

Low numbers of interleukin-12-producing cord blood mononuclear cells and immunoglobulin E sensitization in early childhood

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Summary

Background Successful pregnancies are associated with skewing towards a Th2 cytokine profile. Cytokine responses to allergens can be detected in cord blood mononuclear cells (CBMC), suggesting allergen priming already *in utero*.

Objective To investigate the cytokine profile in CBMC after *in vitro* stimulation with allergens and to relate the responses to the outcome in terms of allergic disease at 2 years of age.

Methods CBMC were isolated from 82 children. The responses to ovalbumin (OVA), birch, cat and phytohaemagglutinin (PHA) were investigated by the ELISpot technique. The numbers of IFN- γ -, IL-4- and IL-12-producing CBMC were counted for each stimulation. The children were followed prospectively; skin prick test (SPT) and RAST towards food and inhalant allergens were assessed at 24 months of age.

Results Sixteen (19.5%) children were classified as IgE sensitized (positive SPT; ≥ 3 mm and/or RAST; ≥ 0.35 kU_A/L). The numbers of IL-12-producing CBMC after stimulation with birch, OVA and cat were lower among IgE-sensitized children, statistically significant for cat. IFN- γ -producing cells, did not differ in numbers between the sensitized and non-sensitized children. Children who had atopic eczema/dermatitis syndrome (AEDS) during the observation ($n = 53$) had significantly lower numbers of IFN- γ -producing CBMC after stimulation with OVA and cat than their non-AEDS counterparts.

Conclusions Although the numbers of infants in our study are limited our data suggest that a low number of IL-12-producing CBMC is associated with IgE sensitization during early childhood and that a reduced number of IFN- γ -producing CBMC promotes the development of AEDS during the first 2 years of life.

Keywords atopy, childhood, cord blood, cytokines, IFN- γ , IgE, IL-4, IL-12, sensitization

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Introduction

IgE-mediated allergic reactions are complex and involve both humoral and cellular components [1]. T lymphocytes, which have a regulatory influence on the 'allergic inflammation', can be sub-divided according to the profile of cytokines they produce upon activation [2]. Thus, two subsets of cytokines, Th1 and Th2 cytokines, with opposing effects on the induction of IgE synthesis, have been characterized [3]. An increased Th1 activity with synthesis of e.g. IFN- γ has long been believed to dominate among non-sensitized individuals while an increase in allergen-specific Th2 activities with synthesis of e.g. IL-4 dominate among IgE-sensitized individuals [1]. However, it has become evident that allergen-specific responses are not only caused by Th2 cytokines and that the polarization of T helper cells is more complex than what was first believed. It has for example been

shown that Th1 and Th2 type of cytokines can even be co-expressed in the same T helper cell [4].

The increasing prevalence of IgE sensitization and allergic diseases during childhood has stressed the need to find ways to predict allergic disease early in life, but so far the search has been unsuccessful [5, 6]. Better understanding of the immune events during fetal life and early childhood is necessary in order to be able to predict and prevent such diseases.

Fetal intra-uterine environment is characterized by a Th2 cytokine profile [7, 8] and the fetus has been shown to be able to produce IgE already at week 22 of gestation [9]. Several publications have reported that proliferative and cytokine responses to allergens can be detected in cord blood mononuclear cells (CBMC) [10] and allergens like β -lactoglobulin and birch pollen are suggested to be capable of actively crossing the placenta [11]. Based on new knowledge in maternal/fetal/newborn immune regulation, it has been hypothesized that the processes that predispose allergic disease begin in early childhood, maybe already *in utero* [12].

IFN- γ is an important factor for the 'immune deviation' during the early postnatal period of life [13, 14]. We have recently shown that the numbers of IFN- γ -producing cells

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were lower in cord blood from children with two allergic parents than in children with no allergic heredity [14]. Others have shown that low levels of IFN- γ are associated with an increased risk of developing atopic eczema/dermatitis syndrome (AEDS) during childhood [15–17]. Findings that is comparable with the hypothesis stating that early AEDS is a Th2-mediated disease [18].

IL-12 is a key factor, regulating cell-mediated immunity and has strong Th1-inducing activities. Thus, IL-12 induces IFN- γ production in NK and T cells [19]. In a recently published study, we have shown a lower number of IL-12-producing CBMC in infants with atopic heredity [14].

