

Black currant seed oil supplementation of mothers enhances IFN- γ and suppresses IL-4 production in breast milk

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Abstract

Background: The first year of infancy is crucial for the development of atopic immune response. Inadequate early Th1 and Treg responses and increased production of Th2 cytokines are associated with atopy. Breast milk contains several immunomodulatory cytokines and other factors that might influence the maturation of the infant's immune system. We assessed the cytokines in breast milk of mother of newborn infants and their associations with black currant seed oil (BCSO) supplementation during pregnancy, mother's atopic status and the development of infant's atopic dermatitis.

Methods: Mothers and infants from an intervention study by black currant seed oil (n = 31) or olive oil as placebo (n = 30) were included in the study. Breast milk samples were collected during the first 3 months of breastfeeding. Breast milk levels of IL-4, IL-5, IL-10, IL-12, IFN- γ and TNF were measured by Luminex technology.

Results: BCSO intervention group had decreased level of IL-4 (p = 0.044) and elevated level of IFN- γ (p = 0.014) in breast milk as compared to olive oil group. No significant differences were observed in IL-5, IL-10, IL-12 and TNF levels between the BCSO and olive oil groups. Mothers who had atopic dermatitis had significantly decreased levels of IL-10 (p = 0.044) in breast milk. Breast milk of the mothers of the children who developed atopic dermatitis had lower levels of IFN- γ (p = 0.039) as compared to the breast milk of the mothers of the children without dermatitis.

Conclusion: Dietary intervention with BCSO had immunomodulatory effects on breast milk cytokine production towards Th2 to Th1 immunodeviation.

The first year of infancy is crucial for the development of atopic immune response (1–4). The foetal immune environment is weakly Th2 predominated and sensitization to allergens starts *in utero* (1, 2, 5). In children who generate atopy inadequate early Th1 and Treg responses fail to suppress production of Th2 cytokines in the neonatal period (1–4). Breast milk contains factors that influence the maturation of the infant's immune system during the first year of life. Breast milk contains numerous components protecting the infant against infections, including factors that provide specific immunity, like antibodies and lymphocytes (6). Breast milk also contains non-specific protective factors, such as the iron-chelating protein, lactoferrin, lysozyme and oligosaccharides. In addition, several

immunomodulatory cytokines are present in human milk. The concentrations of Th2-type cytokines are higher in breast milk from allergic than non-allergic mothers (7).

Essential fatty acids (EFA) have an important role in the regulation of epidermal inflammation and immune function (8, 9). A clear association has been observed between breast milk TGF- β and breast milk EFA composition suggesting that it may be possible to influence the immunological properties of breast milk by dietary fatty acid intervention of the mother (10). Arachidonic acid (AA)-derived eicosanoids are mostly proinflammatory, whereas eicosapentaenoic acid (EPA)-derived eicosanoids are anti-inflammatory (11). Eicosanoids derived from dihomo- γ -linolenic acid (DGLA) are either anti-inflammatory

or inactive (11). AA-derived PGE₂ has been shown to inhibit production of Th1 cytokine IFN- γ , but not Th2 cytokines IL-4 and IL-5 in human T cells (12). In line with this, AA-derived LTB₄ induces both IL-4 and IL-5 in human T cells (13, 14). Blackcurrant (*Ribes nigrum*) seed oil (BCSO) contains linoleic acid (LA) and γ -linolenic acid (GLA), precursors of DGLA, as well as α -linolenic acid (ALA) and stearidonic acid (SDA), precursors of EPA (15). In addition, dietary supplementation of BCSO increases the content of DGLA in plasma phospholipids (16). The increased production of eicosanoids from DGLA and EPA instead of AA, during BCSO intervention, could thus direct the Th cell balance from Th2 to Th1. It is possible that this shift in balance could also be seen in breast milk (10).

