses to Gram-negative bacteria as primary explanatory variables while adjusting for pregnancy, age, etc. Finally, we used the IgM/IgA responses to Gram-negative bacteria and IgM responses to OSEs and NO-adducts as explanatory variables while indexes of TRYCAT pathway activation were the dependent variables. Consequently, tests for between-subjects effects were employed to examine the effects of the significant explanatory variables on each of the dependent variables. Ln transformations were employed to normalize the data distribution of measures, such as CRP and IgA responses to TRYCATs. All OD values (OSEs, NO-adducts, Gram-negative bacteria, TRYCATS) were transformed into z scores and z unit weighted composite scores were used as pathway indices. Total IgM responses to OSEs (IgM OSEs) or NO adducts (IgM OSE adducts) were computed as the sum of all z scores of all separate OSE or NO measurements, respectively. Total IgM (IgM LPS) or IgA (IgA LPS) responsivity to Gram-negative bacteria (an index of total bacterial load) was assessed as sum of all z scores of IgA or IgM responses to the 6 bacterial antigens. The activity of the TRYCAT pathway was assessed as: sum of the z scores of IgM (IgM TRYCATs) or IgA (IgA TRYCATs) responses to TRYCATs,

the Δ IgA – IgM TRYCAT index (that is IgA TRYCAT – IgM TRYCAT) and IgA KYN/TRY (z score IgA against kynurenine – z score IgA against tryptophan). All statistical analyses were performed using IBM SPSS windows version 22. Tests were 2-tailed and a *p*-value of 0.05 was used for statistical significance.

Results

Descriptive statistics

Table 1 shows the demographic and clinical data of non-pregnant women and pregnant women, divided into those with lower versus higher IgM responses directed against OSEs (median split method). There were no significant differences in age between both groups. Education was somewhat lower in pregnant women than in non-pregnant women. End of term EPDS was significantly greater in pregnant than in non-pregnant women. The STAI and post-natal EPDS values were significantly lower in pregnant as compared to non-pregnant women. There was no difference in the incidence of prenatal depression between the two groups of pregnant women when differentiated by high versus low IgM responses to OSEs. There were no significant differences in IgM responses directed to NO-adducts and IgA responses directed to 6 Gram-negative bacteria or TRYCATs across the three groups. The IgM values directed to OSEs, 6 Gram-negative bacteria or TRYCATs were significantly lower in women belonging to the low IgM group than in the two other groups. The Δ IgA – IgM TRYCAT values did not differ between the subgroups, while the IgA KYN/TRY ratio was significantly higher in non-pregnant females.

Effects of pregnancy and diagnoses on IgM responses to OSEs and NO-adducts

Table 2 shows the results of 2 different multivariate GLM analyses with the IgM responses to OSEs or NO-adducts as dependent variables, and diagnosis-pregnancy (3 groups: pregnant with and without prenatal depression and pregnant women), a lifetime diagnosis of depression and alcohol use during pregnancy as primary explanatory variables, while adjusting for effects of age, BMI, etc. In regression #1 we entered the IgM values against each of the 6 OSE together with IgM OSE as dependent variables. This multivariate GLM analysis revealed significant multivariate effect of diagnosis-pregnancy and use of alcohol. Tests for between-subjects effects revealed a significant effect of diagnosis-pregnancy on all IgM responses directed to OSEs, except azelaic acid, and an effect of alcohol use on IgM response to Pi only. Table 3 shows the estimated marginal mean values (SE) of all OSE values obtained after GLM regression and

Table 1 Demographic data of non-pregnant and pregnant women, divided into those with higher (> −1.8088 z) and lower (<− 1.8088 z) OSE (oxidative specific epitopes) values

