Maternal fish oil supplementation in pregnancy reduces interleukin-13 levels in cord blood of infants at high risk of atopy


School of Paediatrics and Child Health and †University Department of Medicine, University of Western Australia and ‡Western Australian Institute for Medical Research and §Division of Cell Biology, Telethon Institute for Child Health Research, Perth, Australia

Summary

Background and objectives The epidemiological association between higher dietary n-3 polyunsaturated fatty acids (PUFA) and lower prevalence of asthma, has led to interest in the role of early dietary modification in allergic disease prevention. In this study we examined the effects of maternal n-3 (PUFA)-rich fish oil supplementation on cord blood (CB) IgE and cytokine levels in neonates at risk of developing allergic disease.

Methods In a randomized double-blind, placebo-controlled trial, 83 atopic pregnant women received either fish oil capsules (n = 40) containing 3.7 g n-3 PUFA/day or placebo capsules (n = 43) from 20 weeks gestation until delivery. CB cytokine levels (IL-4, IL-5, IL-10, IL-12, IL-13, TNF-α and IFN-γ) and total IgE levels were measured and compared between the two groups. Fatty acid composition of red cell membranes was analysed by gas chromatography and the relationships among PUFA, cytokine and IgE levels were examined.

Results Maternal fish oil supplementation resulted in a significant increase in n-3 PUFA levels (P < 0.001) in neonatal erythrocyte membranes. Neonates whose mothers had fish oil supplementation had significantly lower plasma IL-13 (P < 0.05) compared to the control group. There was also a significant inverse relationship between levels of n-3 PUFA in neonatal cell membranes and plasma IL-13. There was no difference in levels of IgE and the other cytokines measured.

Conclusions This study provides preliminary evidence that increasing neonatal n-3 PUFA levels with maternal dietary supplementation can achieve subtle modification of neonatal cytokine levels. Further assessment of immune function and clinical follow-up of these infants will help determine if there are any significant effects on postnatal immunodevelopment and expression of allergic disease.

Keywords atopy, cord blood, fish oil, IL-13, LCPUFA, pregnancy

Submitted 2 August 2002; revised 17 October 2002; accepted 28 October 2002

Introduction

The events leading to T helper cell 2 (Th2) allergic immune responses are not known, but changing environmental exposures in early life are implicated in the recent increase in allergic diseases [1]. Although not clearly identified, some of these factors may operate before birth to favour persistent Th2 immunity.

Maternal diet in pregnancy represents an important exposure with significant potential to modify immune function and predisposition to allergic disease. One of the most significant recent dietary changes has been decreased consumption of anti-inflammatory omega-3 polyunsaturated fatty acids (n-3 PUFA), with corresponding increased intake of pro-inflammatory n-6 (omega 6) PUFA. In modern Western diets, the ratio of n-6 to n-3 fatty acids ranges from approximately 20–30:1 instead of the traditional range of 1–2:1 [2]. These changes are reflected in parallel changes in the fatty acid composition of breast milk over the last 20 years [3]. This, coupled with inadequate supplementation of milk formulae, has led to a falling intake of anti-inflammatory n-3 PUFA in early life during the critical period of immune maturation. There is preliminary evidence that lower n-3 PUFA levels in breast milk are associated with infant atopy [4].

Other epidemiological and experimental data also provide a plausible link between these dietary changes and altered immune development. The contrasting immunological effects of n-6 and n-3 PUFA are well described. Omega-6 PUFA [18:2n-6 linoleic acid (LA) and 20:4n-6 arachidonic acid (AA)] promote inflammation, by enhancing mononuclear proliferation, prostaglandin (PG) production (particularly PGE2), and 'pro-allergic' Th2 differentiation [5]. In contrast, n-3 fatty acids [including 22:6n-3 docosahexaenoic acid (DHA) and 20:5n-3 eicosapentaenoic acid (EPA)] appear to inhibit allergic immune responses through a number of anti-inflammatory properties, including inhibition of PGE2 [6], lymphoproliferation [7], proinflammatory cytokine responses [8,9] and
expression of MHC class II antigens [10]. These and other anti-inflammatory effects have been utilized in a number of inflammatory conditions with some success (reviewed in [2]). It is proposed that the more 'proinflammatory' Western diets favour immunological reactivity and Th2 differentiation during the critical time of early development. While increased dietary n-3 PUFA intake has been associated with lower prevalence of asthma and allergic inflammation [11,12], the benefits of n-3 PUFA in established allergic disease are less clear [13–17]. However, potential dietary effects are likely to be more significant before immune responses are fully established, and suggest a possible role of early n-3 PUFA supplementation in allergic disease prevention. Therefore, in the current study, atopic women were supplemented with fish oil during pregnancy in order to assess the immunological effects of n-3 PUFA in high risk neonates.

