EPR imaging of dose distributions aiming at applications in radiation therapy

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Th2-like chemokine levels are increased in allergic children and influenced by maternal immunity during pregnancy

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Abstract

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Th2-like chemokine levels are elevated in allergic children and influenced by maternal immunity during pregnancy

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Background: The influence of the intrauterine environment on the immunity and allergy development in the offspring is unclear. We aimed to investigate (i) if the pregnancy magnifies the Th2 immunity in allergic and non-allergic women, (ii) if the maternal chemokine levels during pregnancy influenced the offspring's chemokine levels during childhood and (iii) the relationship between circulating Th1/Th2-associated chemokines and allergy in mothers and children.

Methods: The Th1-associated chemokines CXCL9, CXCL10, CXCL11 and the Th2-associated chemokines CCL17, CCL18 and CCL22 were quantified by Luminex and ELISA in 20 women with and 36 women without allergic symptoms at gestational week (gw) 10-12, 15-16, 25, 35, 39 and 2 and 12 months postpartum and in their children at birth, 6, 12, 24 months and 6 years of age. Total IgE levels were measured using ImmunoCAP Technology.

Results: The levels of the Th2-like chemokines were not magnified by pregnancy. Instead decreased levels were shown during pregnancy (irrespectively of maternal allergy status) as compared to postpartum. In the whole group, the Th1-like chemokine levels were higher at gw 39 than during the first and second trimester and postpartum. Maternal CXCL11, CCL18 and CCL22 levels during and after pregnancy correlated with the corresponding chemokines in the offspring during childhood. Increased CCL22 and decreased CXCL10 levels in the children were associated with sensitisation and increased CCL17 levels with allergic symptoms during childhood. Maternal chemokine levels were not associated with maternal allergic disease.

Conclusions: Allergic symptoms and sensitisation were associated with decreased Th1- and increased Th2-associated chemokine levels during childhood, indicating a Th2-shift in the allergic children, possibly influenced by the maternal immunity during pregnancy.

Keywords: allergy, CCL17, CCL22, chemokines, pregnancy, Th2

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Abbreviations

AD: Atopic dermatitis

ARC: Allergic rhinoconjuntivitis

CB: Cord blood

NFκB: Nuclear Factor κB

SPT: Skin prick tests

Th: T-helper

gw: gestational week

regulatory T cells: Tregs

Introduction

It has long been known that allergy development begins very early in life. The observation of maternal allergy as a greater risk factor for allergy development in the offspring as compared with paternal allergy (1, 2) has shed light on the pregnancy as an important time period for immune development of the fetus (3). Furthermore, maternal exposure to farms and stables during pregnancy protects against development of asthma symptoms, allergic rhinoconjuntivitis (ARC), atopic dermatitis (AD) and allergic sensitisation (4-6) in the offspring, supporting the role of pregnancy in maternal-fetal immune imprinting. Complex interactions between the genetic disposition, environmental exposures and the intra-uterine environment are thought to direct the immune system towards allergy or tolerance.

A normal pregnancy is traditionally described as a Th2-phenomenon (7). The balance between Th1 and Th2 immunity in pregnancy is believed to be a strictly controlled process, as increased Th1-like immune responses have been associated with spontaneous abortions (8), pre-eclampsia (9) and pre-term labour (10). In addition to a Th2 deviated immunity, the suppressive mechanisms by regulatory T cells (Tregs) are also important for a successful pregnancy (11). In contrast, Th17-like immunity has been associated with spontaneous abortions (12) and might be detrimental for the pregnancy in combination with a Th1 deviated immunity.