Variables	Non-pregnant[a]	Pregnant OSE > −1.8088[b]	Pregnant OSE < −1.8088[c]	F/X^2	df	p
Age (years)	28.1 (3.2)	29.3 (6.9)	27.7 (6.6)	0.53	2/70	0.593
Year education	17.7 (1.3)[b,c]	11.7 (4.6)[a]	12.8 (2.7)[a]	24.69	2/67	<0.001
Actual BMI	20.5 (2.7)[b,c]	26.2 (5.7)[a]	27.1 (3.9)[a]	16.57	2/70	<0.001
EPDS end term	4.3 (3.0)[b,c]	9.4 (6.8)[a]	8.1 (6.5)[a]	5.09	2/69	0.009
STAI end term	49.6 (6.3)[b,c]	43.4 (9.3)[a]	41.6 (11.7)[a]	4.88	2/69	0.010
EPDS T2 postnatal	4.3 (3.0)[b,c]	3.4 (5.1)	2.5 (3.1)	1.22	2/62	0.300
STAI T2 postnatal	49.6 (6.3)[b,c]	35.0 (8.2)[a]	33.2 (6.3)[a]	38.27	2/61	<0.001
Prenatal MDD (N/Y)	24 / 0	12 / 13	13 / 11	NA	-	-
IgM OSE (z score)	3.05 (5.69)[c]	1.45 (2.96)[c]	-4.55 (2.19)[a,b]	25.35	2/70	<0.001
IgM NO (z-score)	−0.39 (1.59)	0.40 (1.65)	−0.03 (2.46)	1.01	2/70	0.369
IgA LPS (z-score)	2.60 (4.93)[c]	0.11 (3.64)[c]	-2.72 (4.77)[a,b]	8.49	2/70	0.001
IgM LPS (z-score)	0.21 (1.11)[c]	0.39 (0.94)[c]	-0.61 (0.60)[a,b]	8.30	2/70	0.001
IgA TRYCATs (z-score)	1.52 (8.4)	−0.43 (0.84)	−1.08 (8.37)	0.71	2/70	0.497
IgM TRYCATs (z score)	1.80 (6.61)[c]	2.70 (5.87)[c]	-4.62 (5.28)[a,b]	10.94	2/70	<0.001
ΔIgA – IgM TRYCATs (z score)	−0.28 (10.75)	−3.13 (8.15)	3.54 (9.58)	3.01	2/70	0.056
IgA KYN/TRY (z score)	0.50 (0.82)[b, c]	-0.39 (1.32)[a]	-0.10 (0.66)[a]	7.08	2/70	0.002
hs CRP (mg/dL)	1.57 (2.04)[b, c]	5.76 (5.49)[a]	4.39 (4.03)[a]	16.92	2/69	<0.001

All data are shown as mean (±SD)

BMI body mass index, *EPDS* Edinburgh Postnatal Depression Scale, *STAI* Spielberger's State Anxiety Inventory, PC PS: first principal component subtracted from the physio-somatic symptoms, *IgM OSE* sum of z-scores of the IgM responses to all OSE measurements, *IgM NO* sum of z-scores of the IgM responses to all NO-adduct measurements, *IgM LPS* sum of z-scores of the IgM responses directed against 6 g-negative bacteria, *IgA LPS* sum of z-scores of the IgA responses directed against 6 g-negative bacteria, *IgM TRYCATs* sum of z-scores of the IgM responses directed against tryptophan catabolites, *IgA TRYCATs* sum of z-scores of the IgA responses directed against tryptophan catabolites, *ΔIgA-IgM TRYCATs* sum of z scores of all IgA responses to TRYCATs – sum of z scores of all IgM responses to TRYCATs, *IgA KYN/TRY* z score IgA directed against kynurenine – z score IgA directed against tryptophan

[a,b,c] Denotes pairwise differences between the three categories

protected post-hoc analyses on the group mean values. All IgM values were significantly lower in pregnant women as compared to nonpregnant women, while there was no significant effect of prenatal depression. The use of alcohol significantly increased IgM responses to Pi. In regression #2 we entered the IgM responses directed to 3 NO-adduct values and IgM NO adducts as dependent variables but we could not find any significant effect of diagnosis-pregnancy, while there were significant multivariate effects of alcohol use and a lifetime diagnosis of depression. Tests for between-subject effects and Table 3 show that alcohol use lowers the IgM responses to NO-tryptophan (NOW), while a lifetime history of depression was accompanied by significantly increased IgM responses to NO-cysteinyl and IgM NO adducts.