Although there are ongoing studies examining the merits of giving n-3 PUFA-rich fish oil in the early post-natal period [18], to our knowledge this is the first study examining the effects of fish oil supplements in pregnancy on neonatal cytokine levels. Because immune responses are initiated before birth and neonates at high atopic risk already have differences in Th1/Th2 responses at birth [19], we hypothesized any beneficial effects of n-3 PUFA on immune development may be greater in the antenatal period. Here we report the effects of maternal fish oil supplementation on cord blood (CB) cytokine and IgE levels.

Materials and methods

Overview/study design

In a double-blind placebo-controlled longitudinal study, 98 allergic women were randomized to receive either a fish oil supplement or a placebo (olive oil) from 20 weeks of pregnancy to delivery. CB was collected for analysis of fatty acid levels, cytokines and total IgE levels.

Study population

All women were booked for delivery at St John of God Hospital, Subiaco, Western Australia between January 2000 and September 2001. Women were defined as allergic if they had a history of doctor-diagnosed allergic rhinitis and/or asthma and one or more positive skin prick test (SPT) to common allergens (house dust mite, grasses, moulds, cat, dog, feathers and cockroach; Hollister-Stier Laboratories, Spokane, WA, USA). A well size of ≥3 mm above the negative control was considered positive. Women were ineligible for the study if they smoked, had other medical problems, complicated pregnancies, seafood allergy or their normal dietary intake exceeded two meals of fish per week. Participants were subsequently excluded if their delivery was pre-term (less than 36 weeks' gestation). To minimize potential confounding factors, at randomization the groups were stratified by parity (no previous term childbirth vs. one or more), pre-pregnancy body mass index (BMI), age and maternal allergy (allergic rhinitis or asthma). Ethical approval for this study was granted by the Princess Margaret Hospital and the St John of God Hospital (Subiaco) Research and Ethics Committees. All women signed a written consent form.

Fish oil (n-3 PUFA) supplements

The supplement group received four (1 g) fish oil capsules per day, comprising a total of 3.7 g of n-3 PUFA with 56.0% as DHA and 27.7% as EPA (Ocean Nutrition, Halifax, Nova Scotia, Canada; confirmed by gas chromatography). The placebo group received four (1 g) capsules of olive oil per day (containing 66.6% n-9 oleic acid and less than 1% n-3 PUFA; Pan Laboratories, Moorabbin, NSW, Australia). Twenty weeks' gestation was selected as the 'starting point' for supplementation because the first T cell responses to allergen are not detectable until at least 22 weeks' gestation [20], and earlier supplementation is logistically more difficult. There were no significant changes in background dietary intake in the two groups, assessed by a semiquantitative food frequency questionnaire [21] at 20 and 30 weeks' gestation.

The choice of olive oil as a placebo

Olive oil was chosen as the most suitable lipid for placebo control. Although immunological effects have been reported with olive oil [22], the amount given (4 g) through supplementation does not significantly alter the average daily intake of oleic acid (around 26 g/day) in Australian diets [23].

Samples

CB samples were collected from the placental vessels by venepuncture immediately after delivery, and placed into heparinized Roswell Park Memorial Institute (RPMI, Life Technology, UK) culture medium for transport at room temperature. Plasma samples were isolated within 1 h of collection and stored at −80 °C for IgE and cytokine batch analysis later. The dilution factor of CB in RPMI was recorded, and cytokine and IgE levels adjusted accordingly. Total lipids were extracted from red cell membranes (RBC) with methanol and chloroform. CB was also collected into citrate dextrose (ACD) and K3 EDTA (ethylenediamine-tetraacetic acid) anticoagulated tubes (Becton Dickinson, San Jose, CA, USA) for lymphocyte immunophenotyping and total white cell counts.