Allergies are associated with increased Th2-like responses to allergens and the Th2-deviated immunity of allergic women could be beneficial in a reproductive perspective. On the other hand, pregnancy might magnify the already Th2-biased immunity of allergic women, leading to an exposure of a strong Th2-environment for the fetus, possibly influencing allergy development later in life. A pronounced Th2 deviation during pregnancy in allergic women is supported by our previous finding of increased total IgE levels during pregnancy as compared with postpartum (13). The maternal total IgE levels correlated with cord blood (CB) IgE and CCL22 levels, indicating that a pronounced maternal Th2-shift during pregnancy could promote Th2 responses in the offspring (14). Increased CB CCL17 and CCL22 levels were associated with development of allergic symptoms and sensitisation during the first 6 years of life (15), supporting that the Th2 deviation associated with allergy is established very early in life. The present study expands the current knowledge on the

immunological interactions between mother and child during pregnancy, by studying the IFN-γ induced chemokines CXCL9, CXCL10, CXCL11 the IL-4 and IL-13 induced chemokines CCL17 and CCL22 and the IL-4, IL-10 and IL-13 induced CCL18 (16). Th1 and Th2-like chemokines have previously been associated with established allergic disease (17, 18), but their role in the prenatal priming of the immune system has not been investigated. The aim of the study was (i) to investigate if the pregnancy magnifies the Th2 immunity in allergic and non-allergic women, (ii) if the maternal chemokine levels during pregnancy influenced the offspring's chemokine levels during childhood and (iii) the relationship between circulating Th1/Th2-associated chemokines and allergy in mothers and children.

Methods

Study group

Fifty-six women were included in the study during 2000 – 2002, 20 women with and 36 without allergic symptoms. Seventeen women had ARC, 4 had asthma (of whom 1 also had ARC) and 2 had AD (both of them also had ARC). Allergic sensitisation was determined by the Phadiatop test (Phadia, Uppsala, Sweden), which detects allergen-specific IgE antibodies to inhalant allergens. Thirteen women were sensitised and experienced allergic symptoms and 30 women were non-sensitised and without allergic symptoms. Blood samples were collected at gestational week (gw) 10-12, 15-16, 25, 35, 39 and 2 and 12 months postpartum. Umbilical CB was collected after delivery. Due to practical reasons, it was impossible to perform this study, with a larger number of study participants. A detailed description of maternal and neonatal characteristics can be obtained elsewhere (13, 15).

Follow up of the children was performed with questionnaires, collection of blood samples and medical examinations at 6 and 12 months of age by an experienced allergy research nurse, and at 24 months and 6 years of age by a paediatric allergologist (15). Plasma samples from the mothers were stored at -70°C and the CB plasma/serum and plasma samples from the children were stored at -20°C. A description of the clinical definitions, skin prick tests (SPT) and quantification of total and allergen specific IgE (PhadiatopInfant and Phadiatop test) can be found elsewhere (15, supplementary material 1). Nineteen children reported allergic symptoms, 27 children reported no symptoms of allergic disease and 10 families declined participation at various time points during childhood. Eleven of the 19 children with allergic symptoms were sensitised, according to SPT and/or circulating allergen specific IgE antibodies, and 15 of the 27 children without allergic symptoms were non-sensitised. Detailed information on allergic symptoms and sensitisation in the children is described elsewhere (15). The distribution of allergic symptoms and sensitisation in children of mothers with and without allergic symptoms is described in supplementary table 2.

Analysis of chemokines in plasma from mothers and children

The plasma levels of CXCL9, CXCL10, CXCL11, CCL17 and CCL22 were quantified by an in-house Luminex assay, as previously described (19). The lower detection limits were 41 pg/ml for CXCL9, 3 pg/ml for CXCL10, 17 pg/ml for CXCL11 and 8 pg/ml for CCL18 and 2 pg/ml for CCL17 and CCL22. The CCL18 levels were measured with ELISA (14). The inter-assay variation, determined by two internal control samples was 26% for CXCL9, 12% for CXCL10, 10% for CXCL11, 17% for CCL17, 14% for CCL18 and 12% for CCL22. One internal control sample was analysed in 12 wells on the same plate and the intra-assay variation was ≤ 10 % for all chemokines.

Analysis of cytokines and chemokines in CB

The Beadlyte® Human Multi-Cytokine Beadmaster™ Kit (Upstate, CA, USA) was used on the Luminex platform for quantification of IL-4, IL-5, IL-9, IL-10, IL-12(p70), IL-13, IFN-γ, CCL11, CXCL10 and CCL22 in CB. CB levels of CXCL11, CCL17 and CCL18 was measured by an in-house ELISA, described in detail elsewhere (14).

