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**Cord blood cytokines and chemokines and development of allergic disease**

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### **Abstract**

Exposure to ubiquitous allergens early in life, even before birth, may influence the incidence of allergic diseases later in life. During pregnancy, the fetomaternal interface is surrounded by high levels of Th2-like cytokines, possibly favouring the development of Th2-like immune responses in the offspring. The aim of this study is to evaluate the relation between cord blood (CB) IgE antibodies, Th1- and Th2-like cytokines and chemokines, maternal allergy and development of allergic disease during the first 2 years of life in the offspring. The CB cytokine and chemokine levels from children of 20 allergic and 36 non-allergic women were determined by a multiplexed Luminex assay and ELISA. Total CB and maternal IgE antibody concentrations were quantified using ImmunoCAP technology. The maternal IgE levels during and after pregnancy correlated with CB IgE and Th2-associated macrophage-derived chemokine (MDC (CCL22) levels. Development of allergic disease and sensitisation was associated with increased CB IgE and MDC (CCL22) levels, as well as high ratios of MDC (CCL22) to Th1-associated interferon- $\gamma$  inducible protein 10 (IP-10 (CXCL10) and interferon- $\gamma$  inducible T cell  $\alpha$  chemoattractant (I-TAC (CXCL11) (**n=7 allergic vs. n=25 non-allergic**). The correlations between maternal IgE and CB IgE and MDC (CCL22) levels possibly indicate that the maternal immunity can affect the Th1/Th2 profile in the neonate. Development of allergic disease is associated with a more marked Th2-like deviation already at birth, shown as increased levels of CB IgE and MDC (CCL22) and higher ratios of MDC (CCL22) to IP-10 (CXCL10) and I-TAC (CXCL11).

**Key words:** allergy; cytokines; chemokines; cord blood; IgE; luminex

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**Abbreviations used:**

ARC: allergic rhinoconjunctivitis

BALF: bronchoalveolar lavage fluid

CB: Cord blood

CCL: CC chemokine ligand

CCR: CC chemokine receptor

CXCL: CXC chemokine ligand

CXCR: CXC chemokine receptor

ELISA: Enzyme-linked immunosorbent assay

IP-10: interferon- $\gamma$  inducible protein 10

I-TAC: interferon- $\gamma$  inducible T cell  $\alpha$  chemoattractant

MDC: macrophage-derived chemokine

PBMC: peripheral blood mononuclear cells

PARC: pulmonary and activation regulated chemokine

SPT: skin prick test

TARC: thymus- and activation-regulated chemokine

Th: T-helper

## **Introduction**

Exposure to allergens early in life may have an impact on the incidence of allergy many years later(1-3). The initial exposures to ubiquitous allergens invariably occur during the first year of life, or even before birth(4). In support of an important role for the gestational environment on offspring immune development, maternal exposure to stables during pregnancy protects against allergic sensitisation, whereas exposures during infancy has weaker or no effect at all(5). The priming and the status of the immune system before and at birth and its relation to subsequent allergic disease needs further investigation, however.

During pregnancy, the fetomaternal interface is surrounded by high levels of T helper (Th)2-like cytokines(6), probably in order to divert the maternal immune response away from damaging Th1-mediated immune responses(7). This Th2 polarisation during pregnancy may influence the offspring for variable periods postnatally. As the cytokine milieu at the priming of T cells direct the Th1/Th2 differentiation(8), the gestational environment could be very important for shaping immune responses. Maternal sensitisation to allergens was associated with a decreased ability to produce the Th2 antagonist IFN- $\gamma$  in newborn mice(9) and an elevated production of the Th2-like cytokine IL-13 in the human infant(10). The higher cord blood (CB) IgE levels seen in newborns of allergic mothers as compared to newborns with a paternal or no allergic history(11-13) may depend on a stronger decidual Th2 shift in allergic mothers(14).

In addition to the Th2-dominated cytokine profile, an upregulation of Th2-induced chemokines has been associated with allergic manifestations including atopic dermatitis(15-17) and asthma(18-20). The chemokines comprise a large protein family, which is responsible for the trafficking of leukocytes to the site of inflammation and the regulation of leukocyte

maturation(21). The chemokine receptors are expressed on the surface of several cell types involved in the allergic inflammation, *e. g.* eosinophils, monocytes, Th1 and Th2 lymphocytes(22). Atopic dermatitis is associated with high circulating levels of Th2-associated eotaxin (CC ligand 11 (CCL11)(16), macrophage-derived chemokine (MDC (CCL22))(15, 16, 23), thymus- and activation-regulated chemokine (TARC (CCL17))(15-17) and pulmonary and activation regulated chemokine (PARC (CCL18)(24) in children and adults. Increased levels of MDC (CCL22) and TARC (CCL17) in bronchoalveolar lavage fluid (BALF) have been reported in asthmatics(18) after allergen challenge(20). In contrast, interferon- $\gamma$  inducible protein 10 (IP-10 (CXCL10)) and interferon- $\gamma$  inducible T cell  $\alpha$  chemoattractant (I-TAC (CXCL11)) are associated with Th1-like diseases like sarcoidosis, tuberculosis(19) and Crohn's disease(25).