Table 2 Results of multivariate GLM analysis with the IgM responses directed against oxidative specific epitopes (OSEs) and 3 nitric oxide (NO) adducts as dependent variables

Tests	Dependent variables	Exploratory variables	F	df	p
Multivariate #1	All 6 OSEs	Diagnosis-pregnancy	4.17	12 / 124	<0.001
		Alcohol	3.43	6 / 62	0.006
		MDD-Life	0.77	6 / 62	0.589
		Age	1.54	6 / 62	0.180
Between-subject effects	IgM MDA	Diagnosis-pregnancy	11.14	1 / 67	<0.001
	IgM Pi	Diagnosis-pregnancy	8.11	1 / 67	0.001
		Alcohol	5.03	1 / 67	0.028
	IgM Palmitic acid	Diagnosis-pregnancy	6.37	1 / 67	0.003
	IgM Myristic acid	Diagnosis-pregnancy	9.25	1 / 67	<0.001
	IgM Farnesyl cysteine	Diagnosis-pregnancy	4.23	1 / 67	0.019
	IgM total	Diagnosis-pregnancy	8.84	1 / 67	<0.001
Multivariate #2	IgM 3 NO adducts	Diagnosis-pregnancy	1.59	6 / 130	0.155
		Alcohol	2.88	3 / 65	0.043
		MDD-Life	3.31	3 / 65	0.025
		Age	1.97	3 / 65	0.128
Between – subject effects	IgM NOW	Alcohol	7.25	1 / 67	0.009
	IgM NO-Cysteinyl	MDD-Life	8.27	1 / 67	0.003
	IgM Total NO	MDD-Life	4.13	1 / 67	0.046

MDD-Life lifetime diagnosis of major depression, *Alcohol* alcohol consumption during pregnancy, *MDA* malondialdehyde, *Pi* phosphatidylinositol, *NOW* NO-tryptophan

Table 3 Estimated marginal means (SE; in z-values) of IgM response to oxidative specific epitopes (OSEs) and NO-adducts obtained by multivariate regression listed in Table 2

Variables	Not pregnant[a]	Pregnant[b]	Pregnant + antenatal depression[c]
IgM MDA	0.99 (0.25)[b,c]	-0.05 (0.24)[a]	-0.17 (0.19)[b]
IgM Azelaic acid	0.50 (0.27)	−0.02 (0.26)	0.22 (0.21)
IgM Pi	0.80 (0.26)[b,c]	−0.22 (0.25)[a]	-0.05 (0.20)[a]
IgM Palmitic acid	0.67 (0.27)[b,c]	-0.21 (0.25)[a]	-0.21 (0.20)[a]
IgM Myristic acid	0.83 (0.26)[b,c]	-0.24 (0.25)[a]	-0.14 (0.20)[a]
IgM Farnesyl Cysteine	0.55 (0.27)[b,c]	-0.16 (0.26)[a]	-0.22 (0.21)[a]
IgM Total OSE	4.34 (1.31)[b,c]	-0.90 (1.25)[a]	-0.57 (1.00)[a]
IgM NO-Arginine	−0.34 (0.28)	0.39 (0.26)	−0.19 (0.21)
IgM NOW	−0.36 (0.27)	0.08 (0.26)	−0.37 (0.21)
IgM NO-Cysteinyl	0.36 (0.27)	0.52 (0.26)	0.22 (0.21)
IgM Total NO	−0.35 (0.52)	0.98 (0.50)	−0.34 (0.40)
Variables	No Alcohol	Alcohol	
IgM Pi	−0.15 (0.16)	0.05 (0.27)	
IgM NOW	0.20 (0.17)	−0.63 (0.29)	
Variables	No MDD-Life	MDD-Life	
IgM NO-Cysteinyl	−0.12 (0.16)	0.85 (0.31)	
IgM Total NO	−0.56 (0.31)	0.75 (0.59)	

MDA malondialdehyde, *Pi* phosphatidylinositol, *NOW* NO-tryptophan, *Alcohol* consumption during pregnancy, *MDD-Life* lifetime diagnosis of major depression