Our earlier report showed that dietary supplementation with BCSO significantly reduces the prevalence of atopic dermatitis in infants at the age of 1 yr (17). Dietary rapeseed oil intervention or dietary intervention with probiotics increase n-3 acid fatty acids (ALA, SDA) and the breast milk concentrations of several cytokines (18). On the other hand, increased breast milk levels of several cytokines, including IL-4, predict the development of atopic dermatitis by the age of 6 months (19). A possible mechanism by which dietary BCSO could prevent the development of atopic dermatitis could thus be modulation of breast milk cytokines. In this paper the cytokines in breast milk were analysed to investigate their potential association with the BCSO supplementation, development of infant's atopic dermatitis and maternal atopy.

Methods

Study subjects

The subjects of this study were randomly chosen from the study population of a randomized double-blind placebo-controlled (DBPC) intervention trial (ISRCTN14869647, www.controlled-trials.com) that was carried out during the years 2004–2008 at the public healthcare centres in the Salo and Kaarina–Piikkiö regions in south-western Finland. The trial was conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by the Ethical Committee of the Hospital District of South-West Finland. All subjects participating signed a written informed consent. In the clinical study, altogether 151 mothers were randomly assigned to receive BCSO and 162 olive oil as placebo. The preparation and the fatty acid composition of BCSO were described in the clinical study report (17). The supplementation was first given to the mothers during the 8th to 16th wk of pregnancy and then continued until the end of the exclusive breastfeeding period as previously described (17).

During the mothers' first visit, the study physician, a dermatologist, or the nurse filled in a preliminary information form which contained evaluations of, among other things, the mothers' residential environment, pets, smoking, usage of vitamins and additional nutrients and tendency for atopic diseases in the family. The children were examined at the ages of 3, 12 and 24 months, at which points any occurrences of atopic dermatitis were evaluated. A dermatologist evaluated the skin of each child at each visit. Atopic dermatitis was

defined as a chronic or relapsing itchy dermatitis with a characteristic morphology and distribution (20). The SCORAD index, an internationally used batch of criteria for atopic dermatitis, was used to assess dermatitis severity (21). Atopic dermatitis was also recorded if there was a history of chronic or relapsing itchy dermatitis with a typical localization. At each follow-up visit, a data form was filled in, which charted atopic dermatitis, doctor-diagnosed asthma and self-reported recurrent wheezing, food allergies, allergic nose or eye symptoms, any other diseases and the use of vitamins and additional nutrients by the children and their mothers. Any changes in the residential environment, household animals and smoking were also recorded in the forms.

Thirty-one mothers and their infants from the BCSO group and 30 from the placebo group were randomly selected for this immunological study. The baseline demographic and clinical characteristics of these infants were in proportion to the whole clinical study population and are presented in Table 1 (17). The breast milk samples were collected mainly after delivery, but because of early discharge and/or delayed start of lactation, this was not possible with all mothers. Those mothers gave their samples at the 3-month control visit. There were no significant differences in the cytokine levels or in the demographics of the subjects with regard to the sampling time (Table, online supporting information). The samples were centrifuged and stored in aliquots at -70°C .

Table 1 Baseline demographic and clinical characteristics

	Blackcurrant seed oil	Olive oil	p-value*
Number	31	30	
Parental atopy; n (%)			0.65
Both	6 (19.4)	8 (26.7)	
One	16 (51.6)	16 (53.3)	
None	9 (29.0)	6 (20.0)	
Birth weight (g); mean (SEM)	3557 (86)	3653 (93)	0.32
Parental smoking; n (%)	10 (32.3)	7 (23.3)	0.29
Breastfeeding (months); mean (SEM)	9.0 (1.0)	9.5 (0.9)	0.79
Excl breastfeeding (months); mean (SEM)	3.9 (0.3)	4.2 (0.3)	0.20
Any pets at home; n (%)	14 (45.2)	12 (40.0)	0.68
Older siblings; n (%)			0.67
None	11 (35.5)	14 (46.7)	
One	13 (41.9)	10 (33.3)	
Two or more	7 (22.6)	6 (20.0)	
Atopic dermatitis at; n (%)			
3 months	3 (9.7)	4 (12.9)	0.65
12 months	11 (35.5)	15 (50.0)	0.35
24 months	14 (45.2)	17 (56.7)	0.43
SCORAD at; mean (SEM)			
3 months	2.5 (1.2)	2.6 (0.8)	0.60
12 months	3.5 (0.7)	4.9 (1.1)	0.68
24 months	3.6 (0.9)	4.4 (1.2)	0.94

*Chi-square test or Mann–Whitney *U*-test.