Chemokines have a crucial role in establishing the Th1/Th2 balance and they are widely used as markers for Th1/Th2 immunity. The relation between the occurrence of CB chemokines at birth and the development of allergic diseases in the offspring is poorly understood. We hypothesize that maternal allergy is associated with increased levels of cord blood IgE antibodies, an elevated production of Th2-like cyto- and chemokines and development of allergic diseases in the child. To address our hypothesis, 20 allergic pregnant women and 36 non-allergic pregnant women were recruited, and their children were followed to 2 years of age. Using a multiplexed Luminex assay and sensitive ELISA, CB concentrations of the cytokines interleukin-4 (IL-4), IL-5, IL-9, IL-10, IL-12(p70), IL-13, IFN- $\gamma$  and the chemokines eotaxin (CCL11), IP-10 (CXCL10), I-TAC (CXCL11), MDC (CCL22), TARC (CCL17), PARC (CCL18) were quantified and related to maternal IgE levels during pregnancy.

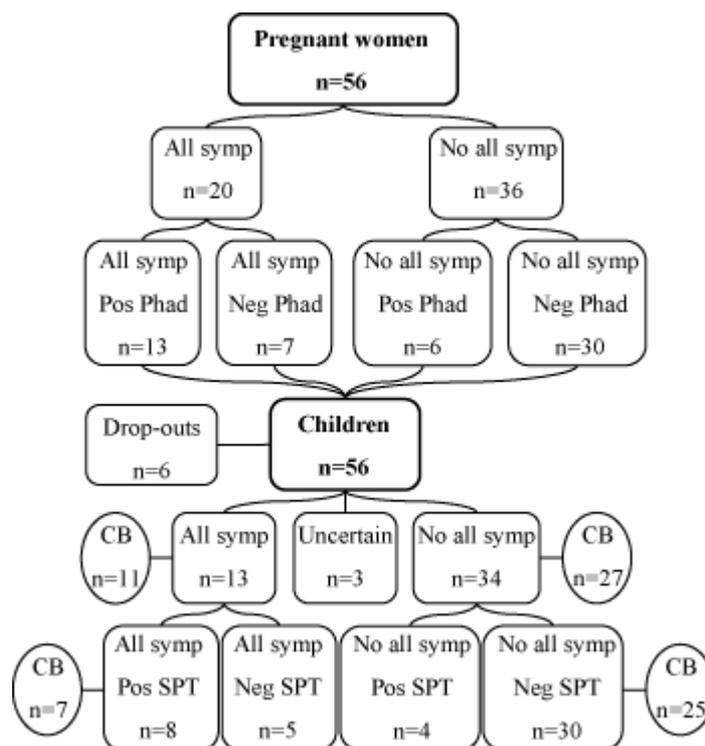
## Methods

### Study group

The study comprised 20 allergic (mean age (range) 30.8 years (24-38), mean number of previous successful pregnancies 0.55 (0-2)) and 36 non-allergic (mean age (range) 30.8 years (22-40), mean number of previous successful pregnancies 0.81 (0-2)) pregnant women from the Linköping area, County of Östergötland, Sweden. **Due to technical and practical reasons, it was not possible to perform this study with a larger number of participants.**

The allergic status was established by a typical clinical history, *e.g.* allergic rhinoconjunctivitis (ARC, n=17), allergic asthma (n=4 of whom 1 also had ARC) or flexural itchy eczema (n=2, both had also ARC). An experienced allergy research nurse interviewed the women using structured questionnaires. The presence of IgE antibodies to common inhalant allergens (**from birch, mugwort, timothy, cat, dog, horse, house-dust mite, Cladosporium and Alternaria**) was measured by Phadiatop<sup>®</sup> (Pharmacia Diagnostics, Uppsala, Sweden). The cut-off for positivity was 0.35 kU<sub>A</sub>/l. Thirteen women were sensitised with allergic symptoms (sensitised according to Phadiatop testing combined with a history of allergic symptoms) and 30 women were non-sensitised without allergic symptoms (**Fig 1**). The pregnant women were recruited by convenience sampling, *i e* among women attending the maternity health care clinic in Linköping. Plasma samples and peripheral blood mononuclear cells (PBMC) were collected at 5 occasions during pregnancy (at gestational week 10-12, 15-16, 25, 35 and 39), as well as 8 weeks and 1 year after the delivery. All plasma samples were frozen and stored at -70°C. Umbilical cord blood (CB) was collected at birth and the plasma and serum samples were frozen and stored at -20°C.

