

Increased activation of GATA-3, IL-2 and IL-5 of cord blood mononuclear cells in infants with IgE sensitization

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Risk of allergic diseases has been linked to abnormal patterns of fetal immune development, suggesting that priming of the immune system may occur *in utero*. The aim of the study was to investigate whether the pattern of immune response in cord blood mononuclear cells (CBMC) shows association with allergic diseases and IgE sensitization at 2 yr of age, and to study the effect of maternal probiotic supplementation on CBMC immune responses. CBMC were isolated from 98 neonates in a randomized double-blinded intervention study. CBMC were stimulated with beta-lactoglobulin, and phytohemagglutinin (PHA). Secretion of interferon-gamma (IFN- γ), interleukin-5 (IL-5), and IL-13 was measured by an ELISA; IL-2, IL-4, and IL-10 by a cytokine bead assay. T-cell polarization-associated IL-4 receptor and IL-12R expressions, and the respective transcription factors GATA-3 and T-bet were analyzed with RT-PCR. The above responses were compared with the development of allergic diseases and IgE sensitization at 2 yr of age, and with the maternal probiotic or placebo supplementation. PHA-stimulated GATA-3 expression and IL-2 secretion in CBMC were higher in IgE-sensitized children at an age of 2 yr than in the non-sensitized, non-allergic children ($p = 0.03$ and 0.026). PHA-induced expression of GATA-3 correlated with IL-5 ($p = 0.003$, $r = 0.300$) and IL-13 ($p = 0.007$, $r = 0.278$) secretion of CBMC, and IL-5 secretion of β -lactoglobulin-stimulated CBMC was higher in IgE-sensitized children at 2 yr of age than in the non-sensitized, non-allergic children ($p = 0.013$). Probiotic bacteria had no effect on CBMC immune responses. In CBMC-enhanced induction of GATA-3, which activates several Th2 cytokines genes, was a risk factor for IgE sensitization. The immune deviation towards Th2-type immunity developed already *in utero* and seemed to modulate the pattern of immune response favoring an IgE response to environmental antigens.

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Previous studies have suggested that priming of the immune system to develop allergic immune responses may occur even before birth (1, 2). Cord blood mononuclear cells (CBMC) are responsive to a variety of environmental allergens. Antigen-specific cytokine responses to allergens in CBMCs have been demonstrated in

several studies (3–5). Normal fetal development occurs in an environment biased towards a T helper-type (Th)2 immune response (6, 7).

Interestingly, pronounced Th2-biased responsiveness to allergens and reduced IFN- γ secretion of CBMC and a reduced number of IFN- γ producing CBMC (8) have been reported in

infants who developed allergic diseases later in life (3, 9, 10).

The transcription factor GATA-3 is necessary for Th-2 cytokine gene expression in CD4 cells. In transgenic mice, elevated GATA-3 in CD4 cells causes IL-4, IL-5, IL-6, IL-10 and IL-13 expression in naive T cells, and GATA-3 binding sites are present in the promoter regions of the Th2 cytokines genes (11). GATA-3 is able to activate IL-4 and IL-5 promoters, possibly in cooperation with other activation-inducible factors (11, 12). In a murine model of asthma, transgenic T-cell expression of the dominant-negative GATA-3 allele attenuates allergic inflammation, leading to decreased eosinophilia, to mucus production, and to the low IgE levels associated with decreased IL-4, IL-5 and IL-13 production (13). By contrast, the transcription factor T-bet controls the expression of the hallmark Th1 cytokine IFN- γ (14), which is secreted upon activation of the IL-12 receptor (IL-12R).

Both environmental and genetic factors influence the development of the fetal immune system *in utero*. The pattern of immunological responses of CBMC may be the intrinsic feature of the newborn infant that predisposes to the development of allergic diseases. IgE-associated allergic disease results from unbalanced acquired immunity involving inappropriate Th2 cytokine responses to environmental antigens. In allergic responses, IL-4 and IL-13 induce B-cell switching to IgE production, whereas IL-5 activates eosinophils (15–17). In the present study, we investigated whether the markers of T-cell polarization, such as activation of GATA-3, T-bet, IL-4R and IL-12R, and cytokine secretion (IL-2, IL-4, IL-5, IL-10, IL-13 and IFN- γ) in cord blood are associated with the development of allergic diseases and IgE sensitization later in life, i.e., by age 2 yr.