Measurement of RBC fatty acid levels

RBC membrane incorporation of n-3 and n-6 fatty acids was measured because this gives a long-term indication of dietary levels over the preceding weeks and months (reflecting the half-life of these cells). Fatty acid analyses were carried out as previously described [24]. Briefly, the phospholipid fraction was obtained from total lipid extracts by thin-layer chromatography and the fatty acid methyl esters were analysed by gas liquid chromatography using a Hewlett-Packard model 5980 A gas chromatograph (Hewlett-Packard, Gaithersburg, MD, USA). The fatty acids were expressed as a percentage of the weight of the total fatty acids measured.

CB cytokine levels

CB plasma levels of IL-1, IL-6, IL-12, TNF-α, IL-5 and IL-4 were measured by enzyme-linked immunosorbent assay (ELISA) as previously described [25]. IL-10, IL-6 and IFN-γ were quantified using time resolved fluorometry (TRF; DELFIA, PerkinElmer Life Sciences, Boston, MA, USA). Briefly, the ELISA method was followed using 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, PerkinElmer Life Sciences). The detection limit was 3 pg/mL for IL-4, IL-5, IL-6, IL-10, IL-12, IL-13 and IFN-γ, and 6 pg/mL for TNF-α. Three plasma samples diluted more than 30% in RPMI at collection were not tested.

CB IgE measurements

IgE measurements were performed on CB plasma by the diagnostic Immunology laboratory at Princess Margaret Hospital using a standardized commercial fluoroimmunoassay (Pharmacia ImmunoCAP for total serum IgE). The limit of detection was 0.35 kU/A/L.

Flow cytometric analysis of lymphocyte phenotypes

Whole-blood samples were stained using a two-tube, four-colour direct immunofluorescence reagent kit (Becton Dickinson Immunocytometry Systems (BDIS) MULTItest™ IMK Kit). Tube A contained 10 μL anti-CD3 fluorescein isothiocyanate (FITC), anti-CD8 phycoerythrin (PE), anti-CD45 peridinin chlorophyll protein (PerCP) and anti-CD4 allophycocyanin (APC); tube B contained 10 μL FITC-labelled anti-CD3, PE-labelled anti-CD16, anti-CD56, PerCP labelled anti-CD45 and APC-labelled anti-CD19. Contaminating RBCs were lysed with fluorescence-activated cell sorting (FACS) lysing solution (Becton Dickinson). Samples were analysed on a flow cytometer (FACSCalibur) and data was acquired and analysed using MultiSET and CELLQuest software (Becton Dickinson). An average of 5000 events falling within the lymphocyte gate were counted in each tube in duplicate. CalibRITE 3 and APC beads (Becton Dickinson) were employed for instrument calibration and the same voltage settings used for each sample analysed. An adult control sample was used as a quality control check of the system and monoclonal antibody staining was evaluated by calculating a lymphosum [T cell%+ natural killer (NK) cells %+ B cells%] and monitoring proximity to 100%. Absolute counts of lymphocyte phenotypes were determined from total white blood cell counts (improved Neubauer haemocytometer, Webely Scientific, West Sussex, UK) and a 500-cell white cell differential was performed on a blood smear stained by modified Wrights stain on an automated slide stainer (Miles HemaTek, Rankin Biomedical Co., Clarkson, MI, USA). The total white cell count was adjusted for the presence of nucleated RBCs.

Statistical analysis

All statistical analyses were performed using SPSS software (Version 10 for Macintosh). Log natural transformed data were used for the statistical analysis of all cytokines, IgE and some fatty acids (EPA and total n-3 PUFA) as these data were skewed. Fatty acid data are expressed as mean and SD of the percentage of total fatty acids measured. Cytokine data were described by the geometric mean (GM) and 95% confidence intervals (CI). The transformed data were found to have a log normal distribution using the Kolmogorov-Smirnov test and probability plots.

Differences in fatty acid composition between the fish oil group and the placebo group were assessed by independent Student's *t*-tests. Linear regression was used to examine relationships between continuous variables, after adjusting for confounding factors. Potential confounding factors were determined by Pearson correlation. Logistic regression was used to examine the relationships between the intervention (using ‘group’ as a binary variable) and immunological outcomes (continuous variables), after accounting for potential confounding factors. A *P*-value < 0.05 was considered statistically significant for all analyses.