Fifty-six children to the women described above were born from August 2000 to November 2002, (32 boys, 24 girls). **Fifty-three babies were delivered vaginally and 3 by cesarean**



*Figure 1. Flow chart of the women and their children included in the study. Fifty-six women were recruited, 20 women with and 36 women without allergic symptoms of whom 13 were sensitised with allergic symptoms and 30 were non-sensitised without allergic symptoms. Thirteen of the 56 children reported allergic symptoms, 3 children fulfilled some, but not all criteria for allergic disease and 34 showed no symptoms of allergic disease. Of the children with allergic symptoms, 8 were also sensitised and 30 of the 34 children without allergic symptoms were non-sensitised. The numbers of available CB-samples for the analysed groups are shown in the ellipses.*

**section.** Due to technical reasons, only 46 CB samples were available. Questionnaires regarding environmental factors and allergic symptoms in the children were answered by the parents at 3, 6, 12, 18 and 24 months. The children were examined by an experienced allergy research nurse at 6 and 12 months and by a paediatrician at 24 months of age. Three children did not attend to the clinical examinations. All of the other children (n=53) attended to the 6 and 12 months examinations and 47 children to the 24 month examination.

Eczema was defined as pruritic, chronic or chronically relapsing non-infectious dermatitis with typical features and distribution. Asthma was defined as three or more episodes of bronchial obstruction, at least once verified by a physician or two episodes of bronchial obstruction combined with eczema or **food allergy**. **In this cohort, all sensitised children**

**with bronchial obstruction episodes had wheezed more than three times, however.**

Allergic rhino-conjunctivitis was defined as rhinitis and conjunctivitis appearing at least twice after exposure to a particular allergen and not related to infection. Urticaria was defined as allergic if it appeared at least twice within one hour after exposure to a particular allergen. Food-related gastrointestinal problems were defined as vomiting and/or diarrhoea on at least two separate occasions after intake of certain offending food. Thirteen children reported allergic symptoms. Ten children had eczema and 4 of them also had asthma. Of the 5 children with asthma, 3 also had eczema, 1 also had urticaria, and 1 child had both eczema and urticaria combined with asthma. Three children fulfilled some, but not all criteria for allergic disease and 34 showed no symptoms of allergic disease (**Fig 1**).

Skin prick tests (SPT) were performed in duplicate on the volar aspects of the forearms. The allergens used were; thawed egg white, fresh skimmed cow's milk (lipid concentration 0.5%) (6, 12 and 24 months), cat (12 and 24 months), and birch and timothy (24 months). If a pet is present at home and if the parents wanted to know if their child was sensitised to that species, an additional allergen was used i.e. cat, dog, horse, bird, and rodent. All extracts were standardised allergen extracts from ALK (Soluprick®, ALK, Hørsholm, Denmark). Histamine hydrochloride (10 mg/ml) was used as positive control and glycerol as negative control. The test was regarded as positive when the mean wheal diameter was at least 3 mm. Thirteen of the children had at least 1 positive SPT, 11 to egg, 3 to milk, 2 to cat, 1 to dog and 1 to timothy. Forty children were not sensitised. Eight of the children with allergic symptoms were SPT positive. Five of these children had eczema, 2 of them also had asthma and 1 child had urticaria and asthma. Thirty children were non-sensitised without allergic symptoms.

### **Determination of total IgE**

The total IgE concentrations in the 46 CB samples were analysed by the immunoassay system ImmunoCAP (Pharmacia Diagnostics, Uppsala, Sweden) using ImmunoCAP Total IgE Low Range to allow detection of very low IgE concentrations. The procedure recommendations were followed and the results were expressed as kU/l.

### **CB cytokine and chemokine concentrations determined by a multiplexed Luminex assay**

The concentrations of IL-4, IL-5, IL-9, IL-10, IL-12(p70), IL-13, IFN- $\gamma$ , eotaxin (CCL11), IP-10 (CXCL10) and MDC (CCL22) in the CB samples were obtained by a multiplex assay using Beadlyte<sup>®</sup> Human Multi-Cytokine Beadmaster<sup>™</sup> Kit (Upstate, CA, USA) according to the manufacturer's instructions. Both CB serum and plasma were analysed with this kit, as the manufacturer stated that this was appropriate. The majority of the CB samples were plasma samples.

