

Allergen-specific T cell reactivity in cord blood: the influence of maternal cytokine production

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Summary

Background Successful pregnancy is dependent upon T helper (Th)2-type-dominated immunological responsiveness in gestation-associated compartments.

Objective In our study we observed the influence of the maternal Th2-associated cytokine pattern on the naive fetal T cell phenotype and asked if circulating Th2 cytokines of atopic mothers affects the Th1/Th2 differentiation of the fetus.

Methods Cord blood mononuclear cells (CBMC) and peripheral blood mononuclear cells (PBMC) of the corresponding mothers were isolated. The proliferative response of CBMC and PBMC to Betalactoglobulin (BLG) was assessed by liquid scintillation counting. The cytokines interferon (IFN)- γ , and interleukin (IL)-5, IL-10 and IL-13 in the cell culture supernatants were measured using the ELISA technique. We then defined two subgroups based on maternal levels of specific IgE against aeroallergens: sensitized mothers (MA^+) and their neonates (NMA^+) ($n = 18$) and non-sensitized mothers (MA^-) and their neonates (NMA^-) ($n = 29$).

Results Nearly all mothers (98%) and neonates (92%) had a positive proliferation response after stimulation with BLG (mean stimulation index (10–90 percentile): neonates: 7 (2–15); mothers 14 (5–29)). In supernatants of BLG-stimulated cell cultures, sensitized mothers showed a significantly lower IFN- γ concentration in comparison to non-sensitized mothers ($MA^+ = 25$; $MA^- = 123$ IU/L; $P < 0.05$), whereas the neonates did not differ significantly ($NMA^+ = 306$; $NMA^- = 224$ IU/L; n. s.). Nor was any difference found in the IL-13 concentration between the two groups of sensitized and non-sensitized mothers ($MA^+ = 48$; $MA^- = 125$ pg/mL; n. s.). CBMC of neonates with a sensitized mother showed significantly higher IL-13 concentrations in response to BLG than neonates of non-sensitized mothers ($NMA^+ = 1442$, $NMA^- = 738$ pg/mL; $P < 0.05$). The IL-5 and IL-10 concentrations did not differ significantly within the neonatal and the maternal subgroups.

Conclusions Our data suggests that maternal sensitization to allergens is associated with the reduced maternal production of the Th2 antagonist IFN- γ and elevated production of the Th2 cytokine IL-13 in the offspring.

Keywords: T cell proliferation; cord blood; allergen; early sensitization; cytokines, materno–fetal interaction

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Introduction

The increasing prevalence of atopic dermatitis, bronchial asthma and allergic rhinoconjunctivitis has led to attempts to prevent the development of allergic diseases in childhood

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[1,2]. Thus, detailed understanding of the immunological process is required if primary prevention strategies are to be developed.

Atopic diseases are associated with elevated serum levels of both total and allergen-specific IgE [3,4]. The production of IgE is controlled by T lymphocytes, in particular by the T helper (Th)2 subsets of the CD4⁺ T cells. These cells secrete various Th2-associated cytokines such as interleukin (IL)-4, IL-5, IL-10 and IL-13, which results in B cell switching to IgE production and modulates the state of effector cells of allergic inflammation [3,4]. In contrast to the Th2-like response leading to allergic disease, non-atopic individuals show mainly Th1 immunity characterized by the production of interferon (IFN)- γ and IL-12, which inhibits the growth of Th2 cells [5]. The balance between the two subsets of T cells is pivotal for allergic sensitization. However, little is known about the mechanism and the timing by which allergens provoke the selective development of a Th2 immune response within the atopic population.

In the last 10 years there has been a growing body of evidence that the immunological changes leading to atopic diseases start very early in childhood or even prenatally, i.e. during pregnancy [6]. Much research has focused on the reactivity of cord blood cells in response to various foods and inhalant allergic stimuli. To date it is clearly demonstrated that cord blood mononuclear cells (CBMC) of atopy-prone as well as non-atopy-prone children [6,7] proliferate in response to betalactoglobulin (BLG) [6,8–10], bovine serum albumin [6,8,11,12], ovalbumin [6,8,10,12–14], birch pollen [6–8], timothy grass pollen [6–8], cat fur [6,8] and house dust mite [6,15,16]. These results have been interpreted as indirect proof that allergen priming must occur before birth.