[a,b,c] Denotes pairwise differences between the three categories

Associations between commensal bacteria and IgM responses to OSEs

Using correlation analyses we detected significant correlations between IgA LPS and IgM OSEs ($r = 0.375$, $p = 0.001$), IgM responses to MDA ($r = 0.401$, $p = 0.001$), IgM responses to azelaic acid ($r = 0.233$, $p = 0.047$), IgM responses to Pi ($r = 0.472$, $p < 0.001$), IgM responses to palmitic acid ($r = 0.279$, $p = 0.017$), and IgM responses to myristic acid ($r = 0.376$, $p = 0.001$). There were also highly significant correlations between IgM LPS and IgM OSEs ($r = 0.760$, $p < 0.001$) and with all separate OSEs ($r > 0.560$, $p < 0.001$). Table 4 shows the outcomes of multivariate GLM analysis #1 and #2 with all IgM responses to OSEs as dependent variables and IgA and IgM LPS as explanatory variables. We found highly significant multivariate effects of IgA and IgM LPS on all OSEs variables. Tests for between-subject effects revealed that all IgM responses to OSEs were positively associated with IgA LPS and IgM LPS. In order to delineate which bacteria are most important we have carried out additional analyses will all 6 Gram-negative bacteria as dependent variables (IgM or IgA). Multivariate GLM analysis #3 shows a multivariate effect of the IgA responses to *Morganella* on the IgM OSE values, while univariate analysis showed an effect on IgM responses to Pi and myristic acid. Multivariate GLM analysis #4 shows a multivariate effect of the IgM responses to *K. pneumoniae* and *C. koseri*. Univariate analyses showed that IgM responses to *K. pneumoniae* were associated with IgM responses to azelaic acid, Pi and all OSEs together, while *C. koseri* was significantly associated with IgM responses to all OSEs.

Associations between commensal bacteria and IgM responses to NO-adducts

Using correlation analyses, we detected significant correlations between IgA LPS and IgM responses to NOW ($r = −0.307$, $p = 0.008$) but not the other NO-adducts. Correlation analyses showed significant correlations between IgM LPS and IgM responses to NO cysteinyl ($r = −0.413$, $p < 0.001$). Table 5, multivariate GLM analysis #1 shows no significant multivariate association between IgA LPS and IgM NO-adducts, while regression #2 shows that there was a significant multivariate association between IgM LPS and IgM responses to NO-adducts. Univariate analysis showed a significant association with IgM responses to NO-cysteinyl only.

Multivariate analysis #3 shows that IgA responses to *C. koseri* had a significant multivariate effect on the IgM against NO-adducts while univariate effects shows a significant effect on IgM NO-cysteinyl only. Multivariate analysis #4 shows that IgM responses to *C. koseri* had a significant multivariate effect, while tests for between-subjects effects showed an effect of IgM responses to *C. koseri* on NO-cysteinyl only.

Associations between the TRYCAT pathway and IgM responses to OSEs and NO-adducts

In order to examine the associations between IgM/IgA against TRYCATs, on the one hand, and IgM/IgA LPS, IgM OSEs and IgM NO-adducts, CRP and a lifetime history of PMS and depression, on the other, we performed a multivariate GLM analysis with the TRYCATs as dependent variables (entered as IgM TRYCATs, IgA TRYCATs, ΔIgA - IgM TRYCATs, and IgA

Table 4 Results of multivariate GLM analysis with IgM response directed against oxidative specific epitopes (OSEs) as dependent variables

Test	Dependent variables	Independent variables	F	df	p
Multivariate #1	IgM all OSEs	IgA LPS	5.37	6/66	<0.001
Between- subject effects	IgM MDA	IgA LPS	10.12	1/71	0.002
	IgM Azelaic acid	IgA LPS	4.71	1/71	0.033
	IgM Pi	IgA LPS	16.13	1/71	<0.001
	IgM Palmitic acid	IgA LPS	7.03	1/71	0.010
	IgM Myristic acid	IgA LPS	14.81	1/71	<0.001
	IgM Farnesyl-Cysteine	IgA LPS	5.20	1/71	0.026
	IgM Total OSEs	IgA LPS	13.31	1/71	0.001
Multivariate #2	IgM All OSEs	IgM LPS	25.23	6/65	<0.001
		Pregnancy	8.59	6/65	<0.001
Between- subject effects	IgM MDA	IgM LPS	33.23	1/70	<0.001
	IgM Azelaic acid	IgM LPS	49.73	1/70	<0.001
	IgM Pi	IgM LPS	130.26	1/70	<0.001
	IgM Palmitic acid	IgM LPS	33.29	1/70	<0.001
	IgM Myristic acid	IgM LPS	65.76	1/70	<0.001
	IgM Farnesyl-Cysteine	IgM LPS	43.50	1/70	<0.001
	IgM Total OSEs	IgM LPS	16.64	1/70	<0.001
Multivariate #3	IgM All OSEs	IgA *Morganella morganii*	4.48	6/64	0.001
Between-subject effects	IgM Pi	IgA *M. morganii*	7.96	1/71	0.006
	IgM Myristic Acid	IgA *M. morganii*	7.81	1/71	0.007
Multivariate #4	IgM all OSEs	IgM *Klebsiella pneumoniae*	3.83	6/65	0.002
		IgM *Citrobacter koseri*	5.51	6/65	<0.001
Between- subject effects	IgM Azelaic acid	IgM *K. pneumoniae*	11.97	1/70	0.001
	IgM Pi	IgM *K. pneumoniae*	16.53	1/70	<0.001
	IgM Total OSEs	IgM *K. pneumoniae*	6.23	1/70	0.015
Between- subject effects	IgM MDA	IgM *C. koseri*	10.25	1/70	0.006
	IgM Azelaic acid	IgM *C. koseri*	5.77	1/70	0.008
	IgM Pi	IgM *C. koseri*	26.86	1/70	<0.001
	IgM Palmitic acid	IgM *C. koseri*	13.65	1/70	<0.001
	IgM Myristic acid	IgM *C. koseri*	25.51	1/70	<0.001
	IgM Farnesyl-cysteine	IgM *C. koseri*	15.77	1/70	<0.001
	IgM Total OSEs	IgM *C. koseri*	28.15	1/70	<0.001