Results

Characteristics of the maternal and neonatal populations

Ninety-eight women were initially recruited to the study. Seven women in the fish oil group and one in the placebo group withdrew from the study because of nausea they attributed to the capsules. Three infants in the fish oil group and one in the control group born before 36 weeks' gestation were excluded from the analysis. Two infants with significant disease were excluded from the analysis. One required chemotherapy for a malignant condition (multisystem Langerhan's histiocytosis) and the other died of a degenerative neurological condition (with progressive leukodystrophy) at several months of age. Although both were in the intervention group, neither condition was linked in any apparent way to the dietary supplementation. CB was inadvertently not collected at one delivery. Characteristics of the 83 mothers and their healthy full-term babies who completed the study (40 in the fish oil group and 43 in the placebo group) are shown (Table 1). There were no significant differences in maternal atopic status, parity, method of delivery or infant gender between the groups. There were no differences in maternal age, or pre-pregnancy BMI or in infant birth weight, birth length, head circumference, gestational age or Apgar scores in the fish oil-supplemented group compared to the placebo group (not shown).

Effects of maternal fish oil supplementation on neonatal n-3 PUFA levels

At baseline there were no significant differences in the red cell n-6 or n-3 fatty acids between the two groups. Following supplementation, there were significantly higher total n-3 PUFA levels and specifically EPA (mean ± SD 1.3 ± 0.52%, *P* < 0.001) and DHA (mean ± SD 10.2 ± 1.07%, *P* < 0.001).

Table 1. Characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Olive oil (n = 40)</th>
<th>Fish oil (n = 40)</th>
<th><em>P</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal allergy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma</td>
<td>21 (52.5)</td>
<td>21 (52.5)</td>
<td>0.768</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>20 (50.0)</td>
<td>20 (50.0)</td>
<td>0.968</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nil</td>
<td>11 (27.5)</td>
<td>11 (27.5)</td>
<td>0.726</td>
</tr>
<tr>
<td>One more</td>
<td>22 (55.0)</td>
<td>22 (55.0)</td>
<td></td>
</tr>
<tr>
<td>Delivery method</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elective caesarean</td>
<td>8 (20.0)</td>
<td>8 (20.0)</td>
<td>0.335</td>
</tr>
<tr>
<td>Delivery after labour</td>
<td>32 (80.0)</td>
<td>32 (80.0)</td>
<td></td>
</tr>
<tr>
<td>Gender of the neonate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22 (55.0)</td>
<td>22 (55.0)</td>
<td>0.429</td>
</tr>
<tr>
<td>Female</td>
<td>21 (52.5)</td>
<td>21 (52.5)</td>
<td></td>
</tr>
</tbody>
</table>

*P*-values are as shown (significance level *P* < 0.05).
levels in the fish oil group compared to the placebo group (EPA mean ± SD 0.37 ± 0.28%; DHA mean ± SD 7.30 ± 0.95%; Fig. 1). Levels of n-6 PUFA AA were significantly lower in the fish oil group (mean ± SD 15.02 ± 1.44, P < 0.001), compared with the placebo group (mean ± SD 17.45 ± 1.17%) with a resulting increase in the ratio of n-3/n-6 PUFA levels in fetal cell membranes compared to the placebo group. There was no difference in the levels of oleic acid (18:1n-9) between the groups, indicating that the olive oil placebo did not have any effect on total dietary intakes of oleic acid.