Apart from proliferation assays, cytokine production at birth of mitogen and allergen-stimulated cord blood cells has been investigated by several groups, of which all showed a Th2-dominated cytokine pattern and reduced Th1 production [13,17,18]. Comparing newborns with the later development of allergic diseases and non-atopic children, IFN- γ production is lower in individuals with an atopic family history and increases more slowly from birth to the age of 5 years [18]. *In vitro* data indicates that antigen-specific Th cell precursors differentiate into Th1 or Th2 cells mainly according to the pattern of cytokines in the microenvironment at the time of antigen presentation [19]. In this context it is important to stress the circumstances of the microenvironment concerning the fetal immune system: based on murine studies and clinical observations, Wegmann *et al.* postulated in 1993 that a successful pregnancy is dependent upon a Th2-type dominated immunological response in gestation-associated compartments [20]. In the light of these findings the question which

arises is whether maternal production of Th2 cytokines influences the differentiation of the naive fetal T cell phenotype. To answer this question we collected umbilical cord blood and corresponding blood samples from sensitized and non-sensitized mothers in order to assess the potential role of maternal cytokine production in the development of specific T cell response in the offspring.

Methods

Subjects and study design

The study involved a cohort of 50 families who were randomly selected from women giving birth at a local hospital between December and June 1999. The study protocol was approved by the ethics committee of the University of Freiburg and written consent was obtained from the parents.

Fifty samples of cord blood were collected. Human umbilical cord blood was drawn on heparin (15 IU Heparin/mL blood) before expulsion of the placenta [21]. The infants were born between the 37th and 42nd week of gestation (median 40th week of gestation). Way of delivery, birth weight and medical as well as family history of atopy were obtained with a questionnaire and only families with uneventful pregnancy and delivery were included in our study.

The maternal venous blood samples were obtained by cubital venipuncture and were transported at once to our laboratory where the preparation of mononuclear cells was carried out immediately.

Atopic sensitization was evaluated by determining specific IgE to the house dust mite allergens *Dermatophagoides pteronyssinus* (Der p 1) and *Dermatophagoides farinae*, cat dander, birch or grass pollen allergen, cow's milk protein and hen's egg protein in the maternal sera using the Fluoro-Enzym-Immuno-Assay (FEIA, Pharmacia CAP System, Pharmacia, Uppsala, Sweden). The sensitivity of the assay was > 0.35 kU/L. Mothers with a specific IgE equal to or greater than 3.5 kU/L (CAP-RAST class ≥ 2) in response to at least one tested allergen were classified as 'sensitized'.

Cell preparation

Cord blood mononuclear cells from umbilical cord blood and peripheral blood mononuclear cells (PBMC) taken from the mothers were isolated by density gradient centrifugation (Pharmacia) and washed three times with a saline buffer containing 3% heat-inactivated fetal calf serum (Biochrom, Berlin, Germany). The cells were frozen gradually to -70°C in a freezing container. Thereafter the tubes were stored in liquid nitrogen at -196°C . Before

use, the cells were thawed quickly in a 37 °C water bath, diluted and washed in Ultra culture medium (Bio Whittaker, Walkersville, MD, USA) supplemented with 1% heat-inactivated human serum, 20 µM 2-mercaptoethanol (Merck, Darmstadt, Germany), 2 mM L-glutamine (Sigma, Taufkirchen, Germany) and 170 mg/L Gentamycin (Sigma).

Proliferation assay

For proliferation assays, CBMC and PBMC were cultured in triplicates in concentrations of 25 000 cells/well in 96 well round-bottom tissue-culture plates (Corning, New York, NY, USA) for seven days [21]. CBMC or PBMC alone served as negative control, while CBMC or PBMC plus IL-2 (5 U/well; Boehringer, Mannheim, Germany) were used as positive control. Betalactoglobulin (Sigma) was used at final concentrations of 25 µg/mL. Major allergen of house dust mite *Dermatophagoides pteronyssinus* Der p 1 was kindly provided by Mr Carsten Shou (ALK, Hørsholm, Denmark). Der p 1 was used at final concentrations of 10 µg/mL.

After 7 days of culture at 37 °C in humidified 5% CO₂/95% O₂, [³H]thymidine ([³H]TdR; 0.5 mCi/well; Amersham Int., Amersham, UK) was added and the incorporated radioactivity was measured by scintillation counting after 16 h. Proliferation was expressed as a stimulation index (SI), which was calculated as the ratio between the mean counts/min values measured in stimulated triplicates and the mean counts/min values measured in unstimulated triplicates (SI = cpm in cultures stimulated with allergen/cpm in unstimulated cultures). In accordance with the literature [11], a proliferative response with a SI > 2 in addition to an absolute value of > 750 cpm was regarded as positive.