MDA malondialdehyde, *Pi* phosphatidylinositol, *NOW* NO-tryptophan

KYN/TRY). Table 6 shows the outcome of this multivariate analysis. We found significant effects of a lifetime history of PMS and major depression, IgM OSEs and IgM NO-adducts, IgA LPS (but not IgM LPS) and CRP. Tests for between-subject effects showed that IgA TRYCATs was strongly associated with IgA LPS and PMS (both positively) and CRP (inversely). IgM TRYCATs were positively associated with IgM OSEs. The ΔIgA - IgM TRYCAT values were significantly predicted by

Table 5 Results of multivariate GLM analysis with IgM response directed against nitric oxide (NO) adducts as dependent variables

Tests	Dependent variables	Independent variables	F	df	p
Multivariate #1	IgM all NO adducts	IgA LPS	2.40	3/67	0.076
		MDD-Life	2.73	3/67	0.051
		Alcohol	2.49	3/67	0.068
Multivariate #2	IgM all NO adducts	IgM LPS	5.33	3/67	0.002
		MDD-Life	3.42	3/67	0.022
		Alcohol	1.81	3/67	0.155
Between- subject effects	IgM NO Arginine	IgM LPS	1.51	1/69	0.223
	IgM NOW	IgM LPS	1.87	1/69	0.176
	IgM NO-Cysteine	IgM LPS	15.32	1/69	<0.001
	IgM Total NO	IgM LPS	0.20	1/69	0.659
Multivariate #3	IgM all NO adducts	IgA *Citrobacter koseri*	3.82	3/67	0.014
		MDD-Life	2.74	3/67	0.050
		Alcohol	2.08	3/67	0.111
Between- subject effects	IgM NO-Cysteinyl	IgA *C. koseri*	8.72	1/69	0.004
Multivariate #4	IgM all NO adducts	IgM *C. koseri*	7.22	1/69	<0.001
		MDD-Life	2.56	3/67	0.062
		Alcohol	1.77	3/67	0.162
Between- subject effects	IgM NO-Cysteinyl	IgM *C. koseri*	20.50	1/69	<0.001

MDA malondialdehyde, *Pi* phosphatidylinositol, *NOW* NO-tryptophan, *Alcohol* consumption during pregnancy, *MDD-Life* lifetime diagnosis of major depression

Table 6 Results of multivariate GLM analysis with the tryptophan catabolite (TRYCAT) data as dependent variables

Tests	Dependent variables	Exploratory variables	F	df	p
Multivariate	ΔIgA-IgM TRYCATs, IgA TRYCATs, IgM TRYCATs, and IgA KYN/TRY	PMS lifetime	4.16	3/63	0.009
		MDD Lifetime	2.95	3/63	0.039
		IgM OSEs	29.66	3/63	<0.001
		IgM NO-adducts	4.13	3/63	0.010
		IgA LPS	8.52	3/63	<0.001
		CRP	5.23	3/63	0.003
Between- subject effects	IgA All TRYCATs	PMS lifetime (+)	9.45	1/65	0.003
		IgA LPS (+)	24.53	1/65	<0.001
		CRP (−)	4.54	1/65	0.037
	IgM All TRYCATs	IgM Total OSEs (+)	88.66	1/65	<0.001
	Δ IgA-IgM TRYCATs	IgA LPS (+)	22.87	1/65	<0.001
		IgM OSEs (−)	44.05	1/65	<0.001
		CRP (−)	4.54	1/65	0.037
	IgA KYN/TRY	MDD lifetime (+)	7.75	1/65	0.007
		IgM NO (−)	12.21	1/65	0.001
		CRP (−)	14.07	1/65	<0.001