The aim of this study was to investigate if the numbers of IFN- γ -, IL-4- and IL-12-producing CBMC were related to IgE sensitization and to the development of clinical symptoms of allergic diseases up to 2 years of age.

Methods

Subjects

Eighty-two infants, 47 girls and 35 boys, were recruited for the study. They are part of a larger prospective study

including 281 infants where the influence of heredity on CBMC cytokine profile has been published elsewhere [14]. The parental heredity for atopic disease was extensively characterized. For 29 children both parents (group dh = double heredity) reported a history of bronchial asthma and/or allergic rhino-conjunctivitis towards animal dander and/or towards pollen. For 24 children only the mother (group mh = maternal heredity) and for 29 children neither of the parents (group nh = no heredity) reported a history of respiratory allergic disease (Table 1). The atopic status of the parents was confirmed before delivery by skin prick test (SPT) and only those parents among whom a positive (dh and mh) or a negative SPT confirmed the parental case history were invited to participate in the study. All infants were born at hospitals in the Stockholm area, were full term (>35 weeks of gestation), had birth weights within the normal range and were postnatally healthy. The time of birth was equally distributed between spring–summer (April–September) and autumn–winter (October–March) (Table 1).

The study was approved by the Human Ethics Committee at Huddinge University Hospital, Stockholm and the parents provided informed consent.

Table 1. Demographic data among infants with positive SPT ≥ 3 mm and/or positive RAST ≥ 0.35 kU_A/L (IgE-sensitized group) and among infants with negative SPT/RAST (non-sensitized group)

	IgE sensitized	Non-sensitized
<i>n</i>	16	66
Gender		
Girl, <i>n</i> (%)	8 (50)	39 (59.1)
Boy, <i>n</i> (%)	8 (50)	27 (40.9)
Heredity		
dh, <i>n</i> (%)	7 (46.7)	22 (32.8)
mh, <i>n</i> (%)	6 (37.5)	18 (27.3)
nh, <i>n</i> (%)	3 (20.0)	26 (38.8)
Obstetric data		
Gestation age, weeks, median (range)	40.0 (38–43)	40.0 (37–43)
Birth weight, g median (range)	3525 (2455–4640)	3645 (2680–4800)
Delivery mode, caesarean, <i>n</i> (%)	1 (6.7)	5 (7.5)
Maternal age at delivery, year, median (range)	32.0 (27–44)	31.0 (22–40)
Paternal age at delivery, year, median (range)	33.0 (26–43)	32.0 (22–50)
Month of birth		
Born April–September, <i>n</i> (%)	9 (56.3)	38 (57.6)
Born October–March, <i>n</i> (%)	7 (43.8)	28 (42.4)
Exposure		
Exclusive breastfeeding, months, median (range)	4.8 (0–6)	4.0 (0–9)
Partial breastfeeding, months, median (range)	8.0 (0–25)	9.0 (0–23)
Maternal smoking, <i>n</i> (%)	0	5 (7.5)
Paternal smoking, <i>n</i> (%)	4 (25.0)	8 (12.1)
Furred pets at home, <i>n</i> (%)	1 (6.3)	15 (22.7)
Cat, <i>n</i> (%)	1 (6.3)	9 (13.6)
Dog, <i>n</i> (%)	0	6 (9.1)
Number of siblings		
0, <i>n</i> (%)	11 (68.7)	33 (50.0)
1, <i>n</i> (%)	5 (31.2)	27 (40.9)
≥ 2 , <i>n</i> (%)	0	6 (9.1)
Day care, <i>n</i> (%)	10 (62.5)	51 (77.3)***
Age at day care start, months, median (range)	17.0 (14–24)	18.0 (12–25)

dh, double heredity; mh, maternal heredity; nh, no atopic heredity; SPT, skin prick test; furred pets at home, furred pets during pregnancy and the first 2 years of life.

*** $P = 0.002$.

Clinical evaluation

The children were followed prospectively from birth to 2 years of age and were clinically evaluated at 6, 12, 18 and 24 months of age by one and the same paediatrician (C. N.). The presence of allergic diseases was defined in each child. The following definitions of the various allergic diseases were used:

AEDS was defined according to Hanifin and Rajka [20].

Bronchial asthma was defined as three or more episodes of wheezing, or any episode of wheezing if combined with a family history of allergic disease or other allergic symptoms in the child [21].

Allergic rhino-conjunctivitis was diagnosed if rhinitis and/or conjunctivitis appeared at least twice after exposure to a particular allergen and was unrelated to infection.

