

Maternal mood scores in mid-pregnancy are related to aspects of neonatal immune function

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ABSTRACT

Background: Although there are recognised associations between psychological and immune function, the effects of maternal depressive symptoms on fetal immune development have not been investigated.

Methods: This study examined the relationship between maternal depression scores as assessed by the Beck Depression Inventory (BDI) in the second trimester and measure of neonatal immune function measured in cord blood. This study was conducted in a cohort of women ($n = 83$) who had received either fish oil containing 3.7 g/day *n*-3 polyunsaturated fatty acid (*n*-3PUFA) or a placebo from 20 weeks gestation as part of a randomised controlled trial.

Results: At 20 weeks gestation, prior to the intervention, 22% of women in the study manifested mild to moderate depressive symptoms ($\text{BDI} \geq 10$). Neonates of these women had higher lymphoproliferative responses to a range of stimuli (including egg ovalbumin and cat allergen) compared with neonates of women with normal BDI scores (<10). These neonates also showed higher spontaneous cytokine production including (IL-6 and IL-10) and higher stimulated cytokine responses to both bacterial antigens and allergens. These patterns were evident after allowing for maternal age and education, parity, gestation, infant gender, delivery method and neonatal *n*-3/*n*-6 PUFA status.

Conclusion: This exploratory study supports the notion that maternal mood in pregnancy may have the potential to influence fetal immune development. Further studies are needed to determine the significance of this.

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1. Background

Patterns of immune programming during early development have implications for subsequent health and disease risk. While there is evidence that a range of environmental exposures in pregnancy can influence immune development in humans (such as maternal smoking (Noakes et al., 2006), diet (Dunstan et al., 2003) and microbial exposure (Hughes et al., 1999; Xu et al., 1999)), there are only preliminary studies examining the potential effects of maternal psychological factors on infant immune function. While interactions during this early period are likely to be complex and multifactorial, to our knowledge this is the first human study to investigate the effects of maternal mood in pregnancy on neonatal cellular immune function.

Maternal psychological factors such as depressive mood stress and anxiety and are known to be associated higher levels of pro-inflammatory cytokines (such as IL-6 and $\text{TNF}\alpha$) in both human pregnancy (Coussons-Read et al., 2007, 2005; Maes et al., 2000) and animal models (O'Mahony et al., 2006). This is consistent with higher pro-inflammatory cytokines in non-pregnant adults with depression (Dentino et al., 1999; Maes et al., 1995). Associated activation of the maternal hypothalamic pituitary adrenal axis (HPA) with increased corticotropin releasing hormone (CRH) reduces progesterone production and has down-stream effects on immunomodulation at the materno-fetal interface (Knackstedt et al., 2005). The resulting increase in T helper type 1 (Th1) and inflammatory cytokines (such as $\text{TNF}\alpha$) at the mater-

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² Abbreviations used: BDI, Beck Depression Inventory; PUFA, Polyunsaturated fatty acid; IFN γ , Interferon gamma; SEB, Staphylococcus aureus enterotoxin B; TH1, T helper cell type 1; TH2, T helper cell type 2; HPA, Hypothalamic pituitary adrenal; IL, Interleukin; $\text{TNF}\alpha$, Tumour necrosis factor alpha; IgE, Immunoglobulin E; CD8, Cluster of differentiation 8; DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; CI, Confidence interval; HDM, House dust mite; OVA, Ovalbumin.

no-fetal interface may be maladaptive in this context foreseeably compromising placental blood flow, fetal growth and many aspects of fetal development, including subsequent immunity. While the long term consequences of this increased low-grade inflammation at the materno-fetal interface are not clear, there is some evidence that activation of the HPA in pregnancy (Kapoor et al., 2006, 2008) and inflammation at the materno-fetal interface (Ashdown et al., 2006) are implicated in adverse neurological and cardiovascular development and behavioural disorders in offspring (reviewed in Knackstedt et al., 2005). It has also been proposed that adverse maternal psychological factors may influence predisposition to allergic disease by modifying both organ development and the relative balance of Th1 and Th2 cytokine responses (Knackstedt et al., 2005). So far, the only study to investigate this in human pregnancies found that perceived psychological factors ('discouragement', 'nervousness', 'exhaustion', 'tiredness', 'working stress' and 'anxiety') were associated with elevated neonatal IgE antibody levels detected in cord blood (Lin et al., 2004).

The main aim of the present study was to examine the relationship between maternal depression scores in pregnancy and neonatal cellular immune function allowing for other potential interactions. We measured lymphoproliferation and a range of cytokines to assess Th1 function (IFN γ), Th2 function (IL-13) regulatory function (IL-10) as well as the production of inflammatory cytokines (IL-6). The stimuli used included innocuous environmental antigens (a food and inhalant allergen) as well as bacterial antigens and a non-specific polyclonal stimulus. We performed the study in an existing pregnancy cohort (Dunstan et al., 2003) who received a potential "mood modifying" intervention during pregnancy. Specifically, pregnant women were randomised to either (i) high dose fish oil from 20 weeks gestation (4 g/day) or (ii) a placebo (olive oil). Fish oil has been of interest as a strategy for reducing the incidence of postnatal depression, although its use in context is still controversial (Browne et al., 2006; Freeman et al., 2006; Marangell et al., 2004; Miyake et al., 2006). Although this study was originally designed to assess the role of fish oil in allergy prevention, the cohort provided a valuable opportunity to examine the relationship between maternal depressive symptoms and infant immune function at birth (after controlling for any effects of the fish oil intervention on this).