**Effects of fish oil supplementation on CB cytokine and IgE levels**

IL-13 levels were significantly lower (GM 9.61, 95% CI 5.46–16.93; P = 0.025) in neonates whose mothers received fish oil supplements in pregnancy compared to the placebo group (GM 26.32, 95% CI 13.44–51.55; Fig. 2). Cord plasma IL-13 was detectable in 64% (n = 27) of plasma samples from the placebo group compared to 44.7% (n = 17) of samples from the fish oil group (P = 0.08). Neonates delivered by elective caesarean were less likely [odds ratio (OR) 0.16, 95% CI 0.05–0.54; P = 0.003] to have detectable cord plasma IL-13 and had lower IL-13 levels (GM 6.39, 95% CI 2.59–15.78; P = 0.02) compared to those whose mothers went into active labour (GM 21.41, 95% CI 12.88–35.59). Female infants were more likely (OR 2.75, 95% CI 1.11–6.82; P = 0.03) to have IL-13 detected in the CB plasma, and had higher levels (GM 25.87, 95% CI 13.79–48.53; P = 0.03) compared to male infants (GM 9.80, 95% CI 5.23–18.35). Because gender of the infant and method of delivery did not differ significantly between the groups, they were unlikely to contribute to the difference in IL-13 between the two groups. This was confirmed with multivariate linear regression (for continuous data) or logistic regression (for categorical data). There were no relationships between IL-13 and neonatal growth parameters.

There were no differences in IFN-γ levels between the groups; however, this cytokine was below detection in most (more than 95%) of the cord plasma samples. Most other cytokines (TNF-α, IL-4, IL-5, IL-6, IL-10, IL-12) were also only detectable in a minority of cases (less than 10% of neonates) and there were no differences in the magnitude or the frequency of detection between the groups (not shown).

Total IgE levels in plasma were only detectable (≥0.35 kU/L) in 5% (n = 4) of this neonatal population, and there were no significant differences between the fish oil group and the placebo group (not shown). There was no relationship between IL-13 and IgE levels.

**Effects of fish oil supplementation on lymphocyte subsets in CB**

There was no significant difference in the frequency of lymphocyte subsets (% and absolute concentration) between the two groups, with respect to total T cells (CD3+), T helper cells (CD4+), T suppressor cells (CD8+), NK cells (CD16+ and CD56+) and B cells (CD19+) (not shown).

© 2003 Blackwell Publishing Ltd, Clinical and Experimental Allergy, 33:442–448
Relationship between neonatal n-3 PUFA levels and CB cytokine levels

The fatty acid data from both groups were combined to achieve a range of DHA levels (5.17-12.77% of the total fatty acids measured) to determine any associations between DHA status and cytokine (IL-13) levels. There was a significant inverse relationship between neonatal n-3PUFA levels and cytokine (IL-13) levels. There was a significant inverse relationship between 22:6n-3 (DHA) and plasma IL-13 after accounting for potential confounding effects (parity, gender and delivery method; P = 0.04; Table 2). The range for 20:5n-3 (EPA) was much smaller (0.18-2.82% of total fatty acids) and, although the same trend was seen, this was not statistically significant. In contrast, n-6 PUFA levels tended to be positively correlated to circulating IL-13 in cord plasma, although this was not statistically significant. There were no other significant relationships between PUFA levels and cytokine or IgE levels in cord plasma.

Relationship between neonatal plasma IL-13 levels and CB lymphocyte subsets

There was a significant positive relationship between plasma IL-13 levels and NK cells (CD56+/CD16+ P = 0.02) and B cells (CD19+ P = 0.02) in CB (Table 3). However, these relationships did not remain statistically significant after controlling for differences in gender, delivery method and parity. There was no relationship between NK or B cell numbers and n-3/n-6 PUFA levels. There were also no relationships between numbers or percentages of CD4 and CD8 T cells and cytokine levels (Table 3) or PUFA levels (not shown).

Discussion

Early studies of immunological changes in pregnancy suggested that 'Th2 skewing' of immune responses at the maternal-fetal interface is important for fetal survival [26]. Although new research suggests more complex immunological changes that are not fully explained by the Th1/Th2 paradigm [27,28], this model has been immensely useful. With recognition that abnormalities of early immune programming may contribute to persistent Th2 responses and allergic disease, there is also growing interest in factors that regulate Th1/Th2 responses in the perinatal period. Although multiple environmental changes are likely to be contributing to the increasing propensity for Th2 responses in infancy, to our knowledge this is the first study examining the relationship between maternal dietary PUFA and neonatal Th1/Th2 cytokine levels.

