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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 (BDI $\geqslant 10$). 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.

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 TNFα[2]) 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 TNFα) at the mater-

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[2] *Abbreviations used:* BDI, Beck Depression Inventory; PUFA, Polyunsaturated fatty acid; IFNγ, 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; TNFα, 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.

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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 (IFNγ), 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 (20:5*n*-3, 22:5*n*-3, and 22:6*n*-3) and *n*-6 PUFAs (18:2*n*-6, 20:3*n*-6, 20:4*n*-6, 22:3*n*-6, and 22:4*n*-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 μg/ml house dust mite (HDM) extract (CSL, Parkville, Australia), 100 μg/ml ovalbumin (OVA; Sigma, Castle Hill, Australia), 30 μg/ml cat hair extract (ALK Abello, Holsholm, Denmark) or 1 μg/ml PHA mitogen (HA16, Murex, Biotech Ltd, Dartford, United Kingdom). For PHA stimulation (1 μg/mL), 10^9 CBMC/L were used. In addition, 10 μg/ml mycobacterial antigen (PPD), tetanus toxoid (0.5 Lf/ml) and 200 ng/ml *Staphylococcus* entero-