Briefly, the CB samples were diluted 1:2 in serum diluent and mixed with a mixture of anti-cytokine/chemokine mouse monoclonal antibodies coupled to different Luminex<sup>®</sup> beads (Beadlyte<sup>®</sup> Anti-Cytokine beads, Upstate, CA, USA). A 7-point standard curve with 3-fold dilutions was used to achieve low-end sensitivity. To increase the overall sensitivity, the plate was incubated over night at 4°. After washing, a Beadlyte<sup>®</sup> anti-cytokine biotinylated reporter (Upstate, CA, USA) was added to the beads and incubated at room temperature (RT). After 1.5 hour, Beadlyte<sup>®</sup> Streptavidin Phycoerythrin was added to each well and incubated 30 minutes at RT. The reaction was terminated with Beadlyte<sup>®</sup> Stop Solution, washed and resuspended in sheath fluid. The samples were analysed on a Luminex<sup>100</sup> instrument (Biosource), and the data were acquired using the StarStation 2.0 software (Applied cytometry systems, Sheffield, UK). The sensitivity limits were 1 pg/ml for IL-10, 2 pg/ml for IL-13, 3 pg/ml for IL-9, 4 pg/ml for IL-4 and IFN- $\gamma$ , 5 pg/ml for IP-10(CXCL10), 8 pg/ml for

IL-5 and IL-12 (p70), 10 pg/ml for Eotaxin (CCL11) and 12 pg/ml for MDC (CCL22). A majority of the samples were analysed in duplicates and the mean coefficient of variance (CV) of the duplicates were 17 %. All samples were analysed in one run.

### **CB chemokine concentrations determined by ELISA**

**A double-antibody sandwich ELISA were developed, optimised and used for quantification of CB chemokines.** Costar 3690-plates (Costar Inc., Corning, NY, USA) were coated with 1 µg/ml monoclonal anti-human CCL11/I-TAC (clone 87328) or 1 µg/ml monoclonal anti-human CCL17/TARC (clone 54026) or 0.5 µg/ml monoclonal anti-human CCL18/PARC (clone 64507) in 50 µl carbonate buffer pH 9.6 per well (R&D Systems, Abingdon, UK). The plates were incubated on a plate shaker for 1 hour and over night without shaking. The coated plates were washed 4 times with a microplate washer (Anthos microplate washer Fluido, Salzburg, Austria) with phosphate buffered saline (PBS) with 0.05% Tween (PBT) and blocked with 100 µl prewarmed (37°) PBS supplemented with 2 % low-fat milk per well. After 1 hour incubation on a plate shaker, the plates were washed 4 times. A 7-point standard curve with 2-fold dilutions in PBS with 1 % bovine serum albumin (BSA, Sigma-Aldrich) was constructed for each chemokine, using recombinant human CCL11/I-TAC (3,9-250 pg/ml), recombinant human CCL17/TARC (7,8-500 pg/ml) and recombinant human CCL18/PARC (7,8-500 pg/ml) (R&D Systems). The samples were diluted in a range of 1/2 to 1/600 in PBS with 1 % BSA. The plates were incubated with 50 µl samples in duplicates on a plate shaker for 1 hour. After washing 4 times, 50 µl biotinylated anti-human CCL11/I-TAC / CCL17/TARC / CCL18/PARC Antibody (R&D Systems), diluted to a concentration of 200 ng/ml in high-performance ELISA- (HPE) dilution buffer (CLB, Amsterdam, the Netherlands), were added to the wells. After a second 1-h incubation on a plate shaker, the plates were washed 4 times and 50 µl streptavidin-poly-horse radish

peroxidase (CLB), diluted 1/10 000 in HPE-buffer, was added to the wells. After 30 minutes on a plate shaker, the plates were washed 4 times and 50  $\mu$ l 3,3',5,5'-tetramethylbenzidine liquid substrate system (Sigma-Aldrich) was added to each well and incubated on a plate shaker for 30 minutes in the dark. The reaction was terminated with 1.8 M H<sub>2</sub>SO<sub>4</sub>. The optical densities (OD) were read at 450 nm with a wavelength correction at 540 nm in a VersaMax tunable microplate reader (Molecular Devices, Sunnyvale, CA, USA). Data acquisition was performed using SOFTmaxPRO Version 3.1.2 computer software (Molecular Devices). All steps were performed at RT unless something else is stated. The lower detection limits were 4 pg/ml for I-TAC (CXCL11) and 8 pg/ml for TARC (CCL17) and PARC (CCL18). The CV between the duplicates was below 15% and the mean CV for all samples were 4 %.