Food allergy/acute urticaria was diagnosed as acute onset of symptoms such as skin reactions, wheezing, vomiting or diarrhoea on more than one occasion after ingestion of or contact with a particular food or allergen.

Skin prick test

The same nurse performed SPT according to the manufacturer's recommendation (ALK, Copenhagen, Denmark) against food and inhalant allergens. The SPT included the following food allergens: egg white [Soluprick (ALK Sverige AB, Sweden) weight to volume ratio 1/100], cod fish (Soluprick 1/20), peanut (Soluprick 1/20), cow's milk (3% fat, standard milk) and soy bean protein (Soja Semp[®] Semper AB, Stockholm, Sweden). The SPT also included perennial and seasonal inhalant allergens: cat, dog and *Dermatophagoides farinae* and birch and timothy, respectively (Soluprick 10 HEP). Histamine chloride (10 mg/mL) served as the positive control and the allergen diluent as the negative control. The SPT was considered positive if the weal diameter after 15 min was ≥ 3 mm in comparison to the negative control.

Specific IgE/RAST

Circulating IgE antibodies against cow's milk, egg white, peanut, cod fish, soy bean, wheat, cat dander, dog dander, birch pollen, timothy pollen and house dust mite *D. farinae* were determined with Pharmacia CAP-FEIA[™] (Pharmacia-Upjohn, Uppsala, Sweden) in plasma collected at 24 months of age. An IgE antibody level ≥ 0.35 kU_A/L was considered significant.

Allergic classification

In accordance with Johansson et al. [22] the child was classified as IgE sensitized if one or more SPT was positive (≥ 3 mm) and/or if RAST against one or more of the selected allergens was ≥ 0.35 kU_A/L.

Blood sampling

Immediately after the delivery, cord blood was collected from the umbilicus vein with aspiration technique. Another venous blood sample was collected when the child was 24 months of age. The blood samples were stored at most 24 h before mononuclear cells were separated and isolated by Ficoll-

Paque (Pharmacia-Upjohn) gradient centrifugation performed twice. The cells were resuspended at 10^7 cells/mL in tissue-culture medium [RPMI 1640-Hepes (Gibco, Paisley, UK) supplemented with gentamycin (25 µg/mL)], 2 mM L-glutamine and 10% heat-inactivated fetal calf serum) supplemented with 10% dimethyl sulphoxide (DMSO), gradually frozen at 1 °C/min to -70 °C in a freezing container (Nalgene Cryo 1 °C, Nalge Company, Rochester, NY, USA) and thereafter stored in liquid nitrogen. The handling of cells did not affect the cytokine balance, neither the viability nor the yield of cells [21]. Plasma from cord blood and from blood collected at 24 months of age was also separated and stored at -20 °C pending analysis.

ELISpot

The numbers of IFN- γ -, IL-4- and IL-12-producing cells in cord blood were measured by the ELISpot method. Cells were thawed on a water bath at 37 °C, slowly diluted 10 times in tissue-culture medium (RPMI 1640-Hepes supplemented with gentamycin (25 µg/mL), 2 mM L-glutamine and 20% heat-inactivated fetal calf serum) and washed twice. The cells were then diluted to a concentration of 10^6 cells/mL in medium alone or in medium containing ovalbumin (OVA, 10 µg/mL Sigma, Stockholm, Sweden), birch, cat (both Aquagen-SQ 8×1000 SQ-U/mL, ALK, Hørsholm, Denmark) or phytohaemagglutinin (PHA, 1 µg/mL, Murex Diagnostics Ltd, Dartford, UK) in 5 ml polystyrene round-bottom tubes. Each tube contained 1.2 million cells.