ELISA

For cytokine analysis, CBMC and PBMC were cultured in the medium alone or stimulated with phytohaemagglutinin (PHA) (20 µg/mL; Sigma) or BLG (25 µg/mL; Sigma) and incubated in concentrations of 100 000 cells/well. The supernatants of the unstimulated and the PHA-stimulated cells were obtained after 24 h of culture at 37 °C in humidified 5% CO₂/95% O₂. Following the data of preliminary experiments where we determined the maximal cytokine expression of BLG-stimulated CBMC and PBMC in a subsample of *n* = 10 individuals, we obtained the supernatants of allergen-stimulated cells after 4 days of culture for the cytokines IL-5, IL-13 and IFN-γ and after 5 days for IL-10. They were then analysed using commercially available ELISA technique (Coulter-Immunotech, Krefeld, Germany) in accordance with the manufacturer's

instructions. All assays of the specifically stimulated CBMC and PBMC were performed in duplicate. The limit of detection was 1 pg/mL (IL-5), 1.5 pg/mL (IL-13), 5 pg/mL (IL-10) and 0.08 IU/mL (IFN-γ), respectively.

Statistical analysis

The analysis was carried out using the Statistical Analysis System. In order to determine the statistical significance of differences between the subgroups of atopy and non-atopy prone newborns *P*-values were calculated and the Wilcoxon-test was employed.

Results

Clinical characteristics of the study population

Fifty cord blood samples from full-term neonates (44% males; *n* = 22) and 47 corresponding blood samples from the mothers were collected. The time of delivery varied between the 37th and 42nd week of gestation (median 40th week of gestation). Normal vaginal deliveries were observed in 64% of the population (*n* = 32) and Caesarean sections in 36% (*n* = 18). The mean birth weight was 3419 g (range 2350–4800 g). No women smoked during pregnancy but 14.3% (*n* = 7) of them were exposed to passive smoke at home. The parents of the neonates were asked about their history of allergy. On that occasion 26% (*n* = 13) of the fathers and 42% (*n* = 21) of the mothers reported a history of asthma, allergic rhinitis or atopic eczema. Based on elevated specific IgE, 38% of the mothers were defined as 'atopics' with a CAP-RAST class ≥ 2 in response to one of the tested allergens. Cord blood total IgE was above the detection limit of 2 IU/L in five newborns.

To analyse the role of maternal atopy for T cell differentiation we defined two groups based on an elevated specific IgE of the mothers: group 1 comprised sensitized mothers and their offspring (mothers: MA⁺, *n* = 18; neonates: NMA⁺ *n* = 18) and group 2 comprised non-sensitized mothers and their offspring (mothers: MA⁻, *n* = 27; neonates: NMA⁻; *n* = 27). The way of delivery, time of delivery and birth weight did not differ between the groups (data not shown).

Proliferation assay: BLG is a privileged allergen recognized by cord blood cells

Proliferation activity was assessed by quantification of [³H]thymidine uptake. All tested samples responded to the unspecific stimulus IL-2 with a mean stimulation index of 26 in the neonatal group and a mean stimulation index of 21 in the maternal group (Table 1). Higher proliferation

Table 1. Proliferative response of maternal and neonatal mononuclear cells. The stimulation index (SI; median 10th and 90th percentile) and the percentage of positive responders (defined as a stimulation index > 2 in addition to an absolute value of > 750 cpm) are presented

		Mothers	Neonates
IL-2	SI	21 5–41	26 8–51
	SI > 2	100%	100%
BLG	SI	14 5–29	7 2–15
	SI > 2	98%	92%
OVA	SI	2 1–4	1.7 1–3
	SI > 2	36%	38%
Der p 1	SI	2 1–5	2 1–3
	SI > 2	17%	41%

indices after stimulation with allergens were observed in response to BLG (mothers SI 14; neonates SI 7) compared to OVA and Der p 1 (OVA: mothers SI 2; neonates: SI 1.7; Der p 1: mothers SI 2; neonates: SI 2).