Cytokine assay

The cytokine levels in the breast milk samples were determined with high-sensitivity human cytokine Lincoplex kits (LINCO Research, St. Charles, MO, USA). The assays were performed in accordance with the manufacturer's protocol by employing Luminex technology.

Statistical analyses

Statistical analyses were performed with multivariate logistic regression analysis and ANOVA without corrections for multiple comparisons using StatView 5.0 statistical software (SAS Institute, Cary, NC, USA). Variables included in the analysis were cytokines, study intervention, maternal atopy, exclusive breastfeeding, number of the newborn's siblings, having pets at home, atopic dermatitis of the mother and the child. In two-group analysis, also chi-square test and Mann–Whitney *U*-test were used. *p*-values < 0.05 were considered to be statistically significant.

Results

Figure 1 presents the effects of BCSO intervention on cytokine responses in breast milk. The level of IL-4 (*p* = 0.044) was lower, and the level of IFN- γ (*p* = 0.014) was higher in the BCSO group as compared to olive oil group. No significant differences were observed in IL-5, IL-10, IL-12 and TNF levels between the BCSO and olive oil groups.

Table 2 shows the cytokine levels of mothers with and without atopic dermatitis. Mothers who had atopic dermatitis had significantly decreased levels of IL-10 (*p* = 0.044) in breast milk at 3 months. No significant differences were observed with other cytokines.

Table 2 Breast milk cytokine concentration (pg/ml) of mothers with and without atopic dermatitis; mean (SEM)

	Mothers with atopic dermatitis		<i>p</i> -value*
	Yes (<i>n</i> = 23)	No (<i>n</i> = 38)	
IL-4	228 (71)	269 (56)	0.091
IL-5	1.6 (0.7)	1.5 (0.4)	0.37
IL-10	36.7 (21.5)	52.5 (28.9)	0.044
IL-12	6.5 (3.9)	3.3 (1.6)	0.064
IFN- γ	9.2 (5.2)	10.8 (5.6)	0.50
TNF	5.1 (0.8)	6.3 (0.7)	0.43

*Multivariate logistic regression analysis.

Breast milk cytokine levels in the mothers of the children with and without atopic dermatitis by 12 months of age are shown in Table 3. The breast milk of the mothers of the children who developed atopic dermatitis had lower levels of IFN- γ (*p* = 0.039) as compared to those of the children without dermatitis. No differences were seen in IL-4, IL-5, IL-10, IL-12 or TNF levels. Having pets at home, the number of the newborn's siblings and mothers atopy status had no statistically significant effects on breast milk cytokine levels.

Discussion

BCSO supplementation, started during early pregnancy, was found to decrease IL-4 and to increase IFN- γ cytokine secretion in breast milk. In addition, the mothers of children who developed atopic dermatitis by the age of 12 months had lower levels of IFN- γ in their breast milk. Furthermore, mothers with atopic dermatitis had reduced levels of IL-10, the main cytokine of regulatory T cells, in their breast milk.