Anti-human IL-4 (M-450, Endogen, Woburn, MA, USA), IL-12 or IFN- γ monoclonal antibodies (IL-12-I, 1-D1K, Mabtech Nacka, Sweden) were added onto nitro-cellulose plates (Millipore Corp., Bedford, MA, USA) at a concentration of 10 µg/mL in sterile, filtered carbonate buffer (pH 9.6). Plates were incubated overnight at 4 °C, washed six times with filtered phosphate buffered saline (PBS, pH 7.2), and during the last wash, the PBS was forced through the membrane with a vacuum control machine (Millipore). The pre-stimulated (4 h) cell suspensions were added in triplicates (100 000 cells/well). The plates were incubated for 40 h at 37 °C, 5% CO₂, where after the cells were washed away, biotinylated monoclonal antibodies (anti-IL-4 = IL4-II-biotin, IL-12 = IL12-II-biotin and IFN- γ = 7-B6-1biotin, 1 µg/mL Mabtech) were added, followed by an incubation for 2–4 h at room temperature (RT). Thereafter the plates were washed and incubated for 90 min at RT with streptavidin-alkaline-phosphatase (diluted 1/1000, Mabtech). After another series of washings, BCIP/NBT substrate solution (Bio-Rad, Richmond, VA, USA) was added and the plates were incubated at RT until dark spots emerged at the site of cytokine-secreting cells. The plates were washed with water to stop the development. After drying, the spots were counted in a dissection microscope ($\times 40$).

Statistics

The numbers of cytokine-producing cells in response to medium alone and to the various allergens and mitogens are presented as (median $\pm$ (75th (q_3)–25th (q_1) percentiles))/100 000 cells. The spontaneous numbers of cytokine-producing cells have been subtracted for all values shown. Since the

numbers of cytokine-producing CBMC were not normally distributed the Mann–Whitney *U*-test (non-parametric one-way ANOVA) was used to compare the cytokine responses in the different study groups. In order to compare demographic data between groups the Mann–Whitney test, or the Student's *t*-test for uncorrelated means, after validation for normal distribution by use of the Shapiro–Wilk test, was used. χ^2 analysis or, in the case of small expected frequencies, Fisher's Exact Test was used to compare data in contingency tables. The Spearman rank correlation test (r_s) was used in the correlation analyses. In addition to descriptive statistics, including use of confidence intervals, graphical methods were used to characterize the data. The data were analysed through the use of Stata (7.0 Stata Corporation, College Station, TX, USA). A *P*-value < 0.05 was considered significant.

Results

Clinical evaluation

The cumulative incidence of bronchial asthma among all children was 28% ($n = 23$) during the 24-month follow-up. Ten of these children were boys. At 24 months of age 17 (21%) children had asthma. In all children, bronchial asthma was associated with infections and/or exercise. No cases of asthma correlated to exposure to food, animal dander and/or pollen were reported. None of the children reported a history of rhino-conjunctivitis during the observation period.

Fifty-three (65%) infants presented clinical symptoms characteristic of AEDS during the first 2 years of life and 30 (37%) of the children had AEDS at 24 months of age.

Symptoms of food allergy were reported in eight children during the first 2 years of life. At 24 months of age symptoms of food allergy was reported in five children. Allergic reactions to cow's milk dominated ($n = 7$) followed by allergic reactions to hen's egg ($n = 1$), and wheat ($n = 1$).

IgE sensitization

SPT was performed on all children and a blood sample was available for 80 (98%) of the children at 24 months of age. Sixteen (19.5%) children had at least one positive RAST and/or one positive SPT towards the selected food and inhalant allergens at 24 months of age and these children constitute the IgE-sensitized group. If the children with positive RAST towards food and/or inhalant allergens were grouped in relation to quantitative IgE-antibody measurements five children had IgE antibodies belonging to RAST-class 1 (within the interval 0.35–0.7 kU_A/L) and seven had IgE antibodies $\geq$ RAST-class 2 (≥ 0.7 kU_A/L).

Fourteen (87.5%) of the children in the IgE-sensitized group had IgE antibodies against food allergens; eight children showed positive SPT and 11 children were RAST positive. Positive tests towards cat, dog and/or birch were only found in four children: four were positive in RAST and two in SPT. Seven infants were sensitized against two or more allergens.

Among the IgE-sensitized children ($n = 16$), nine (56.3%) had AEDS and six (37.5%) had bronchial asthma at 24 months of age. All eight children who showed clinical

symptoms of food allergy were positive in RAST and/or in SPT to the corresponding food allergen.

The non-sensitized group of children, i.e. children with negative RAST and negative SPT, included 66 infants. In this group of children, 21 (31.8%) had AEDS and 11 (16.7%) had bronchial asthma at 24 months of age but there was no case of food allergy during the observation period.

There were no statistically significant differences in gender, parental heredity, parental smoking habits, delivery mode, presence of furred pets at home and number of siblings between IgE-sensitized and non-sensitized children (Table 1). However, non-sensitized children were more often attending day care than sensitized children were ($P = 0.002$).