Methods

Study population

The study population comprised 98 infants participating in a clinical study on the role of probiotics in the prevention of allergic diseases between November 2000 and March 2003, at the Skin and Allergy Hospital of Helsinki University Central Hospital (18). Of 925 infants 98 were randomly selected from maternity hospitals in Helsinki to participate in this immunological study. Deliveries were normal (≥ 37 gestational weeks). All infants were at high genetic risk of allergy; at least one parent had a doctor-diagnosed allergic disease. Mothers were randomized according to a compu-

ter-generated block randomization to receive either two capsules a day of a mixture of four bacterial species [*Lactobacillus rhamnosus* GG (ATCC 53103) 5×10^9 colony-forming units (cfu), *L. rhamnosus* LC705 5×10^9 cfu, *Bifidobacterium breve* Bb99 2×10^8 cfu, and *Propionibacterium freudenreichii* ssp. *Shermanii* JS 2×10^9 cfu in a capsule] or placebo product (an inert microcrystalline cellulose). Their infants received the same capsules as mothers once a day, those given probiotics mixed with sugar syrup containing prebiotic galacto-oligosaccharides (0.8 g) and those in placebo group mixed inert cellulose with sugar syrup. Mothers received these products for 1 month before delivery, and their infants for the first 6 months. Products were supplied by Valio Ltd (Helsinki, Finland). The ethics committee of the Hospital for Children and Adolescents, University of Helsinki, approved the study protocol. An external evaluator made the statistical analyses between the probiotic and placebo groups, as the randomization code was not opened during this study.

Allergen skin prick testing

Skin prick tests (SPTs) were performed with commercial allergen extracts of egg white (Stallergenes, Antony, France); birch, timothy, cat, dog, (ALK-Abellø, Hørsholm, Denmark); fish (Stallergenes); and commercial cow milk and wheat grains (diluted in 0.9% NaCl) at an age of 2 yr according to the standard technique. Any mean wheal diameter ≥ 3 mm greater than the negative control was considered positive.

Specific IgE measurements

Plasma-specific IgE antibodies to egg white, cow milk, cat, dog, timothy and birch were measured in peripheral blood samples by immunoassay (ImmunoCAP® system, Pharmacia diagnostics, Uppsala, Sweden) at an age of 2 yr. A level ≥ 0.7 kU/l was taken as a sign of sensitization.

Clinical evaluation

At 2 yr, a pediatrician – blinded to group assignment – examined the children. Eczema, was defined as an itchy skin condition with dry skin and eczema on typical sites and food allergy as a positive open food challenge after an alleviation of symptoms during an elimination diet. Asthma was defined as two or more doctor-diagnosed wheezy episodes accompanied by persistent cough or exercise-induced symptoms occurring between these. Allergic rhinitis was defined as antigen-

specific sensitization to pollens or animal dander with a history of two or more symptoms (nasal discharge, blockage and sneeze/itch) recurrently during antigen contact. Children having one of the diseases listed above were considered to have an allergic disease ($n = 40$), and children with allergic disease and a positive SPT to any antigen tested or any specific IgE concentration ≥ 0.7 kU/l to have IgE-associated disease ($n = 19$). Children with a positive SPT to any antigen tested or any specific IgE concentration ≥ 0.7 kU/l at an age of 2 yr were considered to be IgE sensitized to allergen ($n = 32$). Those with neither IgE sensitization nor allergic disease were classified as non-sensitized, non-allergic children ($n = 39$) (18).