Statistics

Non-parametric tests, corrected for ties, were used (IBM SPSS Statistics 21.0 for Windows, SPSS Inc, Chicago, IL, USA). Friedman's test was used to evaluate if the chemokine levels changed over time. Wilcoxon's signed rank test was used for paired samples and Mann-Whitney *U*-test for unpaired groups. The correlations were done with Spearman's rank order correlation coefficient test and only clear patterns, *i.e.* repeated statistical significant correlations between several time points during/after pregnancy and during childhood, are reported. A probability level of $p<0.05$ was considered as significant. Undetectable levels 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

Circulating Th1- and Th2-associated chemokines during and after pregnancy

The maternal chemokine levels were not related to presence or absence of maternal allergic disease. Thus, similar levels were observed when comparing women with *versus* without allergic symptoms as well as when comparing sensitised women with allergic symptoms *versus* non-sensitised women without allergic symptoms.

The levels of the Th1-associated chemokines CXCL9, CXCL10, CXCL11 and the Th2-associated chemokines CCL17, CCL18 and CCL22 were modified by the pregnancy (Friedman's test, p=0.02-<0.001), regardless of the allergy status of the mother. The changes in chemokine levels during pregnancy and postpartum are therefore presented collectively, as a characterisation of a normal pregnancy, since maternal allergy status did not modify neither the levels nor the longitudinal changes. CXCL10 and CXCL11 were increased at gw 39 as compared with the first and second trimesters as well as compared with 2 and 12 months postpartum. CCL17, CCL18, CCL22 levels were decreased during pregnancy as compared with 2 and 12 months postpartum and CXCL9 levels were decreased as compared with 2 months postpartum only (Table 1). The levels of CCL17 and CCL22 decreased during pregnancy, the highest levels were found in the first trimester (gw 10-12) and the lowest levels in the third trimester (gw 35 and 39). These patterns remained when a hemodilution of 20 % at gw 25, 35, and 39 was taken into account, except for CXCL9 where the chemokine levels became similar between pregnancy and postpartum samples (data not shown in Table 1).

Increased CXCL10 and total IgE levels, but decreased CCL18 levels were noted at gw 10-12 in a group of 5 women with spontaneous abortions between gw 10-12 and 15-16, as compared with 56 women with a normal pregnancy (Table 2).

Correlations between chemokines in mother and child

Maternal CXCL11, CCL18 and CCL22 levels during and after pregnancy correlated with the corresponding chemokines in the offspring at several time points during childhood (Fig 1, Table 3). In contrast, maternal chemokine levels were not related to the offspring's total IgE levels and the

maternal total IgE levels did not correlate with the offspring's chemokine or total IgE levels during childhood.

An imbalance in Th1- and Th2-associated chemokines during childhood is related to allergic symptoms and sensitisation in the children

The kinetics of the chemokines (regardless of allergic symptoms and sensitisation) followed the same pattern as previously reported (19), *i.e.* increased levels of the Th1-associated chemokines CXCL9, CXCL10, CXCL11 and the Th2-associated chemokine CCL18 during the first year and decreased levels of the Th2-associated chemokines CCL17 and CCL22 during the first 2 years of life, followed by a decrease between 2 and 6 years of age for all chemokines (data not shown).

The levels of CCL22 were higher at birth (15) and at 24 months (Fig 2a), and the CXCL10 levels were lower in the sensitised, than the non-sensitised children at 12 months of age (Fig 2b).

Development of allergic symptoms was associated with increased CCL17 levels at birth (15), 6 and 24 months (Fig 2c) and increased total IgE levels at 12 months of age (p=0.03). Allergic symptoms combined with sensitisation were associated with increased total IgE levels at 12 months (p=0.009), CCL17 and CCL22 levels at 24 months (Fig 3) and CCL18 levels at 6 years of age (p=0.04).