Cord blood

The numbers of IFN- γ -, IL-4- and IL-12-producing cells were measured by ELISpot in all cord blood samples ($n = 82$) after stimulation with birch, OVA, cat and PHA.

IFN- γ -producing cells in cord blood

The number of IFN- γ -producing cells in response to medium alone was 1.3 ± 2.7 (median $\pm$ ($q_3 - q_1$)) cells/100 000 cells and in response to PHA 254.5 ± 237.7 (median $\pm$ ($q_3 - q_1$)) cells/100 000 cells. There was a tendency, however not statistically significant, that the numbers of IFN- γ -producing CBMC after PHA stimulation were lower in children classified as IgE sensitized, 225.7 ± 258.7 (median $\pm$ ($q_3 - q_1$)) cells/100 000 cells than in non-sensitized children 262.6 ± 244.3 (median $\pm$ ($q_3 - q_1$)) cells/100 000 cells ($P = 0.083$). There was no difference between infants with and without AEDS, with and without bronchial asthma, or with and without food allergy at 24 months of age (data not shown). Furthermore, there were no associations between the cumulative incidence of bronchial asthma or food allergy from birth to 2 years of age and the number of IFN- γ -producing CBMC (data not shown).

Interestingly, however, children ($n = 53$) who were classified as having had AEDS ever during 0–24 months of age (i.e. cumulative incidence) had fewer IFN- γ -producing CBMC after stimulation with OVA ($P = 0.017$), cat ($P = 0.010$) and birch ($P = 0.064$) (Fig. 1).

IL-4-producing cells in cord blood

The number of IL-4-producing cells in response to medium alone was 0.7 ± 1.4 (median $\pm$ ($q_3 - q_1$)) cells/100 000 cells. Except after stimulation with PHA the number of IL-4-producing CBMC was very low. The non-sensitized group of children showed an increased number of IL-4-producing CBMC after stimulation with PHA, 211.3 ± 155.3 (median $\pm$ ($q_3 - q_1$)) cells/100 000 cells, when compared with the children in the sensitized group, 148.0 ± 205.3 (median $\pm$ ($q_3 - q_1$)) cells/100 000 cells ($P = 0.038$).

The number of IL-4-producing CBMC, after stimulation with PHA, was neither associated with the cumulative incidence of AEDS, bronchial asthma or food allergy, during the observation period, nor with the presence of the various atopic diseases at 24 months of age (data not shown).

IL-4/IFN- γ

No significant differences in the ratio between the number of cells producing IL-4 and IFN- γ after stimulation with mitogen/allergen were observed between IgE-sensitized and non-sensitized children. Among infants with vs. without AEDS, during the observation and at 24 months of age, no difference was seen in the IL-4/IFN- γ ratio. Nor were there any differences between infants with and without bronchial asthma or between infants with and without food allergy (data not shown).

IL-12-producing cells in cord blood

The number of IL-12-producing cells in response to medium alone was 1.7 ± 6.1 (median $\pm$ ($q_3 - q_1$)) cells/100 000 cells. There were great inter-individual variations. The numbers of IL-12-producing CBMC in children classified at 24 months of age as IgE sensitized (SPT ≥ 3 mm and/or RAST ≥ 0.35 kU_A/L) and non-sensitized are presented in Fig. 2. Children classified as non-sensitized had higher numbers of IL-12-producing CBMC after stimulation with the selected antigens. However, the increase in the numbers of IL-12-