### **Statistics**

Non-parametric tests, corrected for ties, were used. The correlations were analysed with Spearman's rank order correlation coefficient test and comparisons between unpaired groups with Mann-Whitney *U*-test. The calculations were made with the statistical package SPSS 14.0 for Windows (SPSS Inc, Chicago, IL, USA). Undetectable samples were given the value of half the cut-off.

### **Ethics**

The study was approved by the Regional Ethics Committee for Human Research at the University Hospital of Linköping.

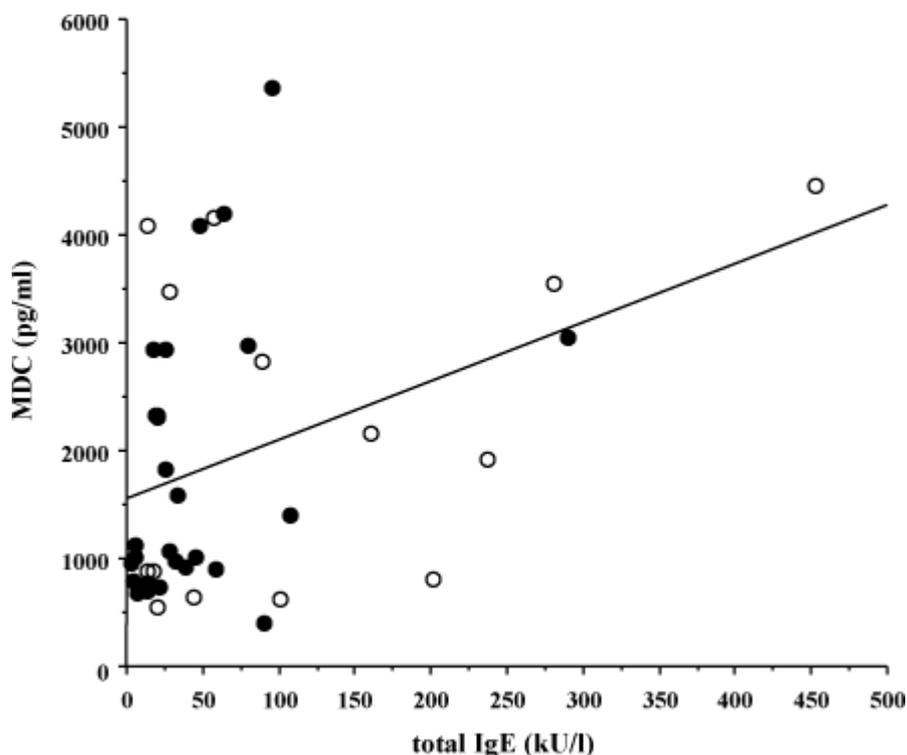
## Results

### CB levels of cytokines and chemokines

A number of the analysed cytokines (IL-4, IL-5, IL-9, IL-10, IL-12(p70), IL-13, IFN- $\gamma$ ) were undetectable, or only sporadically detectable, in the CB samples, even though an overnight incubation of beads and samples was done to achieve higher sensitivity. In contrast, both Th1- and Th2-associated chemokines were readily detectable in umbilical CB. Among these chemokines, Th1-associated I-TAC (CXCL11) and IP-10 (CXCL10) correlated significantly with each other ( $Rho=0.37$ ,  $p=0.01$ ), with a similar tendency for Th2-associated MDC (CCL22) and TARC (CCL17) ( $Rho=0.26$ ,  $p=0.09$ ). Chemokine levels were similar in newborns of sensitised mothers with allergic symptoms ( $n=13$ ) and non-sensitised mothers without allergic symptoms ( $n=30$ ). The CB levels of chemokines and IgE antibodies were not influenced by other factors such as smoking during pregnancy ( $n=7$ ), number of previous pregnancies and neonatal gender (Mann-Whitney U-test).

### Correlations between CB chemokine and IgE levels

The levels of CB MDC (CCL22) correlated with CB IgE levels ( $Rho=0.37$ ,  $p=0.01$ ). Furthermore, we did observe positive correlations between maternal IgE (regardless of diagnosis) and CB IgE levels ( $Rho=0.34-0.35$ ,  $p=0.02-0.04$ ) at week 15 and 39, and 12 months after delivery. CB MDC (CCL22) levels are also related to the maternal IgE levels during pregnancy. **Maternal IgE at gestational weeks 15, 25, 35 and 2 and 12 months after delivery were positively correlated with CB MDC levels ( $Rho=0.31-0.40$ ,  $p=0.01-0.04$ , Fig 2).** No such correlations were observed for the other chemokines, although CB IgE and TARC (CCL17) tended to correlate ( $Rho=0.25$ ,  $p=0.09$ ).



