

## Pro-inflammatory and Th2-type cytokine responses in PBMC in infants are associated with parental smoking

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#### Abstract

**Background** During infancy, a disturbed cytokine balance leads to an atopic immune response. Many risk factors have been associated with the development of atopy. These include parental smoking, elevated cord blood IgE, early exposure to pets and family history of atopy, but the knowledge of their impact on cytokine balance is limited.

**Objective** To assess the cytokines induced by mitogen in peripheral blood mononuclear cells (PBMC) of infants at 3 months and 12 months of age and their potential association with fatty acid (FA) intervention, parental atopy, atopic dermatitis and parental smoking.

**Methods** Infants from an intervention study using black currant seed oil (BCSO,  $n = 34$ ) or placebo ( $n = 34$ ) were included. PBMC samples were taken at the age of 3 and 12 months. Signs of atopic dermatitis and parental smoking were registered. PBMC were isolated from heparinized blood samples, stimulated with ConcanavalinA mitogen and the cytokine responses were detected at 72 h of stimulation by Luminex technology.

**Results** Children of smoking parents had elevated levels of IL-4 ( $P = 0.0004$ ), IL-5 ( $P = 0.0002$ ), IFN- $\gamma$  ( $P = 0.039$ ) and TNF ( $P = 0.0003$ ) at 12 months of age. Children who had atopic dermatitis by the age of 3 months showed elevated levels of IL-5 at 3 months ( $P = 0.0027$ ) and 12 months of age ( $P = 0.022$ ). The production of TNF at the age of 3 months was higher ( $P = 0.010$ ) and the production of IL-12 at the age of 12 months was lower ( $P = 0.025$ ) in infants whose parents were atopic. BCSO intervention did not have any effect on any cytokine production or mRNA expression.

**Conclusion** Children of smoking parents had highly significantly elevated levels of Th2-type cytokines IL-4, IL-5 and pro-inflammatory cytokine TNF. The detrimental effects of parental smoking on the child's immune function should lead us to pay more attention to supporting parents to stop smoking.

**Keywords** atopic dermatitis, black currant seed oil, IL-4, IL-5, parental smoking, TNF

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#### Introduction

Atopic diseases, such as atopic dermatitis, allergic rhinitis and asthma, have become a prevalent public health problem in the industrialized world [1]. The first year of infancy is crucial for the development of immune function with a deviation towards an atopic immune response as a result of an inadequate early Th1 response [2, 3]. Earlier data suggest that the initial sensitization to allergens already starts *in utero* [4]. The selective development

of a Th2 cytokine profile in high-risk children who generate atopy is due to increased production of Th2 cytokines and impaired IFN- $\gamma$  production in the neonatal period. [5, 6]. It has also been shown that in atopic children, the allergen-induced IL-10 production at 12 months of age is lower than in non-atopic children resulting in poor regulatory control of the inflammatory process [5]. Enhanced IL-4 and low IFN- $\gamma$  production has been found already in cord blood CD4<sup>+</sup> T cells of children who developed atopic dermatitis during the first 2 years of life [6].

There are many risk factors that have been associated with the development of atopic disorders. These include parental smoking, elevated cord blood IgE, early exposure to pets and family history of atopy. Parental smoking has been associated with increased serum IL-4 and IgE, increased nasopharyngeal IL-13, lower peripheral blood IFN- $\gamma$ , higher blood eosinophil counts and more frequent respiratory symptoms in children [7–10]. Maternal cigarette smoking has been linked to increased cord blood IgE and lymphoproliferation, allergen sensitization and asthma [8, 11]. It has also been found that mitogen-induced cord blood IL-13 was higher and IFN- $\gamma$  was lower if mothers smoked during pregnancy [12].

The rapid increase in the incidence of atopic diseases has been suggested, in part, to be associated with changing dietary habits such as the intake of essential fatty acids (EFAs)  $\alpha$ -linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6), and with their changed ratios in people's diet [13, 14]. These have an important role in the regulation of epidermal inflammation and immune function [15, 16]. Atopics have been found to have reduced levels of EFAs in some studies with considerable variations in the plasma levels of n-3 and n-6 fatty acid [17]. In our earlier report, it was shown that dietary supplementation with black currant seed oil (BCSO) significantly reduced the prevalence of atopic dermatitis in infants at the age of 1 year [18]. The effects were comparable to those of probiotics administered during pregnancy [19]. BCSO offers an interesting safe alternative for rebalancing the profile of EFAs in atopic patients, and can potentially be a tool for preventing atopic dermatitis at an early stage of life.