In addition to epidemiological associations with allergic disease, the well-recognized anti-inflammatory properties of n-3 PUFA have provided a plausible pathway of influence during early immune development. Some of the anti-inflammatory properties of n-3 PUFA are mediated through competitive inhibition of PGE2 synthesis. Unlike n-6 PUFA, which are preferentially metabolized to produce PGE2 and other inflammatory mediators [29], n-3 PUFA derived metabolites [leukotriene (LT)B3 and PGE3] are less inflammatory. In addition to its inflammatory properties, PGE2 also inhibits Th1 IFN-γ responses [30,31] and favours Th2 differentiation (reviewed in [32]). Neonatal lymphocytes are known to be more sensitive to these 'Th1 inhibitory' effects of PGE2 [33]. and early studies

Table 2. Relationship (regression coefficients) between neonatal red cell membrane fatty acid levels and cord plasma IL-13 levelsa

<table>
<thead>
<tr>
<th>Polysaturated fatty acid</th>
<th>Regression coefficient (95% CI)</th>
<th>Adjusted regression coefficient† (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-6 PUFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linoleic acid (18:2n-6)</td>
<td>-0.53 (-1.57, 0.52)</td>
<td>-0.45 (-1.46, 0.66)</td>
</tr>
<tr>
<td>Dihomo-γ-linolenic acid (20:3n-6)</td>
<td>0.72 (-0.22, 1.66)</td>
<td>0.71 (-0.21, 1.62)</td>
</tr>
<tr>
<td>Arachidonic acid (20:4n-6)</td>
<td>0.07 (-0.30, 0.94)</td>
<td>0.01 (-0.23, 0.26)</td>
</tr>
<tr>
<td>22:3n-6</td>
<td>0.32 (-0.30, 0.94)</td>
<td>0.28 (-0.32, 0.66)</td>
</tr>
<tr>
<td>Docosatetraenoic acid (22:4n-6)</td>
<td>0.87 (-0.15, 1.86)</td>
<td>0.81 (-0.19, 1.86)</td>
</tr>
<tr>
<td>Total n-6†</td>
<td>0.09 (-0.08, 0.25)</td>
<td>0.06 (-0.11, 0.22)</td>
</tr>
<tr>
<td>n-3 PUFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eicosapentaenoic acid (20:5n-3)</td>
<td>-0.40 (-0.98, 0.18)</td>
<td>-0.40 (-0.97, 0.18)</td>
</tr>
<tr>
<td>Docosapentaenoic acid (22:5n-3)</td>
<td>0.22 (-0.83, 0.38)</td>
<td>0.21 (-0.90, 0.27)</td>
</tr>
<tr>
<td>Docosahexaenoic acid (22:6n-3)</td>
<td>-0.27 (-0.52, -0.02)</td>
<td>-0.25 (-0.49, -0.01)</td>
</tr>
<tr>
<td>Total n-3†</td>
<td>-2.73 (-5.45, -0.01)</td>
<td>-2.70 (-5.35, -0.05)</td>
</tr>
</tbody>
</table>


Table 3. Relationship (regression coefficients) between neonatal lymphocyte subsets and cord plasma IL-13 levelsa

<table>
<thead>
<tr>
<th>Cell marker</th>
<th>Regression coefficient (95% CI)</th>
<th>Adjusted regression coefficient† (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+ (T cells)</td>
<td>0.41 (-0.06, 0.89)</td>
<td>0.34 (-0.13, 0.81)</td>
</tr>
<tr>
<td>CD4+ (T helper cells)</td>
<td>0.82 (-0.02, 1.66)</td>
<td>0.53 (-0.10, 1.17)</td>
</tr>
<tr>
<td>CD8+ (T suppressor cells)</td>
<td>0.26 (-1.09, 1.50)</td>
<td>0.29 (-0.96, 1.57)</td>
</tr>
<tr>
<td>CD19+ (B cells)</td>
<td>1.54 (0.31, 2.77)</td>
<td>1.05 (-0.19, 2.29)</td>
</tr>
<tr>
<td>CD16+ 566+ (NK cells)</td>
<td>0.66 (0.15, 1.50)</td>
<td>0.54 (-0.19, 1.28)</td>
</tr>
</tbody>
</table>

*Results of simple and multiple regression analyses with free cord plasma IL-13 and neonatal lymphocyte subsets. †Adjusted for gender, parity and method of delivery. *Indicates significance P < 0.05.