Children with asthma had higher CCL17 and CCL22 levels at birth (15), and higher CCL17 levels at 24 months (p=0.009) and total IgE levels at 6, 12, 24 months and 6 years of age (p=0.03-0.004) than children without allergic symptoms. Asthma and/or ARC was associated with increased CCL17 and CCL22 levels at birth (15) and at 24 months (p=0.001, p=0.03 respectively) and total IgE levels at 6, 12 months and 6 years of age (p=0.03-0.009). AD was associated with increased CCL17 levels at birth (15), at 24 months (p=0.01) and decreased CCL22 levels at 6 years of age (p=0.04).

Accordantly, ratios between the Th1- and Th2-associated chemokines indicated that allergic symptoms and sensitisation were associated with an enhanced Th2-shift during childhood (Supplementary table 1).

Chemokine and total IgE levels were analysed with gender and maternal allergy as potential confounding factors (Mann-Whitney U-test). Boys had lower levels of CXCL9 at 6 and 12 months (p=0.03 for both comparisons) and higher total IgE levels at 12 months of age (p=0.04) as compared

with girls. Maternal allergic symptoms were associated with low offspring CXCL11 levels at 6 months of age ($p=0.04$).

Discussion

This study revealed discrepancies in the immune maturation during childhood in children developing allergic symptoms and sensitisation, shown as increased Th2-like and decreased Th1-like chemokine levels. In addition, a relationship between the maternal immunity during pregnancy and the pre-natal synthesis of chemokines was revealed.

Even though important interactions between the maternal and the offspring's chemokine levels were noted, the idea of an enhanced Th2-shift during pregnancy, with a more pronounced Th2-shift in the allergic women, was not supported by our data. The levels of the Th2-like chemokines CCL17, CCL18 and CCL22 were in fact decreased during pregnancy as compared with postpartum.

It is tempting to speculate that pregnancy hormones influence the levels of the Th2-associated chemokines. Estrogen and progesterone levels are known to increase gradually in the circulation during pregnancy and rapidly decrease postpartum, while our chemokine data revealed gradually decreased CCL17 and CCL22 levels during pregnancy and increased levels postpartum. Progesterone has been suggested to mediate anti-inflammatory effects on the maternal immune system by suppression of Nuclear Factor κB (NFκB) activity (20) and blocking experiments indicate that the CCL17 and CCL22 production rely on NFκB activation (21, 22). The reduced levels of the Th2-like chemokines during pregnancy could also be related to a peripheral consumption of chemokines, but this speculation needs to be investigated in detail.

Studies on mitogen-induced cytokine secretion from peripheral blood mononuclear cells in pre-and postpartum samples have also failed to reveal a Th2-bias during pregnancy, however (23, 24).

It is also important to keep in mind that systemic changes do not always reflect local changes. Thus, studies on Tregs indicate an enrichment of Tregs at the fetal-maternal interface but not in the circulation (11). On the other hand, data on CCL22 levels locally in the cervico-vaginal mucosa support our findings of reduced CCL22 levels during pregnancy (25). Threefold lower CCL22 levels were observed in cervico-vaginal secretions from pregnant women as compared with non-pregnant controls (25).

The highest levels of the Th1-associated chemokines CXCL10 and CXCL11 were found at gw 39 indicating a strong pro-inflammatory response, possibly related to the onset of labour. The assumption

of CXCL10 and CXCL11 as important mediators in parturition is supported by studies on pre-term deliveries where increased levels of CXCL10 and CXCL11 have been observed in serum (26), supernatants from fetal membrane extracts (27) and amniotic fluid (28).

Our additional observation of increased CXCL10 and total IgE levels but decreased CCL18 levels in women with spontaneous abortions indicates an imbalance between Th1/Th2/anti-inflammatory immunity in this group. It is tempting to speculate that CXCL10 and CCL18 could serve as markers for spontaneous abortion risk, but these findings must be interpreted with caution as only five cases of spontaneous abortions were included and none of these pregnancies were verified by ultrasound.

Our inability to reveal any differences in chemokine levels between allergic and non-allergic women might be related to a requirement of a strict regulation between Th1/Th2/anti-inflammatory immunity for the maintenance of pregnancy. Similar levels of circulating chemokines in adults with ARC and healthy controls have been reported (29, 30), indicating that chemokines might be an inappropriate choice of markers for Th1- and Th2-immunity in an adult population with ARC. Increased CCL17, CCL18 and CCL22 levels have been associated with AD (17, 18) and AB (31), clearly reflected in the children in our study with AD as the predominant allergic symptom.