Based on the definition that a response with a SI > 2 in addition to an absolute value > 750 cpm will be classified as positive, a positive proliferative response to BLG was observed in 92–100% with no differences in the two groups (Table 1). As with BLG, the number of positive responses to OVA and to Der p 1 did not differ between atopic and non-atopic mothers and their newborns.

Significantly lower IFN- γ concentration in the supernatants of BLG-stimulated PBMC of sensitized mothers compared to non-sensitized mothers

The principal focus of this study involved detailed analysis of cytokine production by allergen-stimulated CBMC and PBMC in response to stimulation with the unspecific stimulus PHA and the archetypal food allergen BLG. All cytokine concentrations are expressed as the increase above the unstimulated background. Table 2 summarizes the cytokine concentration of PHA-stimulated cells in the maternal and neonatal group. IFN- γ , IL-5 and IL-13 concentrations were similar in the maternal and neonatal cell supernatants. However, the IL-10 concentration in the supernatants in response to PHA was significantly higher in the maternal group (3241 pg/mL) compared to the neonates (307 pg/mL; $P < 0.005$). No differences were detectable

Table 2. Cytokine concentrations of IFN- γ , IL-13, IL-10 and IL-5 in the maternal and the neonatal group in cell supernatants stimulated with PHA. The median and the 5th and 95th percentage values above the unstimulated background are presented.

	Mothers	Neonates
IFN- γ [IU/L]	27 20–56	21 6–49
IL-13 [pg/mL]	203 158–361	163 55–340
IL-10 [pg/mL]	3241 125–10630	307 48–1127
IL-5 [pg/mL]	8 5–56	4 1–7

between the four groups considering the maternal status of allergy (MA⁺ and MA[−]; NMA⁺ and NMA[−]).

In Fig. 1 the IFN- γ concentrations of BLG-stimulated cells of maternal PBMC supernatants and neonatal CBMC supernatants are presented. The mothers with an allergic sensitization showed a significantly lower IFN- γ concentration compared with mothers without allergic sensitization (MA⁺ = 25; MA[−] = 123 IU/L; $P < 0.05$).

Significantly higher IL-13 concentration in the supernatants of BLG-stimulated CBMC of neonates from sensitized mothers

Similarly to the Th1 cytokine IFN- γ a number of Th2 type cytokines (IL-13, IL-10, IL-5 and IL-4) were analysed in the supernatants of BLG-stimulated cell cultures. Using the conventional sandwich ELISA technique, we were not able

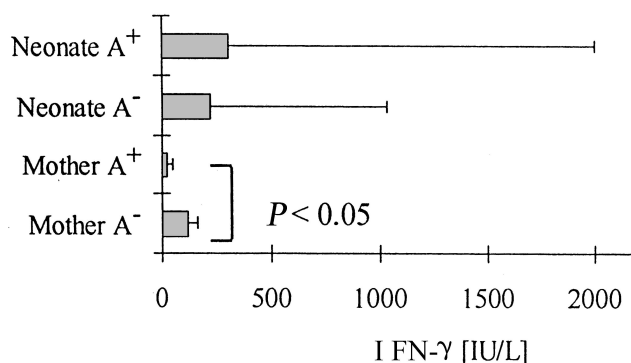


Fig. 1. Cytokine concentrations of IFN- γ in cell supernatants stimulated with BLG. The median and the maxima values above the unstimulated background are presented of the sensitized mothers, the non-sensitized mothers, the neonates with a sensitized mother and the neonates with a non-sensitized mother.

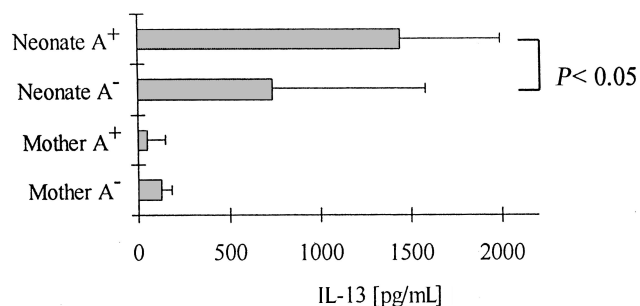


Fig. 2. Cytokine concentrations of IL-13 in cell supernatants stimulated with BLG. The median and the maxima values above the unstimulated background are presented of the sensitized mothers, the non-sensitized mothers, the neonates with a sensitized mother and the neonates with a non-sensitized mother.

to detect IL-4 in supernatants of CBMC. Reports in the literature and our own data suggest that more sensitive techniques are necessary to quantify allergen-specific IL-4 responses by CBMC.