2. Methods

2.1. Participants

The original cohort of 98 pregnant women with allergic disease were recruited prior to 20 weeks of pregnancy as part of a randomised control trial (below) (Dunstan et al., 2003). Maternal atopy was defined as a previous history of doctor-diagnosed allergic rhinitis and/or asthma plus one or more positive (wheal diameter > 3 mm) skin prick tests to common allergens. None of the women had evidence of severe disease (requiring regular corticosteroid prophylaxis) in pregnancy. Women were excluded from the study if they were smokers, had other pre-existing medical conditions, experienced complicated pregnancies, manifested seafood allergies, consumed more than two fish meals per week or were already taking fish oil supplements.

2.2. Study design and intervention

Participants were randomly assigned to receive either a daily fish oil supplement of 4 (1 g) capsules (56% as docosahexaenoic acid (DHA) and 27.7% as eicosapentaenoic acid per day (<4% n-6

PUFA)) (Ocean Nutrition, Halifax, Nova Scotia, Canada) or 4 (1 g) olive oil (placebo) capsules containing 67% oleic acid and less than 1% n-3 PUFA oil (Pan Laboratories, Moorebank, NSW, Australia). The study used a double blind placebo controlled parallel design, undertaken from 20 weeks of pregnancy until delivery (when dietary supplementation ceased). Ethical approval for the study was granted by the Princess Margaret Hospital for Children and St. John of God Hospital Ethics Committees.

2.3. Questionnaires

Individual medical history, family medical history, demographic information and perceived stress information were gathered from participants at the time of recruitment into the study. Medical histories and demographic information were collected at the recruitment interview, at 20 weeks gestation. Participants also completed the Beck Depression Inventory (BDI) questionnaire at 20 weeks gestation, and in the first week after delivery. The BDI is an internationally used 21 item self-report rating inventory that measures characteristic attitudes and symptoms of depressed mood (reliability coefficient = 0.81) (Beck et al., 1961). The highest possible total score on the BDI is sixty-three and the lowest possible score is zero. According to the widespread application of this instrument, a total score of (<10 is considered to reflect 'normal' symptomatology, a score of 10–18 is considered to be indicative of 'mild' to 'moderate' depression, a total of 19–29 is interpreted as reflecting 'moderate' to 'severe' depression, and a score of 30–63 is indicative of severe depression. Therefore, higher total scores indicate more severe depressive symptoms.

2.4. Sample collection and initial processing

Fasting maternal blood samples were collected for lipid measurements after 10 min of seated rest at 20 weeks of gestation. Cord blood samples were collected from the placental vessels by venepuncture immediately after delivery. Peripheral and cord blood mononuclear cells (MC) were assayed using Lymphoprep (Nycomed Pharma, Oslo, Norway) gradient centrifugation, and were cryopreserved for subsequent batch analysis as previously described (Dunstan et al., 2003).

2.5. Maternal fatty acid analyses

Phospholipid fatty acid analyses were carried out by gas-liquid chromatography as previously described (Mori et al., 2000). The fatty acids were expressed as a percentage of the weight of the total fatty acids measured (C14 to C22). The total sum of n-3 PUFAs (20:5n-3, 22:5n-3, and 22:6n-3) and n-6 PUFAs (18:2n-6, 20:3n-6, 20:4n-6, 22:3n-6, and 22:4n-6), as well as the ratio of n-3 to n-6 fatty acids, was also expressed.

2.6. Neonatal cytokine and lymphoproliferation responses

Neonatal immune responses were assessed using cord blood mononuclear cells (CBMC). For cytokine analysis, 2×10^9 CBMC/L were cultured in AIM V (Gibco, Life Technology) serum-free medium (Upham et al., 1995) for 48 h with or without allergens, including 10 μ g/ml house dust mite (HDM) extract (CSL, Parkville, Australia), 100 μ g/ml ovalbumin (OVA; Sigma, Castle Hill, Australia), 30 μ g/ml cat hair extract (ALK Abello, Holsholm, Denmark) or 1 μ g/ml PHA mitogen (HA16, Murex, Biotech Ltd, Dartford, United Kingdom). For PHA stimulation (1 μ g/mL), 10^9 CBMC/L were used. In addition, 10 μ g/ml mycobacterial antigen (PPD), tetanus toxoid (0.5 Lf/ml) and 200 ng/ml *Staphylococcus* entero-

toxin B (SEB) were used as previously described (Dunstan et al., 2006).

Lymphoproliferation responses (after 7 days in culture) were determined by tritiated thymidine incorporation into proliferating cells, as previously described (Dunstan et al., 2003). Disintegrations per minute were measured on a β -counter (Packard Matrix 9600, Conn). Detection of a positive response was defined as an increase of >1000 dpm above unstimulated control cultures.

Cytokines (IL-5, IL-6, IL-10, IL-13 and IFN γ) were measured in the supernatants after 48 h. IL-5 was quantified by enzyme-linked immunosorbent assay (ELISA) and IL-6, IL-10, IL-13 and IFN γ were measured by time resolved fluorometry (TRF; DELPHIA, Perkin-Elmer Life Sciences, Boston, MA, USA) as previously described (Dunstan et al., 2003). Briefly, the ELISA method used paired antibodies (Pharmingen, Sydney, NSW, Australia) and the biotinylated antibody was detected using Europium-labelled streptavidin. Fluorescence dissociated by the addition of low pH enhancement buffer was quantified using a fluorometer (WALLAC VICTOR², Perkin-Elmer Life Sciences). The detection limit for all cytokines was 3 pg/mL.