*Figure 2. Positive correlations between CB MDC and total IgE (regardless of diagnosis) at gestational week 15, 25, 35, 2 and 12 months after delivery were noted. The figure shows a positive correlation between total IgE levels at gestational week 25 and CB MDC ( $Rho=0.36$ ,  $p=0.02$ , Spearman's rank order correlation coefficient test). **Women with allergic symptoms are marked with open circles and women without allergic symptoms with closed circles.***

### **Levels and ratios of CB chemokines and IgE are related to allergic sensitisation in the offspring**

Children with a positive SPT during the first 2 years of life demonstrated significantly higher MDC (CCL22) levels at birth compared to non-sensitised children (median (range) 3809 pg/ml (963-5368) and 1023 pg/ml (405-4455,  $p=0.003$ ). Moreover, the ratios of MDC (CCL22) to IP-10 (CXCL10) and I-TAC (CXCL11) were considerably higher in the sensitised children ( $p=0.001$ ,  $p=0.009$ ). Similar trends were shown for eotaxin (CCL11) / I-TAC (CXCL11) and TARC (CCL17) / I-TAC (CXCL11) ratios ( $p=0.07$  for both ratios). The sensitised, as compared to non-sensitised, children also had higher levels of CB IgE antibodies (median (range) 0.54 kU/l (0.17-1.46) and 0.17 kU/l (0.17-5.36), respectively  $p=0.045$ ).

## Levels and ratios of CB chemokines and IgE are related to allergic disease in the offspring

Children with allergic symptoms (n=11) showed significantly higher TARC (CCL17) levels than children without allergic symptoms (n=27) (Table 1). Furthermore, the MDC (CCL22) / IP-10 (CXCL10) and TARC (CCL17) / IP-10 (CXCL10) ratios were higher (p=0.02 and p=0.01, respectively) in children with, as compared to without, allergic symptoms.

Table 1. CB cytokine and chemokine concentrations (median, range) in children with (n=11) and without allergic symptoms (n=27)

Cytokine/Chemokine	Children with allergic symptoms (pg/ml)	Children without allergic symptoms (pg/ml)	p-values
IL-4	<4	<4	-
IL-5	<8	<8	-
IL-9	<3	<3	-
IL-10	0.5 (0.5-2.4)	<1	0.12
IL-12(p70)	<8	<8	-
IL-13	1 (1-5)	1(1-10)	0.32
IFN- $\gamma$	2 (2-8.8)	<4	0.12
IP-10/CXCL10	214 (33-403)	314 (72-3983)	0.15
I-TAC/CXCL11	26 (2-1081)	42 (2-1050)	0.57
Eotaxin/CCL11	32 (5-101)	34 (5-98)	0.83
MDC/CCL22	2310 (713-5368)	1074 (405-4455)	0.22
TARC/CCL17	653 (220-1855)	412 (4-2062)	<b>0.04</b>
PARC/CCL18	24085 (13808-48126)	23814 (12454-55283)	0.86

*IP-10*, interferon- $\gamma$  inducible protein 10; *I-TAC*, interferon- $\gamma$  inducible T cell  $\alpha$  chemoattractant; *MDC*, macrophage-derived chemokine; *TARC*, thymus- and activation-regulated chemokine; *PARC*, pulmonary and activation regulated chemokine.

Using more strict criteria for allergic disease, sensitised children with allergic symptoms during the first 2 years of life had significantly higher MDC (CCL22) levels at birth compared to non-sensitised children without allergic symptoms (**Fig 3**). Moreover, the ratios of MDC (CCL22) to IP-10 (CXCL10) and I-TAC (CXCL11) were higher in the sensitised children with allergic symptoms (**Fig 4**). Similar trends were shown for TARC (CCL17) / IP-10 (CXCL10) and TARC (CCL17) / I-TAC (CXCL11) ratios (p=0.07 and p=0.08, respectively).