© 2003 Blackwell Publishing Ltd, Clinical and Experimental Allergy, 33:442-448
suggested that the normally impaired IFN-γ Th1 responses in the neonatal period can be reversed by inhibiting PGE2 with indomethacin [32]. This suggests that subtle changes in fatty acid and PG metabolism could alter the Th1/Th2 balance in the neonatal period. Furthermore, diets rich in n-6 PUFA may be directly implicated in the impaired neonatal Th1 responses that have been associated with allergic disease ([19,33-36] and others). A number of studies have described altered fatty acid levels in allergic mothers and their infants ([37,38] and others), but further studies are required.

In this study, we observed that maternal dietary supplementation with n-3 PUFA was associated with significantly lower IL-13 levels in cord plasma. There were also consistent correlations between IL-13 levels and n-3 PUFA levels, in particular DHA. This finding supports a relationship between maternal dietary PUFA and neonatal immune function. The origin of the IL-13 is not clear, but is more likely to be derived from the fetus than the placenta late in pregnancy [39,40]. The predictive value of neonatal Th2 responses, including IL-13, and risk of allergic disease is not clear. Some studies have higher Th2 responses in association with atopic risk [41,42], others have observed lower levels [39,43]. The association between high atopic risk and weaker neonatal Th1 responses appears more consistent [32-36], and may play a role in the reduced ability to develop mature Th1 responses in the post-natal period. In the present study, lower IL-13 levels may reflect a subtle shift in the cytokine balance, favouring Th1 immunity, which is not evident by measuring low-level plasma IFN-γ (below detection in most infants). The mechanism of action is also not clear, but could be mediated through changes in neonatal PGE2 synthesis, which is known to be inhibited by maternal n-3 PUFA supplementation [6]. Omega-3 PUFA have many other immunomodulatory effects (reviewed in [44]), and have been also shown to promote tolerance at the maternal-fetal interface by suppressing MHC Class II expression [10]. It is also possible that immunological effects at the maternal-fetal interface may occur as the secondary result of fatty acid changes on maternal immune responses. Although we have demonstrated a direct relationship between neonatal fatty acid status and neonatal immune function, the effects of this dietary modification on maternal immune response were not assessed in this study.

PUFA may also have effects on NK function and although this is less well documented [44] it appears that high-dose DHA supplementation can decrease NK cell activity [45,46]. Although fish oil supplementation was not associated with differences in NK cell numbers in the present study, function was not assessed. However, the possible relationship between IL-13 and NK cell numbers in this study suggests a potential effect. The significance of this observation is unclear, but highlights the growing need to define the role of NK cells in gestation and immune development. Although initially believed to be a threat to pregnancy, NK cells are now believed to play an immunoregulatory role at the maternal-fetal interface (reviewed in [27]). Depending on the prevailing cytokine and milieu maturation stage, NK cells may also exist in subsets producing type 1 and type 2 cytokines, including IL-13 [47]. We may speculate that variations in NK phenotype may also have implications for immune maturation and atopic risk.

While these immunological changes appear subtle, they may have a significant effect on developing immune responses, which are plastic and vulnerable to influence. Our findings add weight to the speculation that dietary factors can influence the immunological processes that are central to the pathogenesis of atopy, and accordingly that changing dietary patterns may be contributing to the increasing prevalence of allergic disease [5,48]. We have demonstrated preliminary evidence that altered membrane PUFA profiles during gestation may influence immunological function during this critical time of development. Ongoing studies are needed to examine the effects of early n-3 PUFA supplementation on evolving allergen-specific responses and the effects on subsequent allergic disease. These issues will be subsequently addressed in this population.

Acknowledgements

We wish to acknowledge the staff and patients who assisted in this study. We are particularly grateful to the obstetricians and midwives at St John of God Hospital, Subiaco, Western Australia. We thank Professor Scott Weiss, Harvard Medical School for his role in the planning and design of this study. We also wish to thank Ms Lynette McCahon for technical assistance, Ms Elaine Pascoe for statistical advice and Dr Thierry Venaille for his advice and support during the study design. The study was supported by grants from the NH & MRC and Raine Medical Research Foundation, Australia.

References

33 Rinas U, Horneff G, Wahn V. Interferon gamma production by cord blood mononuclear cells is reduced in newborns with a family history of atopic disease and is independent from cord blood IgE levels. Ped Allergy Immunol 1993; 4:60-4.