Development of allergic symptoms, including AD and AB, was associated with increased CCL17 and CCL22 levels and development of sensitisation with increased CCL22 and decreased CXCL10 levels during childhood. Our results indicate a delayed down regulation of the pronounced Th2 deviation established already at birth together with a (less marked) delayed up-regulation of Th1 responses during childhood in children developing allergic symptoms and/or sensitisation. A delayed immune maturation early in life in allergic children have also been suggested in other studies (19, 32).

Increases in total IgE levels were sporadically associated with allergic symptoms with and without sensitisation, but the IgE levels tended to be increased at several time points (data not shown). The confounding effect of gender could influence the data since the total IgE levels were increased and CXCL9 levels were decreased in boys. Dissimilarities in the immunity depending on gender occurs, especially for the IgE synthesis during childhood (33).

The role of circulating chemokines in the pre-natal priming of the immune system has not been investigated previously. The correlations between maternal CXCL11, CCL18 and CCL22 levels and

the corresponding chemokine levels in the offspring during childhood indicate that the maternal immunity during pregnancy can influence the Th1- and Th2-like immunity of the offspring later in life. This is in line with previous findings in this cohort, where total IgE levels during pregnancy correlated with CB IgE and CCL22 levels (14). The influence of the maternal immunity during pregnancy on the developing immune system of the offspring was observed even at 6 years of age, indicating long term effects on the offspring's health. The idea of "fetal programming of diseases" suggests even longer effects of the intra-uterine environment, *i.e.* a link between events *in utero* and development of diseases in adult life (34).

In conclusion, although our data does not support a pronounced Th2 deviation during pregnancy in allergic or non-allergic women, the maternal immunity during pregnancy influenced the immunity of the offspring. Allergy development during the first 6 years of life was associated with a marked Th2 deviation at birth and a delayed down-regulation of this Th2-skewed immunity during childhood.

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Figure legends

Figure 1. Maternal CXCL11, CCL18 and CCL22 levels correlated at several time points during pregnancy/postpartum with the corresponding chemokines in the offspring. The figure shows positive correlations between **A**, CXCL11 levels at gw 25 and CXCL11 levels at 6 months, **B**, CCL18 levels at gw 25 and CCL18 in CB, **C**, CCL22 levels at gw 25 and CCL22 levels at 6 years of age. The CXCL11 and CCL22 levels are presented as pg/ml and CCL18 as ng/ml.

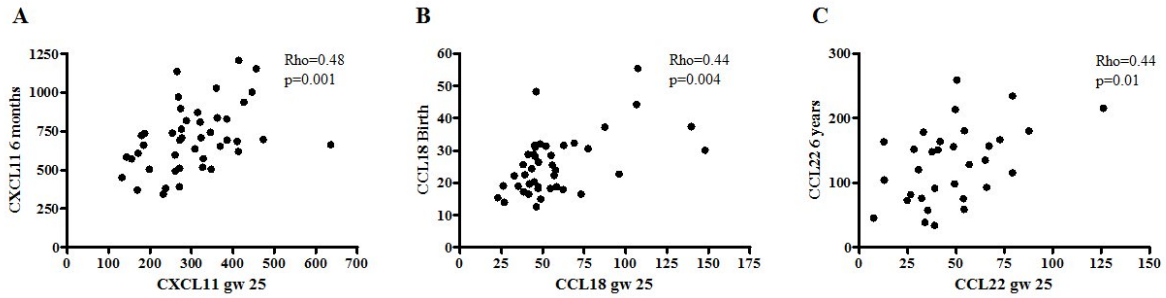

Fig 1

Figure 2A. The levels of CCL22 were increased at 24 months of age in the sensitised children (regardless of allergic symptoms) as compared with the non-sensitised children. Grey bars = sensitised children, white bars = non-sensitised children. Increased CB CCL22 levels were associated with development of sensitisation later in life (15). **B**, The levels of CXCL10 were decreased at 12 months of age in the sensitised children (regardless of allergic symptoms) as compared with the non-sensitised children. Grey bars = sensitised children, white bars = non-sensitised children. **C**, Children with allergic symptoms (regardless of sensitisation) had higher levels of CCL17 at 6 and 24 months of age than children without allergic symptoms. Grey bars = children with allergic symptoms, white bars = children without allergic symptoms. We have previously reported an association between increased CB CCL17 levels and development of allergic symptoms (15). * = $p<0.05$, ** = $p<0.01$