In contrast to IL-4, the Th2 type cytokines IL-13, IL-10 and IL-5 were detectable in all supernatants of stimulated CBMC and PBMC. Figure 2 summarizes the IL-13 concentrations in response to BLG.

CBMC of neonates with a sensitized mother showed significantly higher IL-13 concentrations in response to BLG than neonates without a sensitized mother ($NMA^+ = 1442$, $NMA^- = 738$ pg/mL; $P < 0.05$).

There were no differences in the IL-5 concentration between the four groups (Fig. 3). In response to BLG the IL-10 levels were higher in CBMC of neonates with sensitized mothers and in PBMC of sensitized mothers compared to the neonatal and maternal group with no sensitization of the mothers ($MA^+ = 893$, $MA^- = 686$; $NMA^+ = 152$, $NMA^- = 126$ pg/mL). However, the

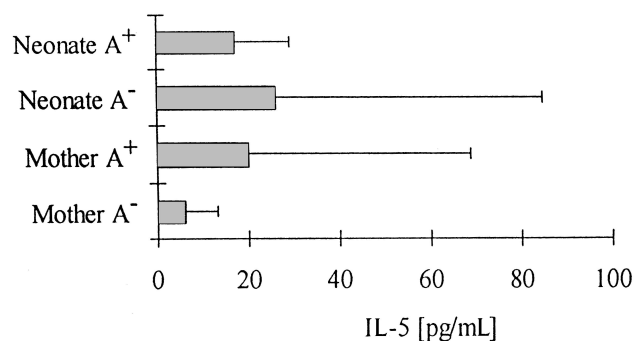


Fig. 3. Cytokine concentrations of IL-5 in cell supernatants stimulated with BLG. The median and the maxima values above the unstimulated background are presented of the sensitized mothers, the non-sensitized mothers, the neonates with a sensitized mother and the neonates with a non-sensitized mother.

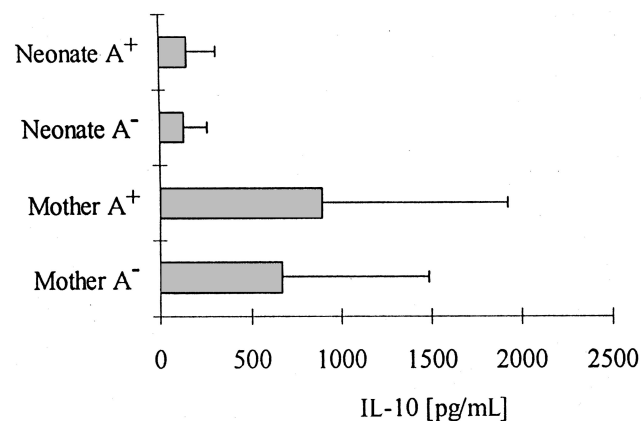


Fig. 4. Cytokine concentrations of IL-10 in cell supernatants stimulated with BLG. The median and the maxima values above the unstimulated background are presented of the sensitized mothers, the non-sensitized mothers, the neonates with a sensitized mother and the neonates with a non-sensitized mother.

differences between the subgroups were not statistically significant (Fig. 4).

We calculated the Spearman correlation coefficient of the different cytokine levels in the maternal and neonatal subgroup (Table 3).

In the maternal group only the two representatives of the Th2 cytokines IL-5 and IL-13 were significantly positively correlated ($r^s = 0.58$, $P = 0.0001$). In the neonatal group we observed the strongest correlation between IL-5 and IL-13, similar to the maternal group. However, in contrast to the mothers, we found a weak but statistically significant correlation between IFN- γ and IL-5, IFN- γ and IL-10, IFN- γ and IL-13 as well as a correlation between IL-5 and IL-10. In addition, the correlation between the maternal and neonatal cytokines was calculated. However, in the case of

Table 3. Correlation analysis between the maternal and the neonatal group. Spearman correlation coefficient (r^s) and P -values are presented

	Mothers		Neonates	
	r^s	P	r^s	P
IFN- γ /IL-5	0.04	$P > 0.05$	0.39	$P = 0.0048^*$
IFN- γ /IL-10	-0.14	$P > 0.05$	0.46	$P = 0.0008^*$
IFN- γ /IL-13	0.25	$P > 0.05$	0.34	$P = 0.015^*$
IL-5/IL-10	-0.28	$P > 0.05$	0.37	$P = 0.0091^*$
IL-5/IL-13	0.58	$P < 0.001^*$	0.52	$P = 0.0001^*$
IL-10/IL-13	-0.27	$P > 0.05$	0.23	$P > 0.05$

*Statistically significant ($P < 0.05$).

all tested cytokines we observed no significant correlation coefficient (data not shown).