PMS lifetime lifetime history of pre-menstrual syndrome, *MDD lifetime* lifetime history of major depression, *IgM TRYCATs* sum of z-scores of the IgM responses directed against tryptophan catabolites, *IgA: TRYCATs* sum of z-scores of the IgA responses directed against tryptophan catabolites, *ΔIgA-IgM TRYCATs* sum of z scores of all IgA responses to TRYCATs – sum of z scores of all IgM responses to TRYCATs, *IgA KYN/TRY* z score IgA directed against kynurenine – z score IgA directed against tryptophan, *IgM OSE* sum of z-scores of the IgM responses to all OSE measurements, *IgM NO* sum of z-scores of the IgM responses to all NO-adduct measurements, *IgA LPS* sum of z-scores of the IgA responses directed against 6 g-negative bacteria, *CRP* C-reactive protein

IgA LPS (positively) and were negatively correlated with CRP and IgM OSEs. The IgA KYN/TRY ratio was predicted by IgM NO-adducts, CRP and a lifetime history of depression.

Best prediction of the TRYCAT pathway biomarkers

Consequently, we have examined which OSEs, NO-adducts and bacteria were most significant in predicting the TRYCAT variables. This analysis showed effects of IgM responses to MDA (F = 6.24, df = 3/63, p = 0.001), myristic acid (F = 6.60, df = 3/63, p = 0.001), and NO-cysteinyl (F = 9.35, df = 3/63, p < 0.001) and IgA responses to *P. putida* (F = 4.46, df = 3/63, p = 0.007) on the TRYCAT variables. Tests for between-subject effects showed that IgM responses to MDA were associated with IgM TRYCATs (F = 17.98, df = 1/65, p < 0.001, positively) and ΔIgA - IgM TRYCATs values (F = 9.12, df = 1/65, p = 0.002, negatively). Also, the IgM responses to myristic acid were associated with IgM TRYCATs (F = 19.98, df = 1/65, p < 0.001, positively) and ΔIgA - IgM TRYCATs values (F = 7.29, df = 1/65, p = 0.009, negatively). The IgM responses to NO-cysteinyl predicted the IgA KYN/TRY ratio (F = 19.74, df = 1/65, p < 0.001), while IgA responses to *P. putida* were positively related to ΔIgA - IgM TRYCATs values (F = 4.46, df = 3/63, p = 0.007).

Discussion

The first major finding of this study is that pregnancy is accompanied by highly significant decreases in IgM responses directed against OSEs, including MDA, Pi and anchorage molecules, such as myristic and palmitoylic acid and farnesyl cysteine. The lowered IgM-mediated autoimmune responses to these molecules expressed on outer and inner membrane may indicate lowered regulatory IgM autoimmune responses, reflective of impaired normal regulatory responses that neutralize cellular debris, such as apoptotic and dying cells expressing MDA and oxidized low-density lipoprotein (LDL), arising from oxidative stress-induced damage (Rahman et al. 2016). Previous data indicates that pregnancy is accompanied by increased levels of advanced oxidation protein products, suggesting increased protein oxidation (Fialová et al. 2003). Thus, the lowered levels of regulatory IgM responses directed to OSEs could contribute to an enhancement of the detrimental consequences of oxidative stress, thereby promoting more inflammation (Frostegård 2013; Rahman et al. 2016). Previously, we have discussed the role of IgM-mediated regulatory responses as part of the compensatory (anti-)inflammatory reflex system (CIRS) in neuro-psychiatric disorders (Kanchanatawan et al. 2017; Maes et al. 2011a, b; Morris and Maes 2013). Moreover, the Th2 cytokine predominance (humoral immunity) in early pregnancy gradually shifts towards a Th1 (cell-mediated immunity) predominance in late gestation (Challis et al. 2009). This together with lowered regulatory IgM-mediated natural autoimmune responses may play a role in the increased incidence of some autoimmune disorders during pregnancy or complicated pregnancies in women who suffer from autoimmune disease (Challis et al. 2009).