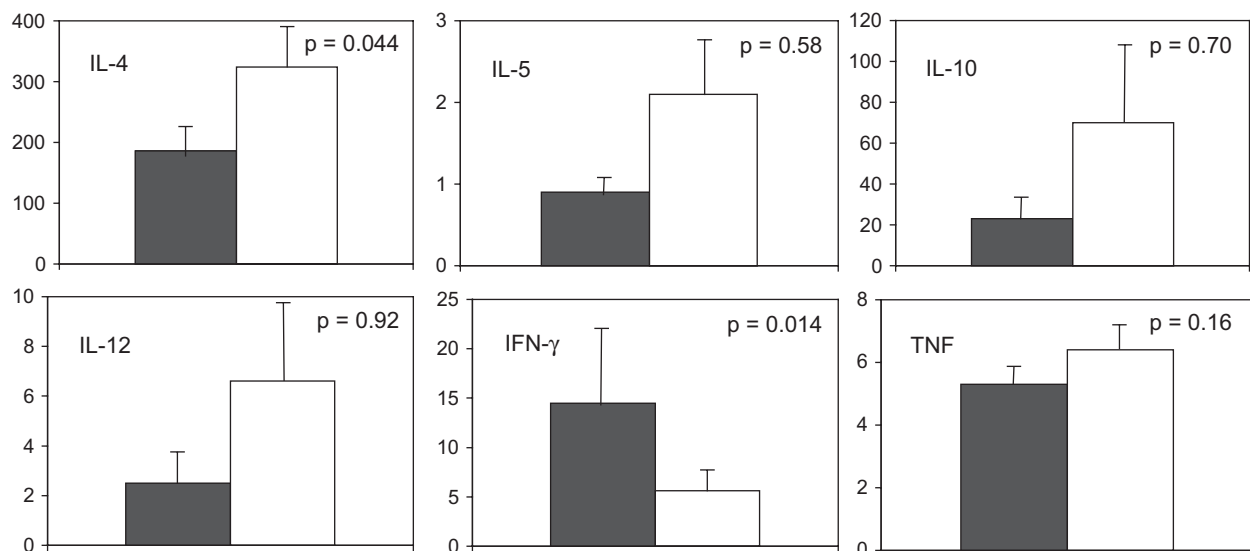


Figure 1 Breast milk IL-4, IL-5, IL-10, IL-12, IFN- γ and TNF concentrations (pg/ml, mean and SEM) of mothers undergoing BCSO (*n* = 31, black column) or olive oil (*n* = 30, white column) intervention analysed by high-sensitivity human cytokine Lincoplex® kits using Luminex® technology.

Table 3 Breast milk cytokine concentration (pg/ml) of mothers of children with and without atopic dermatitis at 12 months; mean (SEM)

	Children with atopic dermatitis at 12 months of age		p-value*
	Yes (n = 26)	No (n = 33)	
IL-4	307 (74)	225 (54)	0.65
IL-5	2.3 (0.8)	0.9 (0.2)	0.56
IL-10	76.5 (44.3)	25.6 (9.4)	0.58
IL-12	7.1 (3.8)	2.7 (1.3)	0.64
IFN- γ	4.8 (1.9)	14.9 (7.1)	0.039
TNF	6.4 (0.9)	5.5 (0.6)	0.38

*Multivariate logistic regression analysis.

Studies on fatty acid intervention of pregnant and breast-feeding mothers have revealed contradictory effects on breast milk cytokine composition. Supplementation with fish oil during pregnancy altered breast milk fatty acid composition but had no effect on breast milk IL-6, IL-10 and IL-13 levels (22). Also supplementation with docosahexaenoic acid-rich tuna oil increased n-3 fatty acid concentration in breast milk, but had no effect on concentrations of IL-1 β , IL-6 and TNF (23). In another study, dietary intervention with rapeseed oil and intervention with probiotics during pregnancy increased the breast milk n-3 fatty acid concentrations as well as the levels of IL-2, IL-4, IL-10 and TNF in breast milk (18). In our DBPC trial, we demonstrated that the use of BCSO in pregnant and breastfeeding mothers and their infants after breastfeeding resulted in a reduced outcome of atopic dermatitis at the age of 12 months (17). This is in agreement with our findings of reduced IL-4 and increased IFN- γ showing immunomodulation towards enhanced Th1 and suppressed Th2 responses.

Breastfeeding as such has been shown to effect the outcome of atopic dermatitis and cytokine responses of infants. Significant reduction in TLR7-mediated IL-10 production in peripheral blood was seen in breastfed infants at the age of only 1 month (24). In a recent study, breastfeeding reduced the risk of asthma but was a risk factor for sensitization to milk nuts and egg (25). In another study, long duration of breastfeeding increased the risk of atopic dermatitis at the age of 18 months (26). In our study, there were no differences in breastfeeding or exclusive breastfeeding between the intervention groups, and breastfeeding was not associated with the cytokine responses or the outcome of atopic dermatitis.