In this article, the cytokines induced by ConcanavalinA mitogen in peripheral blood mononuclear cells (PBMC) of infants at 3 months and 12 months of age were analysed to investigate their potential association with the BCSO supplementation, development of atopic dermatitis, parental atopy and parental smoking.

## 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 (IS-RCTN14869647, [www.controlled-trials.com](http://www.controlled-trials.com)) that was carried out during the years 2004–2008 at the public health care 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. In the clinical study, altogether 151 mothers were randomly assigned to receive BCSO and

162 were assigned to receive placebo. The preparation and the fatty acid composition of BCSO is described in the clinical study report [18]. The doses were first given to the mothers during the 8th–16th weeks of pregnancy and then continued until the end of the exclusive breastfeeding period as previously described [18]. The infants started to receive the same oil in the form of drops when the exclusive breastfeeding was ended, and the children continued receiving the oil until 2 years of age [18].

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 months, 12 months and 24 months, at which points any occurrences of atopic dermatitis were evaluated. Blood samples for cell cultures were taken at the 3- and 12-month visits. 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, 22]. 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 as well as self-reported 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. The skin tests were carried out by an experienced study nurse at the 3-month, 12-month and 24-month follow-up visits of the children according to the EA-ACI guidelines [23]. The tested allergens were standardized allergen extracts of birch, cat, dog, cow's milk, egg, wheat, gliadin and house dust mite (Solu-Prick; ALK-Abellö, Horsholm, Denmark). Histamine hydrochloride (10 mg/mL) was used as a positive control and the ALK-Abellö NSA-diluent as a negative control. Responses were seen to only milk and egg (Table 1) and mainly in children with atopic dermatitis (six out of seven by the age of 1 year).

Thirty-four infants from the BCSO group and 34 infants 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 [18].

Table 1. Baseline demographic and clinical characteristics

|                                           | Black currant<br>seed oil | Olive oil  |
|-------------------------------------------|---------------------------|------------|
| Number                                    | 34                        | 34         |
| Gender; <i>n</i> (%)                      |                           |            |
| Male                                      | 14 (41.2)                 | 16 (47.1)  |
| Female                                    | 20 (59.8)                 | 18 (52.9)  |
| Parental atopy; <i>n</i> (%)              |                           |            |
| Both                                      | 5 (14.7)                  | 5 (14.7)   |
| One                                       | 22 (64.7)                 | 23 (67.6)  |
| None                                      | 7 (20.6)                  | 6 (17.6)   |
| Birth weight (g); mean (SD)               | 3636 (483)                | 3726 (541) |
| Parental smoking; <i>n</i> (%)            | 12 (35.3)                 | 13 (38.2)  |
| Breastfeeding over 4 months; <i>n</i> (%) | 25 (73.5)                 | 25 (73.5)  |
| Any pets at home; <i>n</i> (%)            | 15 (44.1)                 | 14 (41.2)  |
| Older siblings; <i>n</i> (%)              |                           |            |
| None                                      | 13 (38.2)                 | 17 (50.0)  |
| One                                       | 12 (35.3)                 | 12 (35.3)  |
| Two or more                               | 9 (26.5)                  | 5 (14.7)   |
| Atopic dermatitis at; <i>n</i> (%)        |                           |            |
| 3 months                                  | 4 (11.8)                  | 2 (5.9)    |
| 12 months                                 | 7 (20.6)                  | 12 (35.3)  |
| 24 months                                 | 9 (26.5)                  | 13 (38.2)  |
| Sensitization to egg at; <i>n</i> (%)     |                           |            |
| 3 months                                  | 0 (0.0)                   | 1 (2.9)    |
| 12 months                                 | 3 (8.8)                   | 3 (8.8)    |
| 24 months                                 | 0 (0.0)                   | 2 (5.9)    |
| Sensitization to milk at; <i>n</i> (%)    |                           |            |
| 3 months                                  | 1 (2.9)                   | 0 (0.0)    |
| 12 months                                 | 1 (2.9)                   | 0 (0.0)    |
| 24 months                                 | 0 (0.0)                   | 0 (0.0)    |