While the IgM responses to OSEs are significantly suppressed during pregnancy, the IgM responses to NO-adducts were unaffected. S-nitrosylation is one of the mechanisms

underlying uterine smooth muscles relaxation effects of NO (Ulrich et al. 2012). In the guinea pig, the same authors detected protein S-nitrosylation, which plays a role in uterine relaxation and contraction, and are increased independently of raised levels of protein expression. In sheep, pregnancy increases total S-nitrosylation but decreases S-nitrosylation of the endothelial NO synthesizing system (Zhang et al. 2012).

In the current study, perinatal depression was not associated with IgM responses directed against OSEs and NO-adducts. These results, however, do not preclude that perinatal depression may be accompanied by O&NS. No previous studies have investigated oxidative stress in perinatal depression. Nevertheless, a hypothesis had been proposed describing a possible relationship between perinatal depression, oxidative stress, and risk for pregnancy-associated cardiovascular diseases (CVD) (Nicholson et al. 2016). An animal study showed an association of lowered maternal care, a possible indicator of maternal emotional problems, and increased oxidative stress in the brain of nursing mothers (Nicholson et al. 2016). It is not unlikely that lowered IgM-mediated regulatory responses during pregnancy may play a role in the increased incidence of cardiovascular complications during pregnancy and in women of child-bearing age (Mohamed 2014). Indeed, attenuation of the regulatory autoimmune responses, which neutralize apoptotic cells expressing oxidized LDL and MDA (Rahman et al. 2016), may be associated with increased expression of oxidized LDL in pregnancy (Ghaneei et al. 2015).

Our results show that alcohol abuse affects IgM responses to Pi. This is consistent with a study reporting higher antibodies directed against Pi in patients with alcoholic liver disease, especially in alcoholic patients with more severe liver damage (Chedid et al. 1994). Moreover, chronic alcohol consumption may enhance reactive oxygen species (ROS) in mitochondria, thereby affecting mitochondrial functions, including raising levels of mitochondrial cytochrome P450 (CYP2E1) (Adam-Vizi 2005; Albano 2006; Manzo-Avalos and Saavedra-Molina 2010; Ronis et al. 1996), thereby contributing to pathological swelling and mitochondrial dysfunction (Wakabayashi 2002). Interestingly, Pi may be found in the mitochondria outer membrane (Wakabayashi 2002) and, therefore, when exposed to alcohol-induced ROS, the outer membrane of mega mitochondrial complexes may rupture (Manzo-Avalos and Saavedra-Molina 2010; Wakabayashi 2002), leading to increased IgM responses to Pi.

Another major finding of this study is that the natural IgM mediated autoimmune responses directed against OSEs are associated with indices of increased IgA/IgM responses to Gram-negative bacteria, especially *M. morganii*, *C. koseri*, and *K. pneumoniae*. This may suggest that natural IgM-mediated immune responses to OSEs are driven by increased bacterial translocation of Gram-negative bacteria and that suppressed IgM-mediated immune responses to OSEs in pregnancy are a consequence of lowered bacterial translocation in pregnancy. In

general, the gut microbial composition changes drastically from early to late pregnancy and the gut mucosal surface may present low grade inflammation (Koren et al. 2012), while less inflammatory responsivity may be present at the placental interface (Mor and Cardenas 2010). Future research should examine the associations between bacterial translocation, gut-inflammation, O&NS, IgM-mediated autoimmune responses and placental interface immune system activity.

Another major finding of this study is that IgM responses to nitrosylated proteins (NO-adducts) are strongly associated with a lifetime diagnosis of depression and increased bacterial translocation, as measured with IgM/IgA directed to Gram-negative bacteria, especially *C. koseri*. Importantly, major depressive disorder is associated with increased IgM responses directed against SNO-cysteinyl, a potential neurotoxic compound (Maes et al. 2011a, b). This finding is in agreement with previous research showing increased IgM responses to NO-adducts in general and SNO-cysteinyl in depression (Maes et al. 2013a, b). Studies among MS patients found significantly increased IgM-mediated autoimmune responses directed against SNO-cysteine, which is proposed to play a role in the demyelination that characterizes MS (Boullerne et al. 1996, 1995). Previously, we have shown that increased bacterial translocation (as assessed by increased levels of IgA directed against Gram-negative bacteria) is associated with increased IgM response to NO-adducts (Maes et al. 2013a, b).