Sample collection and cell culture

Cord blood samples were collected from placental veins with venepuncture at birth into sterile sodium-heparinized tubes. CBMCs were separated by means of density centrifugation (Ficoll-Paque, Amersham Biosciences, Uppsala, Sweden). The CBMCs were suspended (10^6 cells/ml) in RPMI-1640 (Gibco, Paisley, Scotland) supplemented with 5% AB serum (SPR, Helsinki, Finland), and L-glutamine (2 mmol/l) (Gibco) and incubated (at 37°C and 5% CO₂) in 96-well cell-culture plates (Nunc, Roskilde, Denmark) (200 μ l per well) with phytohemagglutinin (PHA) (5 μ g/ml) (Sigma, St Louis, MO, USA), β -lactoglobulin (β -LG) (200 μ g/ml) (Sigma) or medium alone. The culture supernatants were collected after 72 h incubation and stored at -70°C for subsequent analyses.

ELISA

Quantities of IFN- γ and interleukin-5 (IL-5) in the PHA-, and β -LG-stimulated and non-stimulated culture supernatants were evaluated by means of an ELISA, as previously described (19). Sensitivities of the ELISAs were 8 pg/ml for IFN- γ , and 15.5 pg/ml for IL-5. IL-13 concentrations in the PHA stimulated; and non-stimulated culture supernatants were measured with a commercial human IL-13 ELISA Kit (Pelikine CompactTM, Amsterdam, the Netherlands) (detection limit 0.5 pg/ml). The assays were performed in duplicate, and the color intensity was measured with multiscan MS version 8.0 (Labsystems Oy, Helsinki, Finland).

Cytokine bead assay

Interleukin-2 (IL-2), IL-4, IL-6, IL-10, tumor necrosis factor- α (TNF- α) and IFN- γ concentra-

tions were measured by cytokine bead assay (CBA) from PHA-stimulated and non-stimulated CBMC supernatants. The cytokine capture beads were incubated with culture supernatants or standards and mixed with the PE-conjugated detection antibodies according to the manufacturer's instructions (BD Biosciences Pharmingen, San Diego, CA, USA). Cytokine concentrations were determined on a FACScan flow cytometer (CELLQUEST software, BD Biosciences Pharmingen) with the detection limit 2.6 pg/ml for IL-2 and IL-4, 3.0 pg/ml for IL-6, 2.8 pg/ml for IL-10 and TNF- α , and 7.1 pg/ml for IFN- γ . Results were analyzed with BD CBA analysis software MAC OS version 9.

Real-time (RT) PCR

The mRNA expression of GATA-3, T-Bet, IL-4R and IL-12R was detected by RT-PCR from PHA-stimulated and non-stimulated CBMCs. Total RNA was extracted from frozen (-70°C) lysed cells by the Total Gen Elute Mammalian RNA Kit (Sigma). A reverse transcription reaction was carried out in a final volume of 20 μ l by use of TaqMan Reverse Transcription reagents (Applied Biosystems, Foster City, CA, USA). The reaction mix contained 10x RT-buffer (2 μ l), 5.5 mmol/l MgCl₂ (4.4 μ l), 500 μ mol/l of each deoxyNTP (4 μ l), 2.5 μ mol/l Random Hexamers (1 μ l), 0.4 U/ μ l RNase-inhibitor (0.4 μ l), and 1.5 μ l RNase-free water. This solution was treated with 0.01 U/ μ l DNase (0.2 μ l) (Boehringer Mannheim, Mannheim, Germany). A Multi-scribe Reverse Transcriptase enzyme (1.25 U/ μ l, 0.5 μ l) was added, and the reverse transcription reaction was performed. cDNA was stored at -20°C. RT PCR was performed by automated fluorometer, ABI Prism 7700 Sequence Detection System (Applied Biosystems). Amplification was repeated for 50 cycles. FAM-labeled TaqMan Gene Expression Assays were used to measure transcription levels of the selected genes (T-bet, cat. no. Hs00203436_m1, GATA-3 cat. no. Hs00231122_m1, IL-12R cat. no. Hs00234651_m1 and IL-4R cat. no. Hs00166237_m1). Ribosomal 18S (cat. no. Hs99999901_s1) was served as the endogenous control. PCR reactions were run in triplicate wells with 5 ng of template cDNA/well (25 μ l). Expression of each cytokine was measured also from a calibrator cDNA [PHA (Sigma)-stimulated pooled peripheral blood mononuclear cells of healthy subjects]. The comparative Ct method served to quantitate the gene transcription in cord blood samples. Δ Ct (Ct of target gene - Ct