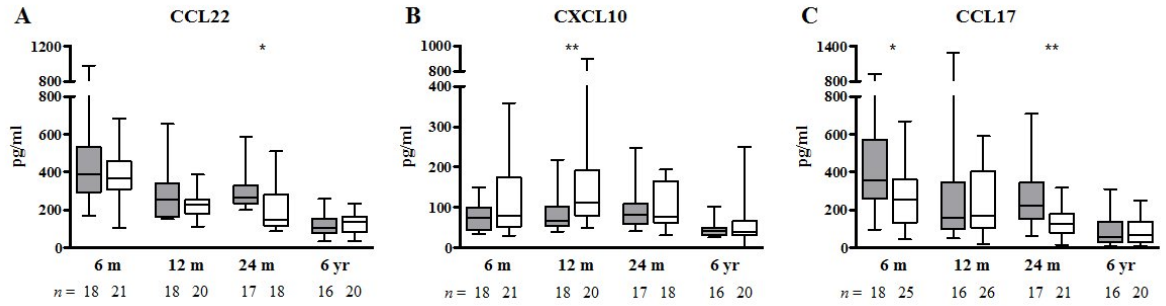

Fig 2

Figure 3A. The CCL17 levels and the **B**, CCL22 levels were increased in the sensitised children with allergic symptoms as compared with the non-sensitised children without allergic symptoms at 24 months of age. The median is shown as a black line. * = $p<0.05$

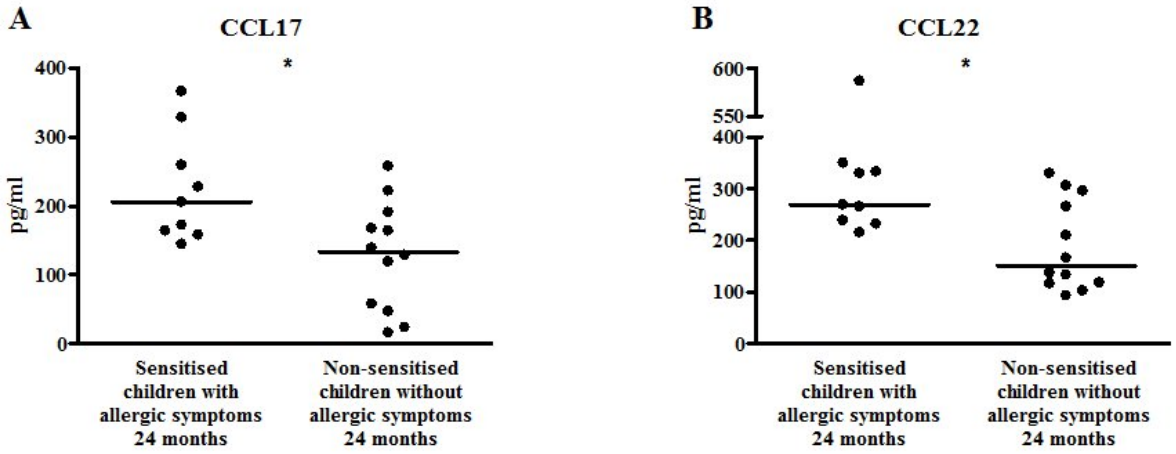

Fig 3

Table 1 Chemokine concentrations (median, range) during and after pregnancy in 56 women with a normal pregnancy.