A further major finding of this study is that the activation of the TRYCAT pathway is strongly predicted by the combined effects of lifetime diagnoses of depression and PMS, bacterial translocation (IgA responses to Gram-negative bacteria) (all three positively) and IgM responses to OSEs and NO adducts and CRP (all three negatively associated). This indicates that major depressive disorder and PMS may be accompanied by a chronic activation of the TRYCAT pathway. The findings are also in agreement with the activation of the TRYCAT pathway by LPS directly, or indirectly by LPS-induced Th1 and M1 macrophagic responses (Martinez et al. 2008). The inverse association between TRYCAT pathway activation and IgM responses to OSEs may indicate that the more general regulatory functions exerted by these autoimmune responses also modulate the TRYCAT pathway, for example by lowering inflammatory and oxidative responses and/or by regulating the activity of the pathway.

Likewise the inverse association between TRYCAT pathway activity and IgM responses to NO-adducts may be explained by direct negative regulatory effects of NO on IDO production (Hucke et al. 2004) or by regulatory effects of nitrosylation on inflammatory responses, for example by inhibiting nuclear factor-κB (Prasad 2007). We found also a negative correlation between TRYCAT pathway activation and CRP, although some previous studies detected a positive correlation between elevated CRP and increased IDO activity in patients with ischemic stroke (Mo et al. 2014). Nevertheless, CRP also displays anti-
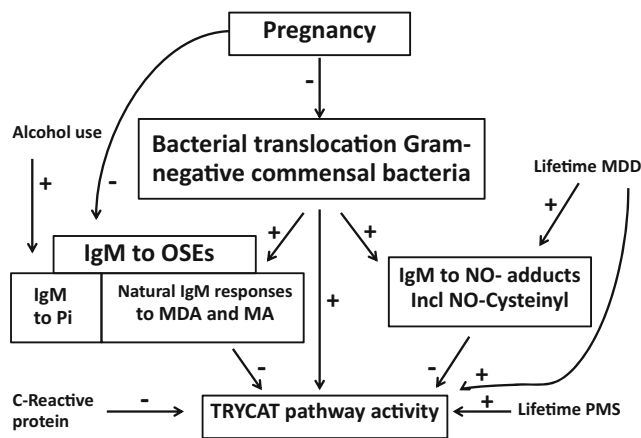
Fig. 1 Summary of our findings

inflammatory effects (Ridker and Lüscher 2014) for example, by preventing neutrophil-endothelial adhesion (Thiele et al. 2014) or decreased production of pro-inflammatory and anti-inflammatory cytokines in target areas (Mold and Clos 2013). All in all, it appears that the TRYCAT pathway is regulated in a complex manner by many different processes. It should also be borne in mind that the placenta is a significant source of melatonin for mother and foetus, which has antioxidant and anti-inflammatory effects, as well as protecting mitochondria at the consequences of CYP2E1 induction (Liang et al. 2012).

This paper has some limitations and strengths that should be considered when interpreting the results. A limitation is that this study is a case-control study and thus no inferences can be made on causality. It would have been more informative if we had measured alterations in serum IgM responses to OSEs and NO-adducts 4–6 weeks after delivery. A first strength is that in this study multivariate GLM analyses were employed and that univariate associations were only interpreted when there were significant overall multivariate effects, thereby reducing type I errors. Secondly, we adjusted for many different background variables and examined the cumulative effects of the predictor variables using multivariate GLM analyses.

Figure 1 summarizes the findings of this study. Natural IgM-mediated autoimmune responses directed against OSEs are significantly decreased in pregnancy. It is hypothesized that bacterial translocation may drive the IgM-mediated immune responses and that since bacterial translocation is lowered during pregnancy the IgM responses may be attenuated. Alcohol use during pregnancy may increase IgM responses to phosphatidylinositol (Pi). Increased IgM-mediated autoimmune responses directed against NO-adducts and especially NO-cysteinyl are associated with lifetime major depressive disorder (MDD). Tryptophan catabolite (TRYCAT) pathway activity is regulated by many processes including bacterial translocation and lifetime histories of premenstrual syndrome (PMS) and MDD (upregulation), while different factors may downregulate TRYCAT pathway activity, namely IgM-mediated natural autoimmune responses to malondialdehyde (MDA) and myristic

acid (MA) (possibly via natural regulatory effects), hypernitrosylation (possibly by inhibiting indoleamine-2,3-dioxygenase) and C-reactive protein (CRP) (possibly through its anti-inflammatory effects).

Compliance with ethical standards

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