### Peripheral blood mononuclear cell (PBMC) cultures

The PBMC were isolated from heparinized blood samples by Ficoll-Paque density gradient centrifugation (Ficoll-Paque PLUS; GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and washed twice with Hanks' balanced salt solution (HBSS) buffered with NaHCO<sub>3</sub> (pH 7.4). After washings, the cells were resuspended in RPMI-1640 culture medium (Invitrogen Co., Carlsbad, CA, USA) supplemented with 5% autologous serum, 2.5 mM L-glutamine (Sigma-Aldrich Co., St Louis, MO, USA) and 100 µg/mL gentamycin sulphate (Biological Industries Ltd., Kibbutz Beit Haemek, Israel) and applied on 48-well flat-bottomed cell culture plates (Costar, Corning Inc., Tewksbury, MA, USA) at a density of 10<sup>6</sup>/mL. The cells were stimulated in the presence of ConcanavalinA mitogen (Pharmacia, Uppsala, Sweden) (10 µg/mL). Medium alone served as unstimulated control. All incubations were performed at 37°C in humidified atmosphere with 5% CO<sub>2</sub>. After 72 h, the cells and the culture supernatants were collected, and the cells were resuspended in TRIzol reagent (Gibco Life Technologies, Paisley, UK) for total RNA extraction.

### Total RNA extraction and RT-PCR

The RNA isolation and the TaqMan<sup>®</sup> RT-PCR were performed as previously described [24]. Briefly, RNA was isolated according to Trizol instructions and the extracted RNA was stored in 75% ethanol at -20°C. The RT reaction was performed using First-strand cDNA Synthesis Kit (Pharmacia) using oligo (dT) primers, and cDNA was stored at -70°C. The amplification of IL-4, IL10 and IFN-γ was performed in MicroAmp<sup>®</sup> optical 96-well reaction plate (PE Applied Biosystems, Foster City, CA, USA). A negative PCR control without template and a positive PCR control with a template of known amplification were included in each assay. The reaction was performed in ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems). For analysis of IL-10, Predeveloped Assay Reagents (PDAR) kits were purchased from PE Applied Biosystems. The primer and probe sequences for detection of β-actin, IFN-γ and IL-4 mRNA expression were as follows: forward primer TTG CCG ACA GGA TGC AGA A; reverse primer TCA GGA GGA GCA ATG ATC TTG AT; and probe TGC CCT GGC ACC CAG CAC AA for β-actin; forward primer CTC GAA ACA GCA TCT GAC TCC TT; reverse primer TGT CCA ACG CAA AGC AAT ACA; and probe TGC TGG CGA CAG TTC AGC CAT CAC for IFN-γ; and forward primer AAG TGC GAT ATC ACC TTA CAG GAG AT; reverse primer TGT TCT TGG AGG CAG CAA AGA T; and probe ACA GAG CAG AAG ACT CTG TGC ACC GAG TT for IL-4, (IFN-γ and IL-4 kindly provided by Prof. Riitta Lahesmaa, Centre for Biotechnology, Turku, Finland). For analysis of IL-10, cDNA-specific PDAR (Pre-Developed Assay Reagents) kit was purchased from Applied Biosystems. The reactions were carried out in ABI PRISM 7700 Sequence Detection System (Applied Biosystems) at 10 µL volumes according to the TaqMan Universal Master Mix protocol (Applied Biosystems), using 300 nm of primers and 200 nm of dual-labelled fluorescent probe [6-carboxyfluorescein (FAM) at the 5' end and 6-carboxytetramethylrhodamine (TAMRA) at the 3' end] and 1 µL of cDNA. The data analysis was performed according to the manufacturer's instructions (User Bulletin #2, P/N 4303849, Applied Biosystems) using comparative Ct (2-ΔΔCt) method, where β-actin served as an endogenous reference gene and unstimulated cell culture as a calibrator. β-actin is not regulated in PBMC cultures [24]. The resulting 2-ΔΔCt value was used to indicate the fold change in cytokine expression relative to unstimulated cultures.

### Cytokine assay

The cytokine responses (IL-4, IL-5, IL-10, IL-12p70, IFN-γ and TNF) from PBMC were detected 72 h after beginning of the stimulation by measuring cytokine

secretion using 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 using StatView 5.0 (SAS Institute, Cary, NC, USA) statistical software. Variables included in the analysis were cytokines, study intervention, birth weight, breastfeeding, parental atopy, parental smoking, number of siblings, having pets at home and atopic dermatitis. *P*-values < 0.05 were considered to be statistically significant.

### Results

BCSO intervention did not have any effect on any cytokine production or mRNA expression. No significant differences were observed between the BCSO and olive oil groups in cytokine production, IL-4, IL-5, IL-10, IL-12, TNF and IFN- $\gamma$  of PBMC at 3 or 12 months of age (data not shown).