	w 10-12	**w 15-16**	**w 25**	**w 35**	**w 39**	**2 m pp**	**12 m pp**
CXCL9	124* (20.5-1378)	95* (20.5-411)	108* (20.5-1079)	110* (20.5-985)	99* (20.5-2035)	131 (20.5-856)	116 (20.5-859)
CXCL10	42$^{\sigma}$ (18-122)	36*$^{Q\sigma}$ (14-72)	34*$^{Q\sigma}$ (11-81)	47$^{\sigma}$ (25-110)	54$^{\Psi\Delta}$ (25-166)	44 (17-115)	41 (15-126)
CXCL11	369 (134-1039)	318$^{\sigma}$ (99-731)	288*$^{\sigma}$ (133-637)	347 (153-985)	354$^{\Psi\Delta}$ (152-1135)	331 (138-663)	339 (144-848)
CCL17	15*^{Q} (1-234)	9*$^{Q\varepsilon}$ (1-136)	6*$^{Q\varepsilon}$ (1-119)	4*$^{Q\varepsilon}$ (1-32)	4*$^{Q\varepsilon}$ (1-58)	23 (1-268)	19 (1-299)
CCL18	62*^{Q} (26-450)	53*$^{Q\varepsilon}$ (20-450)	49*$^{Q\varepsilon}$ (23-148)	53*$^{Q\varepsilon}$ (22-450)	55*^{Q} (29-450)	89 (37-450)	83 (33-450)
CCL22	62*^{Q} (14-154)	59*$^{Q\varepsilon}$ (12-146)	43*$^{Q\varepsilon}$ (8-126)	40*$^{Q\varepsilon}$ (9-97)	41*$^{Q\varepsilon}$ (9-89)	85 (19-209)	78 (20-197)

The table shows median and range in pg/ml for CXCL9, CXCL10, CXCL11, CCL17, CCL22 and in ng/ml for CCL18 in 56 women with a normal pregnancy. The allergic status of the mother did not affect the chemokine levels or the longitudinal changes during and after pregnancy.

w=week, m pp =months postpartum

*= decreased chemokine level as compared with 2 months postpartum, $p<0.05$

Q= decreased chemokine level as compared with 12 months postpartum, $p<0.01$

σ= decreased chemokine level as compared with gestational week 39, $p<0.05$

Δ= increased chemokine level as compared with 2 months postpartum, $p<0.05$

Ψ=increased chemokine level as compared with 12 months postpartum, $p<0.05$

ε= decreased chemokine level as compared with gestational week 10-12, $p<0.05$

Table 2 Chemokine and IgE concentrations (median, range) at gw 10-12 in women with spontaneous abortions and a normal pregnancy

	Spontaneous abortion, (n=5)	**Normal pregnancy, (n=56)**	**p-values**
CXCL10 (pg/ml)	60 (48-152)	42 (18-122)	0.03
CCL18 (ng/ml)	33 (26-91)	62 (26-450)	0.04
Total IgE (kU/l)	126 (26-464)	30 (2-282)	0.03

Table 3 Correlations between maternal chemokine levels during and after pregnancy and the offspring's chemokine levels during childhood (Spearman's rank order correlation coefficient test, Rho, p-value)

	w 15-16	**w 25**	**w 35**	**w 39**	**2 m pp**	**12 m pp**
CXCL11						
6 m	0.40 **	0.48 **	0.37 *		0.49 **	0.53 ***
12 m						0.36 *
6 yr				0.53 **	0.36 *	
CCL18						
Birth		0.44 **				
24 m	0.34 *				0.51 **	
6 yr		0.41 *			0.41 *	
CCL22						
6 yr	0.42 *	0.46 **	0.37 *		0.41 *	0.37 *

w=week, m pp =months postpartum, m = months, yr = year, * = $p<0.05$, ** = $p<0.01$ *** = $p<0.001$

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Supplementary table 1. Ratios between the Th2- and Th1-associated chemokines in relation to development of sensitisation (S), allergic symptoms (AS), asthma bronchiale (AB) asthma bronchiale and/or allergic rhinoconjuntivitis (ABARC), atopic dermatitis (AD) and allergic symptoms combined with sensitisation (SAS).