Discussion

The prevalence of atopic diseases has increased over the past 30 years and is still on the increase [22–24]. Therefore current research focuses on risk factors in early childhood and particularly around birth which might influence the development of allergic sensitization. In this context it is worthy of note that babies of allergic mothers are significantly more likely to develop a positive skin prick test reaction, an atopic eczema or a food intolerance at the age of one to two years compared to babies of allergic fathers [25,26]. These findings support the role of the mother in programming the immune response of the infant prior to birth. Because pregnancy itself is a Th2 phenomenon, we studied the potential influence of maternal cytokine expression on cord blood. Our main interest was to investigate differences between mothers with specific sensitization to common allergens who might have constitutionally higher levels of Th2 cytokines, and mothers without specific sensitization.

In this study increased concentrations of Th2 cytokines IL-13 and IL-10 in response to the common food allergen BLG were found in neonates of sensitized mothers. These differences reached statistical significance for IL-13, but not for IL-10. Parallel to the observed Th2 shift in neonates of sensitized mothers we found increased concentrations of IL-10 and significantly decreased concentrations of the Th1 cytokine IFN- γ in the sensitized mothers themselves in response to the specific stimulus BLG, but not in response to the unspecific stimulus PHA. Our findings support the hypothesis that *in utero* influences may be fundamental in the development of neonatal T cell reactivity and that maternal sensitization might direct the neonatal cytokine concentrations towards a Th2 pattern.

In the maternal and neonatal groups we observed a significant correlation between the two representatives of the Th2 cytokine family IL-13 and IL-5. In the maternal group no further significant relationships between the tested cytokines were seen. In contrast to the mothers, we calculated in the neonates a weak but significantly positive correlation between IFN- γ and IL-5, IFN- γ and IL-10, IFN- γ and IL-13 as well as between IL-5 and IL-10. This data strongly suggests that in contrast to maternal PBMC the T cell of the newborn, even if already biased in a Th2 direction, is still a pluripotent cell with the ability to synthesize both Th1 and Th2 cytokines. In contrast to the neonates, the T cells of the mothers seems to be more strongly polarized in a Th1 or Th2 direction.

In previous studies a clear shift of neonatal cytokines in a Th2 direction was observed. Comparing newborns with the

later development of allergic diseases and non-atopic children, IFN- γ production is lower in individuals with an atopic family [10,17,18]. In contrast to these studies we only had information about the family history of sensitization but not about a later development of allergic sensitization of these individuals. This might explain the fact that we observed no differences in IFN- γ production between neonates of atopic and non-atopic mothers.

Furthermore, the choice of antigen could explain the discrepancy in the cytokine levels between our and previously published data. We used BLG as an allergen-specific stimulus, because cow's milk proteins are usually one of the first nutritive allergens to which neonates are exposed in early life. Moreover, Szépfalusi showed very recently that BLG can cross the human placenta [27]. Based on the observations of this study comparing BLG and birch pollen allergen, it is possible that different inhalant or nutritive allergens have different abilities to cross the human placenta and might therefore provoke variable immunological reactions.

There were no significant relationships between maternal and neonatal cytokine expression. This might be explained by the fact that the maternal cytokine profile changes during pregnancy and materno–fetal interaction might start early during gestation. T cell precursors migrate from the epithelial thymus between 7 and 9 weeks of gestation [28]. A proliferative response of fetal T cells to mitogenic stimuli can be detected in the second trimester which increases with progression through gestation [29]. Therefore we suspect that the analysis of maternal cytokine concentration in the second trimester might add further information to materno–fetal interaction and might better mirror the potential immunological influence on the fetus than maternal cytokine levels around birth do.

Furthermore, several confounders might influence the maternal cytokine concentrations around birth. We evaluated the method of delivery, birth weight and tobacco smoke exposure [30] as possible factors, all of which are able to direct the cytokine levels either in a Th2 or a Th1 direction. Maternal disorders during pregnancy like preeclampsia [31], autoimmune disease [32,33], chronic or acute infectious disease [34–36], neoplasia or the consumption of medication such as corticoids [37], which possibly influence the Th1/Th2 balance, were ruled out by considering information on the questionnaire (data not shown).