2.7. Statistical analysis

The Student's *t*-test was used for comparing means of normalised continuous data and chi-square analysis was applied for proportions. BDI scores were analysed as continuous and dichotomous data: (normal mood (BDI <10) and depressed mood (BDI $\geq$ 10). Associations between fatty acid levels and dichotomous BDI scores in mid-pregnancy were assessed using logistic regression. Continuous cytokine and lymphoproliferation data were logarithmically (naturally) transformed to obtain a log-normal distribution, determined by the Kolmogorov-Smirnov test and probability plots. The associations between BDI scores in mid-pregnancy and the continuous log-transformed neonatal cytokine/lymphoproliferative data were determined by generalised linear modelling and included adjustment for potential confounders. All statistical analyses were performed using SPSS software (version 15). A correction factor for multiple testing (such as a Bonferroni factor) was not applied due to the exploratory nature of this study (Perneger, 1998), however, appropriate caution was taken interpreting the results.

3. Results

3.1. Population characteristics

At 20 weeks gestation (prior to dietary intervention), 22.2% of all the women were experiencing mild to moderate depressive symptoms (BDI scores >10). The BDI in the second trimester was correlated with BDI in the perinatal period ($r=0.43$, $p<0.0001$), supporting it as a consistent and reproducible measure in this population, and that it is likely to represent the prevailing mood in these women during gestation up to and including the time of birth. The characteristics of the population are shown on Table 1.

Women with BDI scores >10 showed a significant improvement in their mood scores after pregnancy (paired samples T-test, mean BDI drop of -4.9 , $p=0.001$) but still had more depressive symptoms (higher mood scores) in the postpartum than the women with no depressive symptoms at 20 weeks ($p=0.001$, Table 1), who did not show any significant change in mood scores from 20 weeks to the post partum period (paired samples T-test, mean BDI drop of -0.6 , $p=0.12$). Although there was a trend for women with depressive symptoms to be older ($p=0.052$) there was only an average difference of 2 years (Table 1), and did not have any ef-

Table 1

Characteristics of the women that completed the study.

Characteristics	BDI <10 <i>n</i> = 63	BDI $\geq$ 10 <i>n</i> = 18	<i>p</i> -value
Mean age, years (SD)	31.3 (3.3)	33.3 (4.9)	0.052
Mood scores, mean BDI (SD)			
– At 20 weeks gestation	4.8 (2.4)	12.2 (2.0)	<0.001
– At 3 days post partum	4.1 (2.6)	7.3 (4.5)	0.001
Mean Δ BDI, 20 weeks gestation to postpartum (SD)	-0.6 (3.0)	-4.9 (4.8)	0.003 ^a
Mean BMI at entry, kg/m ² (SD)	25.5 (4.2)	26.7 (4.7)	0.32
Previous children, <i>n</i> (column %)			
– Nullipara at entry	31 (49.2)	8 (44.4)	
– One or more children	32 (50.8)	10 (55.6)	0.72 ^b
Mean duration of gestation, days (SD)	274 (8.2)	275.3 (7.0)	0.53
Delivery method, <i>n</i> (column %)			
Labour	52 (82.5)	11 (61.1)	
Caesarean	11 (17.5)	7 (38.9)	0.10 ^c
Mean fatty acids levels at 20 weeks,% total red cell fatty acids (SD)			
Total <i>n</i> -3 PUFA	16.1 (1.6)	15.4 (2.0)	0.12
Total <i>n</i> -6 PUFA	25.3 (1.3)	26.0 (1.2)	0.051
Ratio <i>n</i> -3/ <i>n</i> -6 PUFA	0.64 (0.09)	0.60 (0.09)	0.07
Mean fatty acid levels after birth,% total red cell fatty acids (SD)			
Total <i>n</i> -3 PUFA	17.2 (2.7)	17.0 (2.8)	0.80
Total <i>n</i> -6 PUFA	24.8 (1.9)	25.5 (2.7)	0.21
Ratio <i>n</i> -3/ <i>n</i> -6 PUFA	0.71 (0.17)	0.69 (0.19)	0.67

BDI were not recorded for two participants at 20 weeks gestation.

^a There was a significant difference in the “change in BDI” between the groups (due to the greater improvement in the women with mood scores >10).

^b Pearson chi-square test.

^c Fisher's exact test.

fect on the relationships examined in the study (when examined as a possible confounding factor in statistical analysis). There was also a trend of higher caesarean section rates in the women with depressive symptoms at 20 weeks of age, although this was not statistically significant ($p=0.10$). It is not known how differences in mood may have contributed to a requirement for caesarean section, however, women with significant pregnancy or obstetric complications were excluded from the study, and most caesareans were elective. Possible effects of delivery method on immune function and the relationship between immune function and maternal mood in the second trimester were assessed in statistical modelling (below).

3.2. The relationship between maternal BDI scores and fatty acid levels

At 20 weeks, prior to the intervention, women with depressive symptoms (BDI >10) had higher total *n*-6 PUFA levels ($p=0.051$) and hence lower *n*-3/*n*-6 ratios ($p=0.069$) than women with no depressive symptoms (BDI <10) (Table 1), and this was not altered after allowing for potential confounding factors (such as maternal age and education, and previous pregnancies) by logistic regression (Table 2).