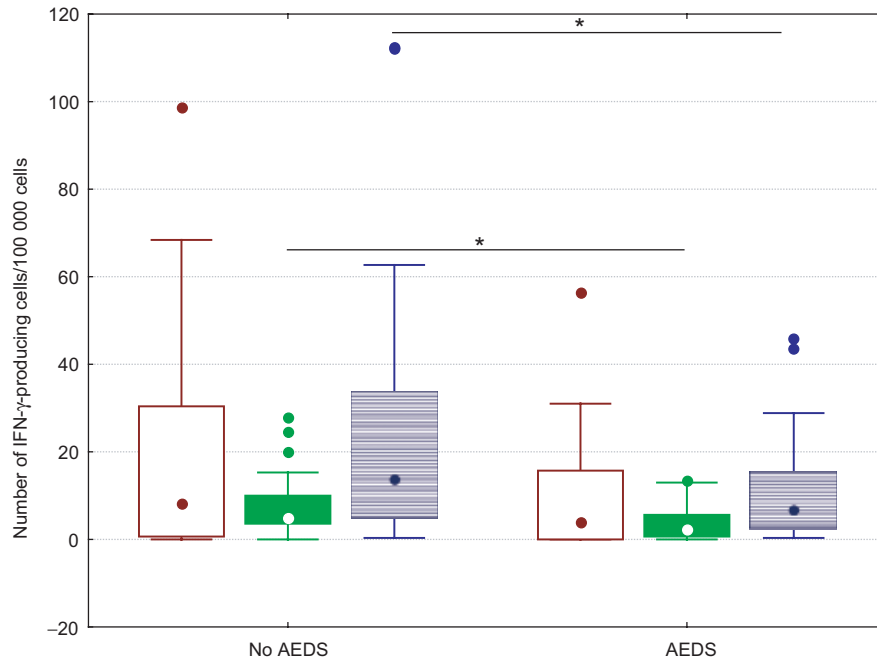


Fig. 1. Frequency of IFN- γ -producing cells/100 000 cord blood mononuclear cells after stimulation with birch pollen (white bar); ovalbumin (green bar) and cat (blue bar) among infants with ($n = 53$) and without atopic eczema/dermatitis syndrome (AEDS) ($n = 29$) during the first 2 years of age. Results are illustrated using the box-plot model. Boxes cover the middle 50% of the data values, between the 25th (q_1) and 75th (q_3) percentiles, the central dot being the median. Lines extend out to non-outlier max and non-outlier min. Outliers are included. * $P < 0.05$.

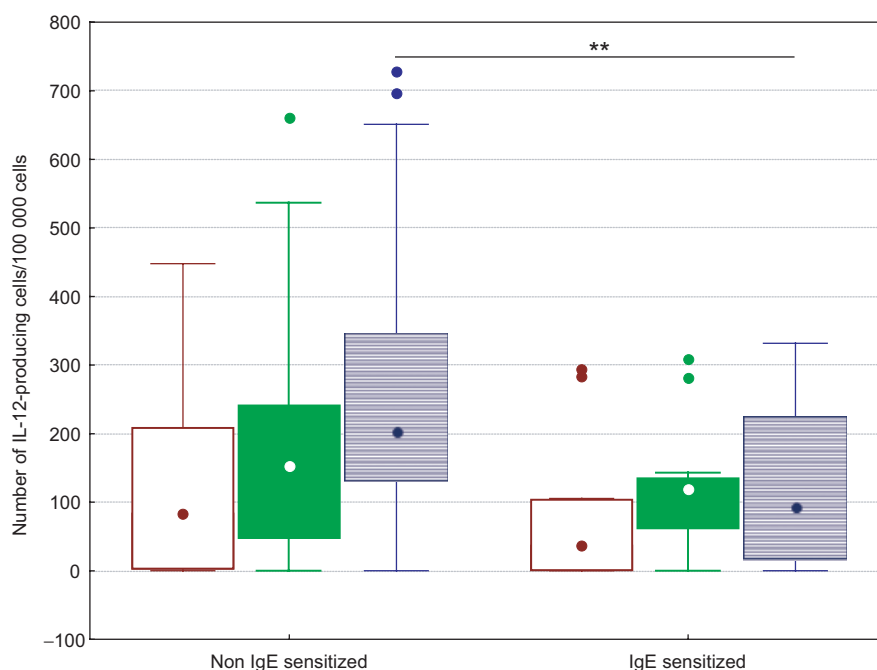


Fig. 2. Frequency of IL-12-producing cells/100 000 cord blood mononuclear cells after stimulation with birch pollen (white bar); ovalbumin (green bar) and cat (blue bar) among IgE sensitized ($n = 16$) and non-sensitized ($n = 66$) at 2 years of age. For explanation of the box-plot model see the legend to Fig. 1. ** $P < 0.01$.