	6 m	p	12 m	p	24 m	p	6 yr	p
CCL17/CXCL9					↑AS vs NAS	0.03		
					↑ABARC vs NAS	0.01		
CCL17/CXCL10					↑AS vs NAS	0.03		
					↑AB vs NAS	0.04		
					↑ABARC vs NAS	0.006		
CCL17/CXCL11	↑AS vs NAS	0.04			↑AS vs NAS	0.02		
					↑AD vs NAS	0.03		
					↑AB vs NAS	0.04		
					↑ABARC vs NAS	0.008		
CCL18/CXCL9	↑AS vs NAS	0.04						
CCL18/CXCL10			↑ S vs NS	0.02				
CCL18/CXCL11							↑ SAS vs NSNAS	0.04
CCL22/CXCL9			↑ S vs NS	0.03				
CCL22/CXCL10			↑ S vs NS	0.005	↑ ABARC vs NAS	0.04		
			↑ SAS vs NSNAS	0.03				
CCL22/CXCL11					↑ SAS vs NSNAS	0.01		
					↑ S vs NS	0.004		

High Th2/Th1 ratios were associated with development of sensitisation, allergic symptoms and allergic symptoms combined with sensitisation. S = sensitised children, NS = non-sensitised children, AS = children with allergic symptoms, NAS = children without allergic symptoms, SAS = sensitised children with allergic symtoms, NSNAS = non-sensitised children without allergic symptoms, AB = asthma bronchiale, ABARC = asthma bronchiale and/or allergic rhinoconjuntivitis, AD = atopic dermatitis, p = p-value

Supplementary table 2. Distribution of allergic symptoms and sensitisation in children of mothers with and without allergic symptoms

Children (n=56)	Women with allergic symptoms (n=20)	Women without allergic symptoms (n=36)	Total
Allergic symptoms	n=8	n=11	n=19
No allergic symptoms	n=11	n=16	n=27
Drop-outs	n=1	n=9	n=10
Sensitised	n=11	n=10	n=21
Non-sensitised	n=7	n=14	n=21

Supplementary material 1

Clinical definitions

Atopic dermatitis (AD) = pruritic, chronic or chronically relapsing non-infectious dermatitis with typical features and distribution. Asthma (at 2 years of age) = doctor verified bronchial obstruction with at least three episodes since birth or two episodes of bronchial obstruction combined with AD or food allergy. Asthma (6 years of age) = one or more episodes of doctor verified bronchial obstruction after two years of age. ARC = seasonal itching and running eyes and nose, appearing at least twice after exposure of an inhalant allergen and not related to infection. Urticaria = allergic if it appeared within one hour after allergen exposure, at least at two separate occasions. Symptoms of food allergy = vomiting and/or diarrhoea, on at least two separate occasions after ingestion of certain offending food. Oral allergy syndrome = allergic if it appeared at least at two separate occasions after intake of certain offending food.

Sensitisation

Skin prick tests (SPT) were performed with egg white and milk at 6, 12, 24 months and 6 years, cat at 12, 24 months and 6 years, and birch and timothy at 24 months and 6 years of age. The allergen extracts and the positive control (Histamine hydrochloride, 10 mg/ml) and the negative control (albumin diluent) were purchased from Allergologisk Laboratorium A/S, (ALK, Soluprick®, Hørsholm, Denmark). A mean wheal diameter of at least 3 mm was considered as positive. ImmunoCAP technology (Pharmacia Diagnostics, Uppsala, Sweden) was used for quantification of IgE antibodies, according to the guidelines provided by the manufacturer. Total IgE levels and presence of circulating allergen specific IgE antibodies to egg, milk, fish, wheat, peanut and soybean (PhadiatopInfant® test, Phadia) were determined at 6, 12, 24 months and 6 years and to birch, mugwort, timothy, cat, horse, dog, house dust mite (Dermatophagoides pteronyssinus and farinae)

and Cladosporium (Phadiatop® test) at 6 years of age. The cutoff for positivity was 0.35 kU_A/l for the PhadiatopInfant® and the Phadiatop® test, and the sensitivity limits were 2 kU/l for the total IgE assay and 0.35 kU/l for the total IgE low-range assay.