Following supplementation, fish oil was associated with a significant increase in *n*-3 PUFA levels and the *n*-3/*n*-6 ratio, together with a proportional fall in *n*-6 PUFA levels, as reported elsewhere in detail (Dunstan et al., 2004). However, the change in fatty acid status (from 20 weeks to the postpartum period) was not correlated with the change in mood scores ($r=0.12$; $p=0.33$), suggesting that fish oil (or maternal PUFA status) did not significantly influence maternal mood in this study. Supporting this, after completing supplementation there was no difference ($p=0.30$) in the mean BDI of women who received fish oil ($n=37$) versus those that did not ($n=38$). Furthermore, wo-

Table 2

The relationship between maternal fatty acid status and dichotomous maternal mood scores in mid-pregnancy (20 weeks) (logistic regression).

Fatty acid status in mid-pregnancy (continuous predictor variable)	BDI as a dichotomous dependent variable (comparing women with BDI <10 vs. ≥10)			
	Coefficient	p-Value	Adjusted coefficient ^a	Adjusted p-value ^a
Total n-3 PUFA	−0.25	0.12	−0.29	0.10
Total n-6 PUFA	0.42	0.06^b	0.45	0.051^b
Ratio of n-3/n-6 PUFA	−5.78	0.07^b	−6.52	0.06^b

Shaded areas show relationships of interest.

^aAdjusting for maternal age, education, and parity.^bDenote significance levels approaching $p = 0.05$.

men in the group with depressive mood scores (BDI ≥10) who received fish oil (8/16) did not show any greater improvement in mood ($p = 0.18$) than the women who received the placebo (8/16). Thus, in this study, supplementation with fish oil did not have any effect on subsequent mean BDI scores in the post-partum period (and there were also no differences in mean BDI scores between the fish oil and placebo groups prior to supplementation at 20 weeks gestation; $p = 0.92$).

Although the fish oil supplementation was unlikely to have confounding effects on maternal mood in this population, neonatal fatty acid status was nevertheless included in statistical models (assessing the relationships between maternal mood and infant immune function) because of the recognised potential effects of fish oil on immune function (Dunstan et al., 2003) (Prescott et al., 2007).

3.3. The relationship between maternal mood scores (BDI) in pregnancy and neonatal immune function

The BDI in mid-pregnancy was examined in relation to a range of neonatal immunological parameters. We measured lymphoproliferation and a range of cytokines including T helper cell type 1 (Th1 IFN γ) and type 2 (Th2 IL-13) cytokines, a regulatory cytokines (IL-10) and an inflammatory cytokine (IL-6). The stimuli used included innocuous environmental antigens (a food and inhalant allergen) as well as bacterial antigens (PPD and SEB) and a non-specific polyclonal stimulus (PHA).

Graphical comparison between continuous BDI scores and log-transformed neonatal immune function showed a distinct change at a BDI of 10 or more for a number of neonatal immune responses. Therefore BDI was used as a continuous predictor and as dichotomous predictor where neonatal immune function was compared in neonates of women with mood scores in the normal range ($n = 63$; BDI <10) with those who had mild depressive symptoms in mid-pregnancy ($n = 18$; BDI ≥10). Significant relationships were identified using generalised linear regression modelling and are shown in on Table 3 and Appendix A (available online). We further examined these relationships after allowing for the effects of potential confounding factors, including maternal age, education level, parity, delivery method, infant gender and neonatal fatty acid status. Both unadjusted and adjusted coefficients are shown in Tables 3 and Appendix A. The only adjusted relationship where BDI is better depicted as a continuous predictor was lymphoproliferation in response to PPD ($p = 0.01$, Table in Appendix A); with BDI as a dichotomous predictor this was not significant $p = 0.17$, Table 3). Apart from this one relationship, all other significant relationships were better represented using BDI as a dichotomous predictor and thus, the data have been represented graphically in two discrete groups after adjustment for confounders.

As summarised below, there was a general pattern of higher cytokine (Figs. 1 and 2) and lymphoproliferative (Fig. 3) responses in neonates of mothers with higher BDI scores in pregnancy.

Table 3

The relationship between dichotomous maternal mood scores (BDI) in mid-pregnancy and neonatal immune function (generalised linear modelling).

	Neonatal immune responses	BDI as a dichotomous predictor variable (comparing women with BDI <10 vs. ≥10)			
		Coefficient	p-value	Adjusted coefficient ^a	Adjusted p-value ^a
Interferon Gamma	HDM	0.19	0.48	0.29	0.30
	OVA	0.54	0.35	0.90	0.12
	CAT	−0.05	0.93	0.30	0.57
	SEB	0.69	0.12	0.85	0.05^d
	PPD	−0.05	0.84	0.03	0.89
	PHA	0.24	0.62	0.40	0.36
Interleukin-13	HDM	0.58	0.046^c	0.70	0.02^c
	OVA	0.31	0.39	0.60	0.08^d
	CAT	0.14	0.67	0.39	0.22
	SEB	0.30	0.22	0.59	0.01^c
	PPD	0.00	1.00	−0.02	0.91
	PHA	−0.07	0.81	0.06	0.82
Interleukin-10	C ^b	0.42	0.14	0.66	0.02^c
	HDM	0.69	0.03^c	0.76	0.008^c
	OVA	−0.00	0.99	0.21	0.46
	CAT	−0.07	0.80	−0.01	0.97
	SEB	−0.06	0.84	0.05	0.87
	PPD	0.02	0.95	0.01	0.98
Interleukin-6	PHA	0.11	0.67	0.24	0.39
	C ^b	0.69	0.08^d	0.79	0.06^d
	HDM	0.29	0.62	0.56	0.32
	OVA	−0.04	0.86	0.05	0.84
	CAT	0.06	0.71	0.14	0.38
	SEB	0.46	0.27	0.78	0.06^d
Interleukin-5	PPD	0.77	0.30	1.12	0.11
	PHA	0.65	0.11	1.14	0.003^c
	HDM	−0.10	0.53	−0.11	0.45
	OVA	0.20	0.54	0.65	0.02^c
	CAT	0.18	0.60	0.54	0.07^d
	SEB	0.02	0.94	0.31	0.33
Lymphoproliferation (Δ DPM)	PPD	−0.10	0.46	−0.14	0.30
	PHA	0.27	0.45	0.58	0.09
	HDM	0.82	0.24	0.23	0.73
	OVA	0.64	0.13	1.10	0.007^c
	CAT	0.71	0.16	1.31	0.007^c
	SEB	0.23	0.07^d	0.14	0.20
	TT	1.06	0.16	1.13	0.14
	PPD	1.39	0.04^c	0.91	0.17
	PHA	−0.90	0.35	−0.03	0.78