Figure 1 presents the effects of parental smoking on cytokine responses in PBMC of the children at 3 and 12 months of age. Children of smoking parents had significantly elevated levels of IL-4 ( $P = 0.0004$ ), IL-5 ( $P = 0.0002$ ), IFN- $\gamma$  ( $P = 0.039$ ) and TNF ( $P = 0.0003$ ) at 12 months of age. Twenty-five fathers smoked during pregnancy and the first year of life. Four mothers smoked during pregnancy and 14 stopped smoking before pregnancy or during the first trimester. All

smoking mothers were in families in which the father smoked.

The mitogen-induced net production of cytokines in the children grouped according to cumulative development of atopic dermatitis by the age of 3 months is shown in Table 2. Children who had atopic dermatitis by the age of 3 months showed elevated levels of IL-5 at 3 months ( $P = 0.0027$ ) and 12 months of age ( $P = 0.022$ ), although only six children had atopic dermatitis at 3 months. No significant differences were observed in the cytokine production when children were grouped according to cumulative development of atopic dermatitis by the age of 12 or 24 months (Data not shown).

The production of cytokines in the infants grouped according to parental atopy is shown in Table 3. The production of TNF at the age of 3 months was higher in infants whose parents were atopic ( $P = 0.010$ ). On the contrary, the production of IL-12 at the age of 12 months was lower in infants whose parents were atopic ( $P = 0.025$ ).

The expressions of cytokine mRNA and productions of cytokines in supernatants correlated well when IL-4 ( $r = 0.59$ ,  $P < 0.0001$ ), IL-10 ( $r = 0.62$ ,  $P < 0.0001$ ) and IFN- $\gamma$  ( $r = 0.75$ ,  $P < 0.0001$ ) were measured. In general, the magnitude of the mRNA responses was low, most likely due to the 72 h timepoint, more optimal to measure production of cytokines. This may be the reason why the only significant association was observed with a cytokine with late mRNA expression. The expression of IFN- $\gamma$  mRNA expression (fold increase to unstimulated cells) was higher in infants with pets at home (mean 218; SEM 59.5) as compared to infants with no pets at home (mean 104; SEM 17.4) ( $P = 0.039$ ).

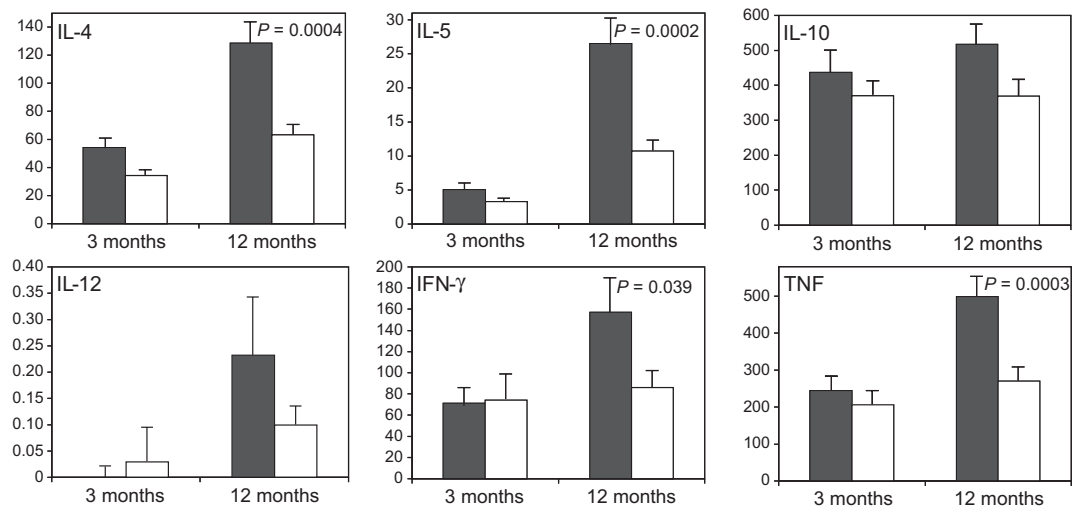


Fig. 1. Mitogen (Concanavalin A) induced IL-4, IL-5, IL-10, IL-12, IFN- $\gamma$  and TNF productions (pg/mL, mean and SEM) in peripheral blood mononuclear cell (PBMC) of infants with smoking ( $n = 25$ , black column) or non-smoking ( $n = 43$ , white column) parents analysed at 3 and 12 months of age by high-sensitivity human cytokine Lincoplex® kits using Luminex® technology.