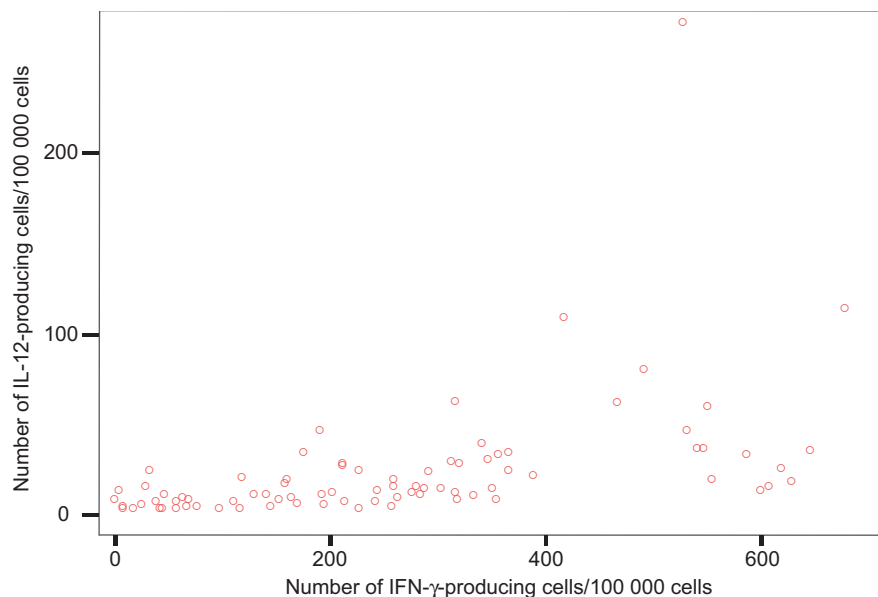


Fig. 3. Spearman rank correlation (r_s) between the number of IFN- γ - and IL-12-producing cells/100 000 cord blood mononuclear cells after stimulation with phytohaemagglutinin among IgE-sensitized and non-sensitized children. Correlation coefficient $r_s = 0.68$ ($P < 0.001$) for the whole study group.

producing cells was statistically significant only for cat ($P = 0.002$). The number of IL-12-producing cells in response to PHA was low. However, there was a tendency that after PHA stimulation IgE-sensitized compared with non-sensitized children had lower numbers of IL-12-producing cells, 8.7 ± 12.2 and 11.4 ± 20.7 (median $\pm (q_3 - q_1)$)/100 000 cells, respectively ($P = 0.071$).

There were no significant differences in the numbers of IL-12-producing CBMC between children who had vs. children who had never had AEDS by 24 months of age. Similarly, the numbers of IL-12-producing CBMC did not differ between children with and without bronchial asthma. Among children with food allergy the number of IL-12-producing CBMC was decreased as in the whole group of IgE-sensitized infants.

Correlation IFN- γ and IL-12

Since IL-12 has been shown to induce IFN- γ production, and IFN- γ , on the other hand, promotes IL-12, it was of interest to evaluate the correlation between the two cytokines.

The correlation between the number of IFN- γ - and IL-12-producing CBMC after stimulation with PHA among all children ($n = 82$) was $r_s = 0.68$ ($P < 0.001$; Fig. 3).

The corresponding correlations for the IgE-sensitized ($n = 16$) and non-sensitized ($n = 66$) children were $r_s = 0.55$ ($P = 0.029$) and $r_s = 0.67$ ($P < 0.001$), respectively.

Discussion

This study shows that the numbers of IL-12-producing CBMC after stimulation with allergen (birch, OVA and cat) and mitogen (PHA) were decreased in children classified as IgE sensitized by positive SPT and/or RAST compared with non-sensitized children (negative SPT and RAST) at 2 years of age. However, this difference was statistically significant only for cat. The induction of IL-12 production was not

correlated to the development of clinical manifestations such as AEDS or bronchial asthma during the observation period.

IL-12 is mainly produced by antigen presenting cells (APC) such as dendritic cells and macrophages, in response to microbial products, cytokines from T cells or after antigen presentation and CD40 ligation [23]. In line with that, we observed that the numbers of IL-12-producing cells after allergen stimulation was high. This was in contrast to what was observed for PHA, probably due to that PHA does not directly induce IL-12 production by APC but do so through T cell derived cytokines such as IFN- γ that may stimulate APC to produce IL-12 through a potent positive feedback mechanism, probably involving the up-regulation of CD40 [23]. The fact that we in the present study observed IL-12-producing cells after PHA stimulation is in agreement with such a hypothesis [23].