The mothers of children who developed atopic dermatitis by the age of 12 months had considerably lower levels of IFN- γ in their breast milk. In a recent study, no association could be seen in with breast milk TGF- β 1 and infants atopic dermatitis at the age of 12 months (27). The concentrations of IL-4 have been shown to be higher in breast milk from allergic than non-allergic mothers and similar trends were seen for IL-5 and IL-13 (7). Comparison of Swedish and Estonian mothers'

breast milk showed that Estonian mothers have lower breast milk levels of TGF- β , but higher levels of secretory IgA, IL-10 and IFN- γ possibly because of differences in dietary habits and microbial exposure (28). Children with higher risk of developing atopic dermatitis during the first 2 yr of life have also been shown to have reduced IFN- γ and enhanced IL-4 producing CD4⁺ T cells in the cord blood (4). All these findings are in concordance with our findings on fatty acid intervention and outcome of atopic dermatitis and the association of these with breast milk IL-4 and IFN- γ . In recent studies, maternal atopy has been associated with increased IgA antibodies and decreased TGF- β , whereas probiotic supplementation has been associated with decreased IgA antibodies and increased IL-10 but not with TGF- β (29, 30).

Our findings of breast milk IL-10 and maternal atopic dermatitis support earlier experimental data. The acute skin lesions in atopic dermatitis are characterized by a initial Th2-type cellular response which then, because of IL-12 induction in antigen-presenting cells, keratinocytes and eosinophils, is deviated to a Th1 response (31, 32). Eventually, the cutaneous Th1 cells and the proinflammatory cytokines IFN- γ and TNF induce a Fas-mediated apoptosis of keratinocytes and chronic inflammation (32, 33). The chronic inflammation may persist because the inflammatory reaction of atopic skin is associated with defective regulatory T-cell function and absence of skin infiltrating CD4⁺CD25⁺FoxP3⁺ regulatory T cells (34). This is in accordance with our findings of reduced level of IL-10, the main cytokine of regulatory T cells, in the breast milk of mothers with atopic dermatitis.

Our study showed that dietary intervention with BCSO had immunomodulatory effects on breast milk cytokine production towards Th2 to Th1 immunodeviation. In addition, breast milk cytokines IFN- γ and IL-10 were inversely associated with atopic dermatitis in both mothers and infants. Our findings are in line with our earlier observations of reduced incidence of atopic dermatitis during BCSO intervention, suggesting that fatty acid intervention may provide a tool for prevention of atopic diseases.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Demographic data and cytokine levels in subjects grouped according to timepoint of the breast milk sample.

Table. Supplemental information: “Demographic data and cytokine levels in subjects grouped according to timepoint of the breast milk sample”

	Colostrum	Three-month	P-value*
Number	39	22	
BCSO (%)	20 (51.3)	11 (50.0)	0.92
Olive oil (%)	19 (48.7)	11 (50.0)	
Maternal atopy; n (%)	26 (66.7)	15 (68.2)	0.90
Maternal atopic dermatitis; n (%)	13 (50.0)	10 (45.5)	0.35
Birth weight (g); mean (SEM)	3540 (68)	3718 (124)	0.22
Breastfeeding (months); mean	9.2 (0.9)	9.4 (1.0)	0.88
Any pets at home; n (%)	21 (53.8)	10 (45.5)	0.84
Older siblings; n (%)			0.26
None	19 (48.7)	6 (27.3)	
One	13 (33.3)	10 (45.4)	
Two or more	7 (18.0)	6 (27.3)	
Atopic dermatitis at; n (%)			
12 months	17 (43.6)	9 (40.9)	0.92
24 months	21 (53.8)	10 (45.5)	0.70
Cytokines (pg/ml); mean (SEM)			
IL-4	251 (55)	258 (72)	0.88
IL-5	1.5 (0.4)	1.5 (0.7)	0.90
IL-10	51 (28)	39 (22)	0.25
IL-12	3.5 (1.7)	6.4 (3.9)	0.14
IFN- γ	8.7 (5.1)	12.8 (6.5)	0.64
TNF	6.7 (0.7)	4.3 (0.7)	0.15

* Chi-square test, Mann-Whitney U-test or multivariate logistic regression analysis (cytokines)