In addition to the considerations about a critical time period of materno–fetal interactions and possible confounders it seems plausible that beside immunological effects environmental factors such as allergen exposure and the maturation process of the fetal immune system must also be taken into account.

Taken together, our hypothesis that maternal sensitization might direct the neonatal cytokine levels towards a

Th2 pattern is weakened by the absence of a clear correlation between the maternal and the neonatal cytokine levels and by the absence of IL-4 data. As stated above we were not able to detect IL-4 in supernatants of CBMC using the conventional sandwich ELISA technique. However, we observed a significant decrease of the Th1 cytokine IFN- γ in the BLG-stimulated PBMC of sensitized mothers which parallels the significant increase of the cytokine IL-13 in BLG-stimulated CBMC of the corresponding neonates. Our data might point to an early influence of maternal cytokine concentrations during gestation on the neonatal cytokine shift towards a Th2 pattern. Nevertheless, further studies are needed to clarify if maternal cytokine concentrations during gestation are potent extrinsic factors programming the immune system of the fetus for the development of atopic disease later in life.

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References

- Barnes PJ. Therapeutic strategies for allergic diseases. *Nature* 1999; 25 (402):31-8.
- von Mutius E. The environmental predictors of allergic disease. *J Allergy Clin Immunol* 2000; 105:9-19.
- Renz H. The central role of T-cells in allergic sensitization and IgE regulation. *Exp Dermatol* 1995; 4:173-82.
- Daser A, Meissner N, Herz U, Renz H. Role and modulation of T-cell cytokines in allergy. *Current Opinion Immunol* 1995; 7:762-70.
- Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 1996; 17:138-46.
- Miles EA, Warner JA, Jones AC, Colwell BM, Bryant TN, Warner JO. Peripheral blood mononuclear cell proliferative responses in the first year of life in babies born to allergic parents. *Clin Exp Allergy* 1996; 26:780-8.
- Van-Duren-Schmidt K, Pichler J, Ebner C et al. Prenatal contact with inhalant allergens. *Pediatr Res* 1997; 41:128-31.
- Jones AC, Miles EA, Warner JO, Colwell BM, Bryant TN, Warner JA. Fetal peripheral blood mononuclear cell proliferative responses to mitogenic and allergenic stimuli during gestation. *Pediatr Allergy Immunol* 1996; 7:109-16.
- Piastra M, Stabile A, Fioravanti G, Castagnola M, Pani G, Ria F. Cord blood mononuclear cell responsiveness to beta-lactoglobulin: T-cell activity in 'atopy-prone' and 'non-atopy-prone' newborns. *Int Arch Allergy Immunol* 1994; 104:358-65.
- Warner JA, Miles EA, Jones AC, Quint DJ, Colwell BM, Warner JO. Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic eczema? *Clin Exp Allergy* 1994; 24:423-30.
- Kondo N, Kobayashi Y, Shinoda S et al. Cord blood lymphocyte responses to food antigens for the prediction of allergic disorders. *Arch Dis Child* 1992; 67:1003-7.
- Szepfalusi Z, Nentwich I, Gerstmayr M et al. Prenatal allergen contact with milk proteins. *Clin Exp Allergy* 1997; 27:28-35.
- Yabuhara A, Macaubas C, Prescott SL et al. TH2-polarized immunological memory to inhalant allergens in atopics is established during infancy and early childhood. *Clin Exp Allergy* 1997; 27:1261-9.
- Prescott SL, Macaubas C, Yabuhara A et al. Developing patterns of T cell memory to environmental allergens in the first two years of life. *Int Arch Allergy Immunol* 1997; 113:75-9.
- Piccinni MP, Mecacci F, Sampognaro S et al. Aeroallergen sensitization can occur during fetal life. *Int Arch Allergy Immunol* 1993; 102:301-3.
- Holt PG, O'Keeffe P, Holt BJ et al. T-cell 'priming' against environmental allergens in human neonates. sequential deletion of food antigen reactivity during infancy with concomitant expansion of responses to ubiquitous inhalant allergens. *Pediatr Allergy Immunol* 1995; 6:85-90.
- Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG. Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 1999; 353:196-200.
- Prescott SL, Macaubas C, Smallacombe T et al. Reciprocal age-related patterns of allergen-specific T-cell immunity in normal vs. atopic infants. *Clin Exp Allergy* 1998; 28 (Suppl. 5):39-44.
- Maggi E, Parronchi P, Manetti R et al. Reciprocal regulatory effects of IFN-gamma and IL-4 on the in vitro development of human Th1 and Th2 clones. *J Immunol* 1992; 148:2142-7.
- Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? *Immunol Today* 1993; 14:353-6.
- Kopp MV, Pichler J, Halmerbauer G et al. Culture conditions for the detection of allergen-specific T-cell reactivity in cord blood: influence of cell number. *Pediatr Allergy Immunol* 2000; 11:4-11.
- Ninan T, Russel G. Respiratory symptoms and atopy in Aberdeen school children: evidence from two surveys 25 years apart. *BMJ* 1992; 304:873-5.
- Rimpelä A. Asthma and allergic rhinitis among Finish adolescents in 1977-91. *Scand J Soc Med* 1995; 1:60-5.
- Wüthrich B, Schindler C, Leuenberger P, Ackermann-Liebrich U, SAPALDIA-Team. Prevalence of atopy and pollinosis in the adult population of Switzerland (SAPALDIA-Study). *Int Arch Allergy Appl Immunol* 1995; 106:149-56.
- Hide DW, Matthews S, Matthews L et al. Effect of allergen avoidance in infancy on allergic manifestations at age two years. *J Allergy Clin Immunol* 1994; 93:842-6.
- Ruiz RG, Kemeny DM, Price JF. Higher risk of infantile