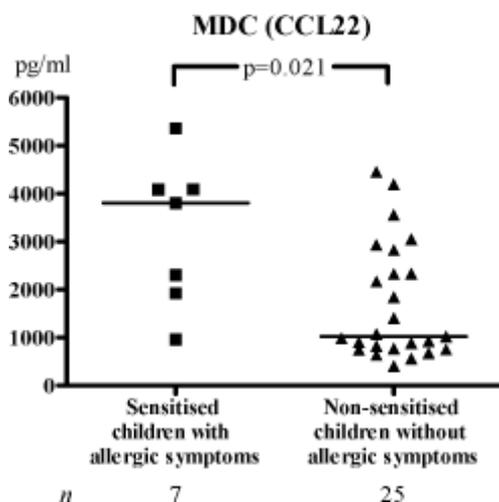


Figure 3. CB MDC (CCL22) levels in 7 sensitised children with allergic symptoms and 25 non-sensitised children without allergic symptoms. The median is represented by a black line. The chemokine levels were analysed using a multiplexed Luminex assay and the groups were compared using the Mann-Whitney U-test.

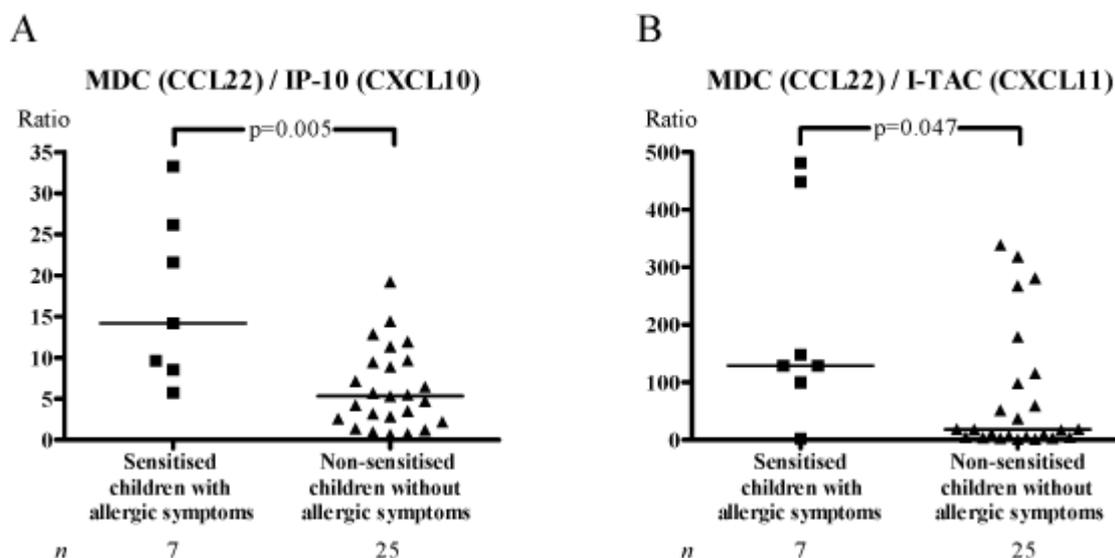


Figure 4. The CB ratios between (A) MDC (CCL22) / IP-10 (CXCL10) and (B) MDC (CCL22) / I-TAC (CXCL11) in 7 children who did develop allergic symptoms and sensitisation during the first 2 years of life and 25 children who did not develop allergic symptoms or sensitisation during the first 2 years of life. The chemokines were detected by a multiplexed Luminex assay and ELISA. The unpaired comparisons were performed using the Mann-Whitney U- test. The median is represented by a black line.

Three children in the group of sensitised children with allergic symptoms were diagnosed with asthma and eczema/urticaria. These children correspond to the highest MDC (CCL22) concentrations and MDC (CCL22) / IP-10 (CXCL10) ratios within the group of sensitised

children with allergic symptoms. Due to the small group size, this finding could not be verified with an appropriate statistical analysis.

The sensitised children with allergic symptoms, as compared to non-sensitised children without allergic symptoms, also had higher levels of CB IgE antibodies (median (range) 0.87 kU/l (0.17-1.46) and 0.17 kU/l (0.17-5.36),  $p=0.01$  respectively). Although CB IgE and maternal IgE levels correlated, we did not observe higher CB IgE levels in newborns of sensitised mothers with allergic symptoms **compared to** non-sensitised mothers without allergic symptoms.