So far, little is known about IL-12 and its influence on morbidity among children. It has been reported that infants who developed severe bronchiolitis after infection with Respiratory Syncytial Virus (RSV) had less circulating IL-12 in cord blood [24]. This is an interesting observation since RSV is known to induce high levels of IgE and has been suggested to be linked to atopy [25]. Other studies with peripheral blood mononuclear cells collected from children with IgE-mediated allergic diseases and stimulated with recombinant human IL-12, have shown a decreased IFN- γ production compared with cells from non-sensitized children, suggesting that IFN- γ and/or IL-12 have a regulatory role in the development of atopic diseases [26]. This is also supported by our recent data that the numbers of IL-12-producing cells are significantly lower in cord blood obtained from children with atopic heredity [14]. In this study IgE-sensitized children exhibited lower number of IL-12-producing cells – a finding that also has been shown by others in a recent publication [27].

The importance of IFN- γ in the development of IgE sensitization and allergic disease has been in focus for several years. We have previously demonstrated that children with two atopic parents have a higher ratio IL-4/IFN- γ -producing

CBMC than children with one or no atopic parent [14]. In the present study, there was increased numbers of IL-4- and IFN- γ -producing cells in the non-sensitized children after PHA stimulation. However, there were no difference in the ratio IL-4/IFN- γ -producing CBMC. The reason for this discrepancy between our two studies is not known but could be due to the fact that in the former study the data were related to heredity while in the present study the data were associated to the development of atopy during early infancy. Alternatively, the cytokine production in cord blood might not reflect the risk of developing atopy or that our time point for evaluating the presence of atopy might be too early. However, our observation is in line with data published by Prescott et al. [13] showing that neonates who subsequently develop atopy initially have reduced capacity to mount both Th1 and Th2 responses. Several publications have shown that the production of IFN- γ is lower in cord blood than in adult blood [28] and that secreted levels of IFN- γ are decreased in cord blood from children who will later develop atopy [15, 17]. Interestingly, the majority of children in these studies developed AEDS as the dominating clinical manifestation of allergic disease [5], which is in line with our data in the present study. Taken together, these observations suggest that IFN- γ -producing CBMC exert an immunomodulatory effect during the early postnatal period. Importantly, the number of IFN- γ -producing CBMC was not correlated to the development of bronchial asthma and IgE sensitization among our children, suggesting that IFN- γ could have different impact on IgE sensitization and on the development of various allergic phenotypes.

Various factors have been found to influence the development of naïve CD4⁺ T cells to become either Th1 or Th2 cells. For the development of Th1 cells IL-12 is considered to be a key factor. IL-12 has been shown to signal through the signal transducer and activator, the transcription factor 4 (STAT4), and to induce IFN- γ production. IFN- γ , on the other hand, activates macrophages and promotes the expression of IL-12 receptor, clearly emphasizing the intricate interplay between IL-12 and IFN- γ [23]. This was also evident in the present study since there was a significant correlation between the numbers of IFN- γ and IL-12, after polyclonal stimulation with PHA, when evaluated among all children (IgE sensitized and non-sensitized).

Although, the numbers of children are limited our study demonstrates decreased numbers of allergen-induced IL-12-producing CBMC in IgE-sensitized children. The decreased numbers of IL-12-producing cells could eventually lead to less IFN- γ production, which in turn could explain the postnatal immune deviation towards IgE sensitization/allergic disease in young children. The reasons for the lower IL-12 production in our IgE-sensitized children are not known. However, defects at several levels have been shown to affect the IL-12 and IFN- γ production.

These include a genetic mutation in the IL-12 receptor β_2 chain, which recently has been shown to be linked to reduced production of IFN- γ [29], or the lack of or a defect in the signalling (β_2) subunit of the receptor, which results in reduced IL-12 production [30, 31]. However, defects at other levels are also possible. For example IL-12 is known to be induced after stimulation with bacterial products via Toll-like and/or CD14 receptors on macrophages [32]. Recently

Baldini et al. [33] reported that a polymorphism in the promoter region of the CD14 gene was associated with elevated levels of soluble CD14 and negatively linked to allergic disease and total serum-IgE levels, suggesting a regulatory role of CD14 in the IL-12 production. Another genetic polymorphism, that might affect the IL-12 and IFN- γ production, is that described for the CD40 ligand [34]. However, other explanations like factors transferred from the mother to the child already *in utero* have also been shown to influence cytokine profiles [35].

Furthermore, the observation that the number of IFN- γ -producing CBMC is decreased in children with AEDS but not in children with bronchial asthma and/or sensitization might indicate that different cytokine profiles in cord blood are connected to different allergic phenotypes. This proposal will be further evaluated at the 5 years follow-up when the number of children with respiratory allergies (i.e. bronchial asthma and allergic rhino-conjunctivitis) most certainly has increased.

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