Shaded areas show relationships of interest.

^aAdjusting for maternal age and education, parity, gestation, infant gender, delivery method and cord blood ratio n-3/n-6.^bSpontaneous cytokine production was only seen for IL-10 and IL-6 (i.e. in unstimulated control cultures).^cDenote significance levels $p \leq 0.05$.^dDenote significance levels approaching $p = 0.05$.

(a) Comparison of spontaneous neonatal cytokine production:

Only IL-6 and IL-10 were present in detectable levels in the supernatants of unstimulated cells in culture for 48 h. The group

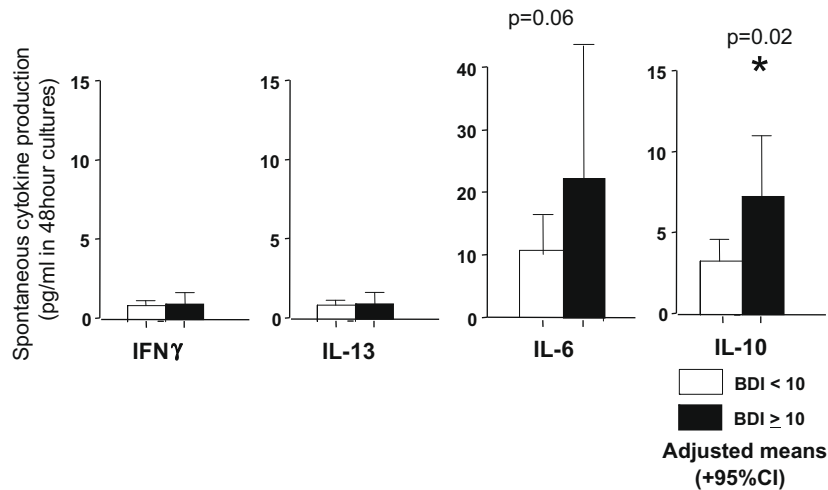


Fig. 1. Comparison of spontaneous cytokine production by neonates according to the maternal mood scores assessed by the BDI in the second trimester after adjustment for potential confounders. Spontaneous (unstimulated) cytokine production but CBMC after 48 h in culture is shown for IFN γ , IL-13, IL-6 and IL-10. Levels are compared for neonate of women with BDI mood score of ≥ 10 (mildly depressive—dark bars) with those in the normal range (< 10 —clear bars) and are shown as the geometric mean and 95% confidence intervals with significant p -values (< 0.05) depicted as “*” (as derived from generalised linear models of log-transformed data after adjustment for confounders as listed in Table 3).

with depressive maternal mood scores (BDI ≥ 10) tended to have higher spontaneous production of both of these cytokines: IL-6 ($p = 0.06$) and IL-10 ($p = 0.02$) as shown in Fig. 1.

(b) Comparison of stimulated neonatal cytokine production:

There were trends for higher IFN γ responses to most stimuli (Fig. 2a), although this only approached statistical significance for SEB ($p = 0.05$). Neonatal IL-10 responses to HDM were also higher ($p = 0.008$) in association with higher maternal BDI scores (Fig. 2b). Neonatal IL-13 Th2 responses also tended to be higher if mothers had higher BDI scores. While this was significant for HDM ($p = 0.02$) and SEB ($p = 0.01$), there were similar trends for responses to OVA ($p = 0.08$) (Fig. 2c). IL-6 pro-inflammatory responses were also higher in response to PHA ($p = 0.003$) and similar trends were seen for SEB ($p = 0.06$) (Fig. 2d). Finally, IL-5 Th2 responses (to OVA; $p = 0.02$ and cat; $p = 0.07$) were also higher in the group with higher BDI scores, after adjusting for confounding influences (Table 3).

(c) Comparison of neonatal lymphoproliferation:

There was a general pattern of higher lymphoproliferative responses in neonates of mothers with higher BDI scores in pregnancy (BDI ≥ 10) has significantly higher lymphoproliferative responses to OVA ($p = 0.007$) and cat ($p = 0.007$) extracts (Fig. 3). Furthermore, though there were clear univariate trends for higher responses to both SEB and PPD (Table 3), these were not significant after adjusting for confounding factors (Table 3 and Fig. 3).