Table 2. Atopic eczema at 3 months and mitogen (ConA)-induced net cytokine production (pg/mL); mean (SEM)

|               | Cytokine production at 3 months |                     |                 | Cytokine production at 12 months |                     |                 |
|---------------|---------------------------------|---------------------|-----------------|----------------------------------|---------------------|-----------------|
|               | Atopic dermatitis at 3 months   |                     |                 | Atopic dermatitis at 3 months    |                     |                 |
|               | Yes ( <i>n</i> = 6)             | No ( <i>n</i> = 62) | <i>P</i> -value | Yes ( <i>n</i> = 6)              | No ( <i>n</i> = 62) | <i>P</i> -value |
| IL-4          | 59.6 (12.6)                     | 41.7 (3.3)          | 0.41            | 128.5 (41.7)                     | 90.5 (9.5)          | 0.14            |
| IL-5          | 9.2 (3.2)                       | 3.5 (3.9)           | 0.0027          | 30.1 (10.9)                      | 16.9 (2.1)          | 0.022           |
| IL-10         | 565 (270)                       | 376 (28)            | 0.17            | 391 (139)                        | 431 (36)            | 0.78            |
| IL-12         | 0.03 (0.03)                     | 0.01 (0.05)         | 0.97            | −0.10 (0.13)                     | 0.19 (0.05)         | 0.11            |
| IFN- $\gamma$ | 39.7 (15.0)                     | 74.7 (15.5)         | 0.39            | 63.0 (16.8)                      | 116.6 (16.3)        | 0.18            |
| TNF           | 232 (51)                        | 219 (23)            | 0.25            | 323 (65)                         | 365 (31)            | 0.11            |

Table 3. Parental atopy and mitogen (ConA)-induced net cytokine production (pg/mL); mean (SEM)

|               | Cytokine production at 3 months |                      |                       |                 | Cytokine production at 12 months |                      |                       |                 |
|---------------|---------------------------------|----------------------|-----------------------|-----------------|----------------------------------|----------------------|-----------------------|-----------------|
|               | Parental atopy                  |                      |                       |                 | Parental atopy                   |                      |                       |                 |
|               | No ( <i>n</i> = 13)             | One ( <i>n</i> = 45) | Both ( <i>n</i> = 10) | <i>P</i> -value | No ( <i>n</i> = 13)              | One ( <i>n</i> = 45) | Both ( <i>n</i> = 10) | <i>P</i> -value |
| IL-4          | 41.9 (7.3)                      | 43.2 (4.0)           | 45.3 (9.3)            | 0.60            | 71.2 (13.6)                      | 104.2 (13.0)         | 77.1 (16.3)           | 0.52            |
| IL-5          | 3.5 (0.9)                       | 4.3 (0.7)            | 3.3 (0.9)             | 0.83            | 14.3 (3.4)                       | 20.8 (2.9)           | 10.7 (4.6)            | 0.98            |
| IL-10         | 377 (47)                        | 391 (45)             | 419 (105)             | 0.82            | 425 (59)                         | 437 (47)             | 385 (88)              | 0.44            |
| IL-12         | −0.05 (0.03)                    | 0.04 (0.06)          | −0.03 (0.03)          | 0.53            | 0.34 (0.16)                      | 0.12 (0.05)          | 0.00 (0.02)           | 0.025           |
| IFN- $\gamma$ | 58.1 (18.7)                     | 72.6 (20.1)          | 84.5 (26.1)           | 0.34            | 150 (519)                        | 104 (15.9)           | 97.5 (31.0)           | 0.33            |
| TNF           | 180 (49)                        | 207 (194)            | 331 (100)             | 0.010           | 314 (66)                         | 379 (37)             | 346 (71)              | 0.71            |

## Discussion

In this study, we showed that children of smoking parents had significantly elevated levels of IL-4, IL-5, IFN- $\gamma$  and TNF in mitogen-stimulated PBMC at 12 months of age. In addition, elevated levels of IL-5 were associated with early onset of atopic dermatitis, and high levels of TNF and low levels of IL-12 were associated with parental atopy. BCSO supplementation did not affect the studied cytokine secretion or cytokine mRNA expression in the children's PBMC.