## Discussion

In support of the idea of prenatal priming of the immune system, we found that the maternal IgE levels during pregnancy correlated with CB MDC (CCL22). The association between maternal IgE and CB IgE and MDC (CCL22) levels suggests that the maternal immunity can shape the Th1/Th2 profile in the neonate. A more Th2-skewed response during pregnancy, characterized by high IgE levels, may induce the expression of Th2 chemokines, and could thereby be responsible for the high levels of MDC (CCL22) and the high ratios between MDC (CCL22) and the Th1 associated chemokines. High levels of CB MDC (CCL22) may strongly influence the offspring postnatally since sensitisation and allergic disease was associated with higher CB levels of MDC (CCL22). In agreement with this finding, Leung et al 2004 reported that increased CB MDC (CCL22) concentrations are associated with the occurrence of wheezing during infancy(26). The children were only followed by telephone interviews to 12 months of age, however. Our finding of an association between high MDC (CCL22) levels, MDC (CCL22) / IP-10 (CXCL10) ratio and allergic disease was strengthened by the additional observation that the three children diagnosed with asthma and eczema/urticaria showed the highest MDC (CCL22) levels and MDC (CCL22) / IP-10 (CXCL10) ratios within the group of sensitised children with allergic symptoms. Even though this finding needs to be confirmed in a larger **number of samples**, allowing for statistical analysis, it suggests that MDC levels and the MDC (CCL22) / IP-10 (CXCL10) ratio could potentially be valuable predictors for the development of allergic diseases later in life. To the best of our knowledge, there are no other studies on the relation of neonatal chemokines and possible allergy related outcomes.

The ratios between MDC (CCL22) and the Th1 associated chemokines IP-10 (CXCL10) and I-TAC (CXCL11) were significantly higher in sensitised and allergic children, clearly

demonstrating the importance of the balance between Th1 and Th2 immunity at birth. Increased ratios of the Th2-like chemokine TARC (CCL17) to IP-10 (CXCL10) and I-TAC (CXCL11) also tended to be associated to sensitisation and allergic disease. Furthermore, children with allergic symptoms showed higher TARC (CCL17) levels and higher TARC (CCL17) / IP-10 (CXCL10) ratio than children without allergic symptoms. These findings strongly support the hypothesis that a more pronounced Th2 deviation at birth is related to allergic disease in the offspring. **Although a study on neonatal house dust mite induced mRNA expression did not support a stronger Th2-skewing among children developing allergic disease, a decreased production of allergen-induced IFN- $\gamma$  by CBMC:s in allergic compared to non-allergic children has been reported by several groups, suggesting an impaired allergen-induced IFN- $\gamma$  production in the neonatal period(27, 28). Furthermore, circulating CB chemokines may better reflect the *in vivo* Th1/Th2 balance.**

In contrast, no differences in PARC (CCL18) levels or ratios were observed between the allergic and non-allergic children, although this Th2 associated chemokine has shown to be related to atopic dermatitis(29). We observed much higher CB levels of PARC (CCL18) than of the other chemokines, however, possibly indicating a less strict regulation of this chemokine. **Our inability to determine differences in CB PARC (CCL18) and eotaxin (CCL11), IP10 (CXCL10), I-TAC (CXCL11) levels between allergic and non-allergic children could be related to the size of the study group, as our population may have not been large enough to reveal such differences.**

The chemokines function as attractants for several leukocytes directly involved in the allergic inflammation *e.g.* Th2 lymphocytes, mast cells and eosinophils. The strong connection

between these cells and the chemokines indicate that high levels of Th2 chemokines at birth could promote allergic diseases later in life. As MDC (CCL22) binds to the CCR4 receptor expressed on mast cells, dendritic cells, NKT and Th2 lymphocytes, high neonatal levels of MDC (CCL22) could affect postnatal migration of these cells, possibly influencing the immunity in the offspring several years later. **The levels of TARC (CCL17) and MDC (CCL22) have been shown to decrease by age(15, 30), suggesting a role for these chemokines in the early immune development.** Also, high levels of MDC (CCL22) may reflect enhanced maternal Th2-like responses, influencing offspring immune development. We did observe correlations between maternal IgE and CB MDC (CCL22) levels. The rho-values of 0.3-0.4 indicate moderate correlations and many other potential factors might affect the levels of CB IgE and chemokines.

Similar to other studies, we found that high CB IgE levels were associated with allergic disease in the offspring(31, 32). In addition, we found that the maternal IgE levels during pregnancy influence the CB IgE levels, since significant correlations occurred at gestational week 15, 39 and 12 months after delivery. This is in agreement with a previous report(12) and indicates that the maternal immunity during pregnancy does have an impact on the prenatal IgE synthesis.

In conclusion, we found that the maternal IgE levels during and after pregnancy correlated with CB MDC (CCL22) and IgE levels, suggesting that the gestational environment may be very important for shaping immune responses in the neonate. Development of sensitisation and allergic disease was associated with a more marked Th2-like deviation already at birth, shown as increased levels of CB IgE and MDC (CCL22), and higher ratios of MDC (CCL22) to IP-10 (CXCL10) and I-TAC (CXCL11).

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