When statistical analyses were repeated with mid-pregnancy BDI as a continuous (rather than a dichotomous) predictor of neonatal immune function, there were also significant positive associations between neonatal lymphoproliferation to PPD ($p = 0.01$), OVA ($p = 0.048$) and cat ($p = 0.049$) after allowing for the same potential confounders (Table in Appendix A).

4. Discussion

To our knowledge this study has provided the first evidence that maternal depressive scores in mid-pregnancy may be associated with increased neonatal immune responsiveness, as detected

by a number of different measures of immune function. Specifically, newborns of women who had higher depressive scores showed higher production of IL-6 inflammatory cytokine (spontaneous and to polyclonal stimulation), stronger lymphoproliferative responses and stronger Th2 responses (IL-13 and IL-5) to allergens. IL-10 production was also higher in women with depressive symptoms (BDI ≥ 10), including both spontaneous production and stimulated responses (although only in response to HDM). Our other recent studies suggest that excessive inflammatory (IL-6 and TNF α) responses in the neonatal period may be associated with increased risk of allergic disease (Prescott et al., 2008), and may therefore be potentially maladaptive. Similarly, although the significance of increased neonatal Th2 IL-13 responses (also noted in association with higher BDI scores) is not clear, some studies have also associated increased neonatal IL-13 responses with subsequent allergic disease (Ohshima et al., 2002; Spinozzi et al., 2001). Further studies are needed to investigate these functional consequences of altered neonatal immune function.

The pattern of effect seen in the present study were consistent with the inflammatory “immune activation” (increased IL-6 and IL-1 receptor antagonist levels) that is seen in depressive and anxious mothers (Maes et al., 2000), and suggest parallel rather than divergent (Knackstedt et al., 2005) effects of stress on the mother and child, at least in this context. This not only included increased spontaneous production of inflammatory IL-6 but also increased production of anti-inflammatory IL-10, suggesting a more general immune activation. This increase in both inflammatory and anti-inflammatory activity has also been noted in other studies (O'Mahony et al., 2006). The mechanism of these effects is not fully established. While stress and anxiety are typically associated with activation of the HPA, the associated increase in CRH and cortisol does not necessarily suppress inflammatory responses, suggesting other pathways of effect. This may be because CRH also has a direct role in the inflammatory immune process (Webster et al., 1998) and may be responsible for the stress-induced activation of allergic/autoimmune phenomena via mast cell degranulation. Furthermore, in pregnancy CRH mediates placental inflammation by suppression of progesterone and progesterone induced blocking factor (PIBF) (Knackstedt et al., 2005).

Previously studies in animals studies have shown that adverse maternal events in pregnancy can induce immuno-suppressive