To date, some findings indicate that maternal cigarette smoking during pregnancy can modify immune function of children in favour of Th2 response over Th1 responses. Noakes *et al.* observed that neonatal IL-13 was higher and IFN- $\gamma$  was lower if mothers smoked during pregnancy [12]. In another study, it was shown that children who were exposed to parental smoking, from pregnancy to 11 years of age, had less mitogen (Concanavalin A)-induced production of IFN- $\gamma$  in PBMC than children of non-smoking parents. In contrast, no significant effects of parental smoking were found on IL-4 production [7]. However, El Nawawy *et al.* observed a significant increase in IL-4 in children of smoking parents [8]. These findings are in line with our findings, although we found, in addition to increased levels of Th2-type cytokines IL-4 and IL-5, some increase also in Th1-type cytokine IFN- $\gamma$ . As a novel

finding, we demonstrated a highly significant increase of pro-inflammatory cytokine TNF in infants of smoking parents. The TNF increase parallel to cytokines of allergic inflammation implies that smoking can be harmful in inducing inflammation of different types. Another association of TNF with allergic responses was seen as we demonstrated that increased TNF was associated with parental atopy. On the other hand, parental atopy was associated with low IL-12 levels reflecting Th2 dominance over Th1 in infants with atopic parents.

Our study demonstrated the effect on cytokines post-natally 12 months of age, suggesting a more prolonged effect of parental smoking on the immune function of the newborn child. Out of the 68 mothers in our study, only four reported having smoked during pregnancy, whereas 14 reported having stopped smoking upon noticing the pregnancy. Of the fathers, 25 smoked. These results show that even passive smoking affects the developing immune system of the newborn infant. This is a surprising result, especially when considering that according to the mothers, most fathers smoke outside and it is in fact quite rare for anyone to smoke indoors nowadays in Finland.

Children who developed atopic dermatitis by the age of 3 months had considerably elevated levels of Th2 cytokine IL-5 at the age of 3 and 12 months. Also, Herberth *et al.* [6] noticed that children with reduced IFN- $\gamma$  and enhanced IL-4 producing CD4<sup>+</sup> T cells in the cord

blood had a higher risk of developing atopic dermatitis during the first 2 years of life. It has been shown that the selective development of a Th2 cytokine profile in children with high risk of atopy is due to increased production of Th2 cytokines, possibly caused by impaired allergen-induced IFN- $\gamma$  production in the neonatal period [5, 6]. Also, decreased allergen-induced IL-10 production at 12 months of age has only been observed in non-atopic children. That may result in a lack of down-regulation of the inflammatory process [5].

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 [18]. However, this intervention study could not demonstrate any significant effect of BCSO on the cytokine production or mRNA expression in PBMC. It is noteworthy that there was no association with cytokine production and outcome of atopic dermatitis either. Human intervention studies concerning FA and fetus immune development are so far rare, and the results are inconclusive. Fish oil supplementation during pregnancy has been shown to reduce both production and mRNA expression of Th2-type cytokines in cord blood [25, 26]. Furthermore,  $\omega$ -3 fatty acid supplementation has been shown to decrease the chemokine ligand CCL17/CXCL11 ratio in cord blood indicating increased Th1 predominance over Th2 [27]. We also found a trend of increased IFN- $\gamma$  and IL-12 in the BCSO group (data not shown). The multivariate analysis failed to show any statistical significance, suggesting that confounding factors, such as parental smoking and parental atopy had a stronger effect on the cytokine production than the BCSO intervention itself. The results of both prenatal and antenatal studies suggest that for successful modulation of the immune system, a prenatal supplementation of diet is beneficial [18, 19, 25–28]. Polyunsaturated FA are precursors for several lipid mediators involved in the reso-

lution of inflammation [29]. BCSO supplementation leads to increased levels of  $\gamma$ -linolenic acid and dihomogamma-linolenic acid, and consequently to reduced production of prostaglandin E2 (PGE<sub>2</sub>) in elderly people [30]. However, in murine models of asthma, PGE<sub>2</sub> has been shown to reduce allergic inflammation and expression of Th2-type cytokines IL-4 and IL-5, thus showing anti-inflammatory properties [31, 32].

Our study sheds new light on the detrimental effects of parental smoking on the child's immune function. This study also shows that fathers' smoking poses a risk for the newborn immune system by enhancing pro-inflammatory cytokines. This enhanced production of Th2-type and pro-inflammatory cytokines may well be connected to increased risk of asthma later in life. Consequently, even more attention should be paid by the maternal health centres to supporting both mothers and fathers in their efforts to stop smoking.

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**Conflict of interests:** Baoru Yang is a former employee of Aromtech, all other authors declare no conflicts of interest.

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