of 18S) value of the calibrator was subtracted from the ΔCt (Ct of target gene – Ct of 18S) values of the analyzed samples, and the difference was determined as the $\Delta\Delta Ct$ value. Results are expressed as relative units based on calculation of $2^{-\Delta\Delta Ct}$, which gives the relative amount of cytokine normalized to the endogenous control (18S) and compared with the calibrator.

Statistical analysis

The non-parametric Mann–Whitney *U*-test served for comparisons of variables, as no variable was normally distributed. The Spearman's rank correlation test served to analyze correlations between variables. All statistical tests were two-tailed. All distributions of continuous data were skewed; comparisons between the probiotic and placebo groups were made after logarithmic transformation, with an analysis of variance (ANOVA) used for the comparisons. The data in probiotic vs. placebo comparisons are presented with geometric means and 95% confidence intervals (CI). Data were analyzed with SPSS for Windows (version 10.0). Values of $P < 0.05$ were considered statistically significant.

Results

Transcription factors and IL-4- and IL-12 -receptors in CBMC

GATA-3 expression was higher in PHA-stimulated CBMC in children with IgE sensitization at an age of 2 yr than in the non-sensitized, non-allergic children ($p = 0.03$) (Table 1, Fig. 1). PHA-induced GATA-3 expression correlated positively with PHA-stimulated IL-5 ($p = 0.003$, $r = 0.300$) and IL-13 ($p = 0.007$, $r = 0.278$) secretion of CBMCs. GATA-3 expression in PHA-stimulated CBMC tended to be higher also in children with IgE-associated disease than in non-sensitized, non-allergic children ($p = 0.05$) (Table 1, Fig. 1). No differences in expression levels of T-bet, IL-4R or IL-12R in PHA-stimulated CBMC, however, appeared in

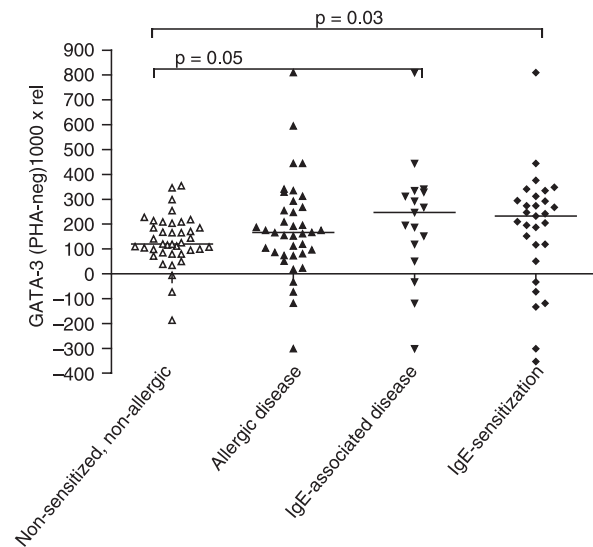


Fig. 1. Expression of transcription factor GATA-3 in PHA-stimulated cord blood mononuclear cells in children with allergic disease, IgE-associated disease, IgE sensitization and in non-sensitized, non-allergic children at an age of 2 yr. Results are presented as 1000× relative transcription (PHA stimulation–no stimulation). Horizontal lines show median values. Values of p by Mann–Whitney *U*-test.

IgE-sensitized children, or in children with IgE-associated disease when compared with levels in non-sensitized, non-allergic children (Table 1). When all children with any allergic disease were compared with non-sensitized, non-allergic children, no differences in CBMC GATA-3, T-bet, IL-4R or IL-12R expressions emerged (Table 1). Probiotic bacteria given to mothers had no effect on transcription factor GATA-3 on T-bet, on IL-12R, or on IL-4R expression of CBMC (data not shown).