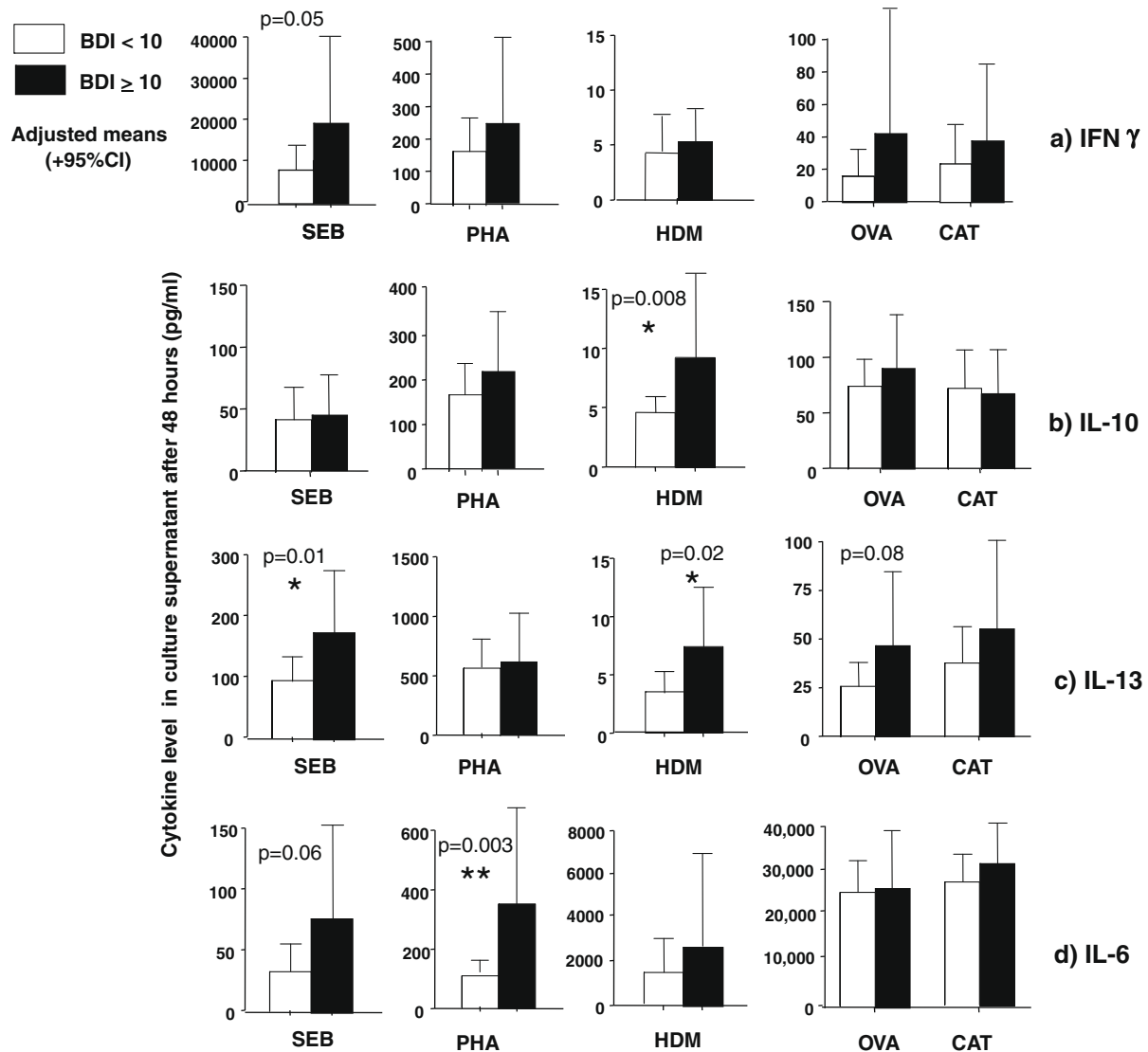


Fig. 2. Comparison of cytokine responses in neonates according to the maternal mood scores assessed by the BDI in the second trimester after adjustment for potential confounders. Cytokine production in response to polyclonal activation (SEB and PHA) and allergen-specific activation (HDM, OVA and cat extracts) are shown for (a) IFN γ , (b) IL-10, (c) IL-13 and (d) IL-6. Levels are compared for neonate of women with BDI mood score of > 10 (mildly depressive—dark bars) with those in the normal range (< 10 —clear bars) and are shown as the geometric mean and 95% confidence intervals with significant p -values (< 0.05) depicted as “*” (< 0.05) and as “**” (< 0.01) as derived from generalised linear models of log-transformed data after adjustment for confounders as listed in Table 3.

effects (rather than immune activation) in primates (Coe et al., 2002, 1996) and rodents (Gotz and Stefanski, 2007; Gotz et al., 2007). However, these were “stress” animals models, and although stress, anxiety and depression are inter-related, the immunological effects may depend on the severity and dominant psychological trait (Lemieux et al., 2008). Furthermore, the immune effects of stress in these models may also be tissue specific, as offspring of stressed rats do have increased pro-inflammatory cytokines in lymphoid tissue (Laviola et al., 2004). Finally, the immunological effects of the mild depressive symptoms in the present study are likely to be different from those seen with overt syndromal depression (Appels et al., 2000; Kiecolt-Glaser et al., 2002). Based on our observations, we speculate that more subtle variations in maternal mood and anxiety could also influence infant immune function, and that it is unsurprising that these effects may differ from animal models that have induced different and potentially more profound psychological stress. Perhaps most importantly, our findings provide further support for the hypothesis that maternal psychological

factors (in this case mood) may also have effects on fetal immune responses.

The functional consequences of variations in neonatal immune function need to be investigated further. Although alterations in perinatal immunity may have more direct implications for immune disorders (such as allergy) (Prescott, 2003), there are additional implications for other aspects of metabolic, physiological and even neuropsychological development (Knackstedt et al., 2005). For example, inflammatory neonatal cytokines are associated with adverse neurological outcomes (Shalak et al., 2002) and blocking effects of these cytokines reduces neurological injury (Hagan et al., 1996) suggesting that this is more than just an epiphenomenon (Foster-Barber and Ferriero, 2002). The effects of more subtle variations in immune activation on neurodevelopment are not known, although there is preliminary evidence that conditions such as schizophrenia are associated with perinatal inflammatory events (reviewed in (Ashdown et al., 2006)). Thus, although these inflammatory pathways are

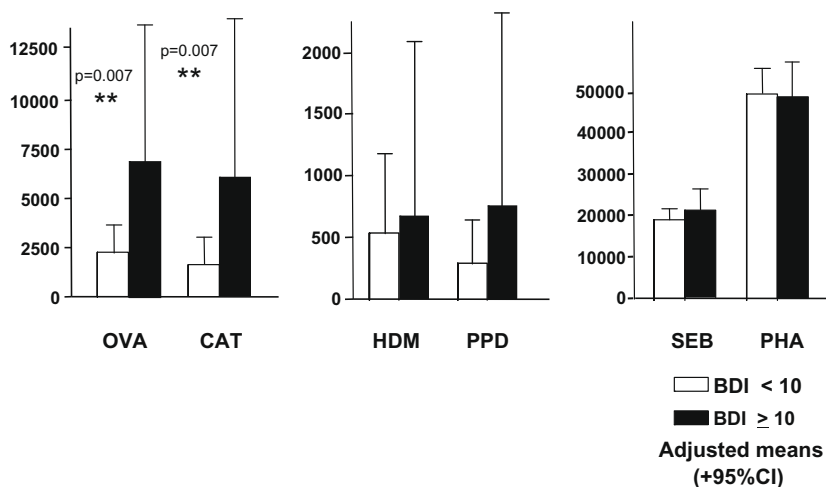


Fig. 3. Comparison of lymphoproliferative responses in neonates according to the maternal mood scores assessed by the BDI in the second trimester after adjustment for potential confounders. Lymphoproliferation in response to polyclonal activation (SEB and PHA) and antigen-specific activation (HDM, OVA, PPD and cat extracts) are shown. Levels are compared for neonate of women with BDI mood score of > 10 (mildly depressive—dark bars) with those in the normal range (<10—clear bars) and are shown as the geometric mean and 95% confidence intervals with significant *p*-values (<0.01) depicted as “**” (as derived from generalised linear models of log-transformed data after adjustment for confounders as listed in Table 3).

important for host defense excessive production of these cytokines is maladaptive and can increase disease risk (Maes et al., 2000). Collectively these observations support the notions that there are important interactions with the developing immune system have the capacity to shape many aspects of subsequent function.