- atopic dermatitis from maternal atopy than from paternal atopy. *Clin Exp Allergy* 1992; 22:762–6.
- 27 Szepefalusi Z, Loibichler C, Pichler J, Reisenberger K, Ebner C, Urbanek R. Direct evidence for transplacental allergen transfer. *Pediatr Res* 2000; 48:404–7.
- 28 Haynes BF, Denning SM, Singer KH, Kurtzberg J. Ontogeny of T-cell precursors: a model for the initial stages of human T-cell development. *Immunol Today* 1989; 10:87–91.
- 29 Warner JA, Jones CA, Jones AC, Warner JO. Prenatal origins of allergic disease. *J Allergy Clin Immunol* 2000; 105:493–8.
- 30 Petro TM, Schwartzbach SD, Zhang S. Smokeless tobacco and nicotine bring about excessive cytokine responses of murine memory T-cells. *Int J Immunopharmacol* 1999; 21:103–14.
- 31 Gratacos E, Filella X, Palacio M, Cararach V, Alonso PL, Fortuny A. Interleukin-4, interleukin-10, and granulocyte-macrophage colony stimulating factor in second-trimester serum from women with preeclampsia. *Obstet Gynecol* 1998; 92:849–53.
- 32 Niessner M, Volk BA. Altered Th1/Th2 cytokine profiles in the intestinal mucosa of patients with inflammatory bowel disease as assessed by quantitative reversed transcribed polymerase chain reaction (RT-PCR). *Clin Exp Immunol* 1995; 101:428–35.
- 33 Singh VK, Mehrotra S, Agarwal SS. The paradigm of Th1 and Th2 cytokines: its relevance to autoimmunity and allergy. *Immunol Res* 1999; 20:147–61.
- 34 Bertolotti A, D'Elia MM, Boni C et al. Different cytokine profiles of intrahepatic T cells in chronic hepatitis B and hepatitis C virus infections. *Gastroenterology* 1997; 112:193–9.
- 35 Else KJ, Finkelman FD, Maliszewski CR, Grencis RK. Cytokine-mediated regulation of chronic intestinal helminth infection. *J Exp Med* 1994; 1:347–51.
- 36 Berner R, Niemeyer CM, Leititis JU et al. Plasma levels and gene expression of granulocyte colony-stimulating factor, tumor necrosis factor- α , interleukin (IL) -1 β , IL-6, IL-8, and soluble intercellular adhesion molecule-1 in neonatal early onset sepsis. *Pediatr Res* 1998; 44:469–77.
- 37 Kavelaars A, van der Pompe G, Bakker JM et al. Altered immune function in human newborns after prenatal administration of betamethasone: enhanced natural killer cell activity and decreased T cell proliferation in cord blood. *Pediatr Res* 1999; 45:306–12.