Another interesting finding of this study is that, prior to any intervention, higher depressive symptom scores were associated with higher *n*-6PUFA levels and lower *n*-3/*n*-6PUFA ratios. This could be consistent with the hypothesis that this fatty acid profile, associated with lower consumption of fish oils may increase the risk of depression (Hibblen, 1998). The effects of fish oil on mood are still unclear as some studies have reported protective effects of supplementation (Freeman et al., 2006; Hibblen and Salem, 2001; Silvers and Scott, 2002) while others have not (Browne et al., 2006; Marangell et al., 2004; Miyake et al., 2006). This is currently being examined in much larger pregnancy studies.

This is an exploratory study and the results need to be interpreted with appropriate caution. While we have shown an association between BDI scores in mid-pregnancy and neonatal immune function these results need to be replicated in preferably larger observational studies. We acknowledge that our “cut-off” of 10 for BDI with this population may over-represent those with depressed mood, due to endorsement of somatically-oriented items as a result of pregnancy-related discomfort.

Furthermore, although we have controlled for a number of potential confounders the association could still be subject to bias and confounding. In particular women in this study only had mild depressive symptoms and ideally women with major depression need to be investigated.

Together, these findings support the general notion that neuro-endocrine pathways have an important influence on the developing immune system and have the potential to alter immune programming and disease susceptibility. Possible “psycho-neuro-immunological” interactions may also have as yet unmeasured effects on other aspects of development. While preliminary, these findings make a case for investigating these interactions further, including better molecular markers or stress, causal pathways and functional consequences.

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Appendix A. (available for an online repository).

The relationship between maternal mood scores (with BDI as a continuous predictor) in mid-pregnancy and neonatal immune function (generalised linear modelling)

	Neonatal immune responses	BDI as a continuous predictor variable			
		Coefficient	p-value	Adjusted coefficient ^a	Adjusted p-value ^a
Interferon Gamma	HDM	0.01	0.82	0.01	0.67
	OVA	−0.01	0.86	0.02	0.81
	CAT	−0.03	0.62	−0.01	0.93
	SEB	0.03	0.55	0.06	0.28 ^a
	PPD	−0.02	0.51	−0.01	0.67
	PHA	0.02	0.68	0.05	0.29
Interleukin 13	HDM	0.00	0.90 ^e	0.01	0.88 ^e
	OVA	−0.02	0.57	0.02	0.57 ^e
	CAT	−0.03	0.35	0.00	0.95
	SEB	−0.03	0.23	0.00	0.97 ^e
	PPD	0.00	0.99	−0.00	0.86
	PHA	−0.04	0.23	−0.02	0.44
Interleukin 10	C ^b	0.03	0.37	0.06	0.09 ^d
	HDM	0.07	0.06 ^d	0.06	0.05 ^d
	OVA	−0.03	0.27	−0.00	0.89
	CAT	−0.02	0.38	−0.02	0.55
	SEB	−0.02	0.48	−0.01	0.78
	PPD	0.00	0.92	−0.00	0.93
Interleukin 6	PHA	−0.00	0.94	0.02	0.48
	C ^b	−0.00	1.00 ^e	−0.00	0.97 ^e
	HDM	−0.03	0.60	−0.02	0.80
	OVA	−0.04	0.16	−0.02	0.55
	CAT	−0.01	0.66	0.01	0.66
	SEB	0.01	0.81	0.06	0.19 ^e
Interleukin 5	PPD	−0.03	0.71	−0.00	0.97
	PHA	0.01	0.76	0.10	0.02 ^c
	HDM	−0.00	0.94	−0.00	0.90
	OVA	−0.03	0.36	0.04	0.20 ^e
	CAT	−0.03	0.44	0.03	0.36 ^e
	SEB	−0.05	0.15	−0.01	0.82
Lymphoproliferation (ΔDPM)	PPD	−0.00	0.84	−0.01	0.59
	PHA	−0.04	0.25	0.01	0.86
	HDM	0.19	0.009 ^{c,f}	0.10	0.17
	OVA	0.04	0.42	0.09	0.048 ^c
	CAT	0.02	0.69	0.11	0.049 ^c
	SEB	0.02	0.12	0.01	0.68
	TT	0.06	0.44	0.07	0.41
	PPD	0.24	<0.001 ^c	0.19	0.01 ^{c,f}
	PHA	−0.02	0.03 ^{c,f}	−0.02	0.14

Shaded areas show relationships of interest.

^aAdjusting for maternal age, education, parity, gestation, infant gender, delivery method and cord blood ratio $n-3/n-6$.

^bSpontaneous cytokine production was only seen for IL-10 and IL-6 (i.e. in unstimulated control cultures).

^cDenote significance levels $p < 0.05$.

^dDenote significance levels approaching $p = 0.05$.

^eSignificant association or approaching significance using dichotomous BDI (<10 vs. ≥ 10) but not continuous BDI (graphing shows a discrete increase or decrease from a BDI of 10+ confirming these as dichotomous rather than continuous relationships).

^f $p = 0.07$ with dichotomous BDI (graphing shows a continuous rather than dichotomous relationship).

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