Cytokine activation in CBMC

The IL-5 response of β -lactoglobulin-stimulated CBMC was higher in children with IgE sensitization at an age of 2 yr than in the non-sensitized, non-allergic children ($p = 0.013$) (Fig. 2); and also in PHA-stimulated CBMC cultures, IL-5 secretion tended to be higher in IgE-sensitized children than in non-sensitized,

Table 1. Transcription factors GATA-3, T-bet, IL-12R and IL4r in PHA-stimulated cord blood mononuclear cells

	<i>n</i>	GATA-3	<i>n</i>	T-bet	<i>n</i>	IL-12R	<i>n</i>	IL-4R
Allergic disease	38	166 (–302–809)	39	13 (–395–164)	39	266 (–1634–905)	39	226 (–306–455)
IgE-associated disease	17	247 (–302–809)*	17	13 (–395–48)	17	232 (–1634–905)	17	254 (–306–455)
IgE sensitization	29	233 (–353–809)*	30	11 (–395–287.9)	30	232 (–1634–905)	30	227 (–306–736)
Non-sensitized, non-allergic children	39	120 (–187–354)	39	15 (–149–106)	39	232 (–1483–1087)	39	201 (–7339–1335)

Results are presented as 1000× relative transcription (PHA stimulation–no stimulation). Medians (ranges). *n*, number of samples. * $p < 0.05$ in comparison with non-sensitized, non-allergic children.

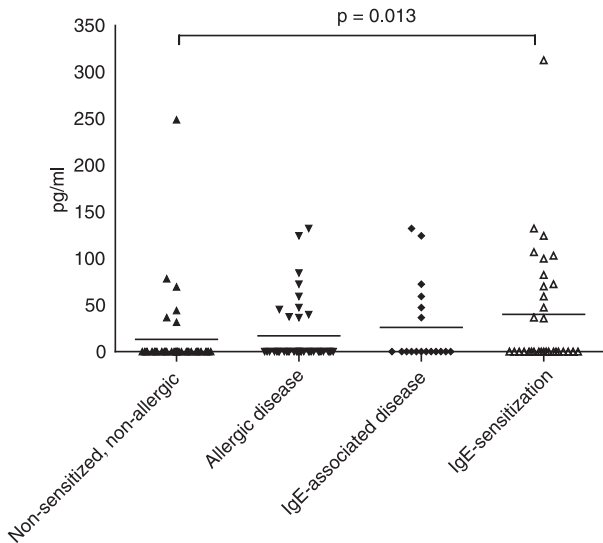


Fig. 2. Secretion of IL-5 in β -lactoglobulin-stimulated cord blood mononuclear cells in children with allergic disease, IgE-associated disease, IgE sensitization and in non-sensitized, non-allergic children at an age of 2 yr. Horizontal lines show mean values. Values of p by Mann–Whitney U -test.

non-allergic children ($p = 0.073$) (Table 2). Neither IL-5, IFN- γ secretion in β -lactoglobulin nor PHA-stimulated CBMC differed between allergic children or between children with IgE-associated disease and non-sensitized, non-allergic children (Table 2, Figs 2 and 3). Neither did IFN- γ response differ between children IgE sensitized or not (Fig. 3). IL-2 and IL-5 secretion in PHA-stimulated CBMC correlated positively ($p = 0.004$, $r = 0.317$). IL-2 secretion of PHA-stimulated CBMC was higher IgE sensitized than non-sensitized, non-allergic children ($p = 0.026$) (Table 2), whereas no difference emerged between allergic children or children with IgE-associated disease when compared with non-sensitized, non-allergic children (Table 2). Neither IL-4, IL-10 nor IL-13 secretion in PHA-stimulated CBMC differed between allergic or IgE-sensitized infants, or infants with IgE-associated disease when compared with non-sensitized, non-allergic children (Table 2). IL-13 and

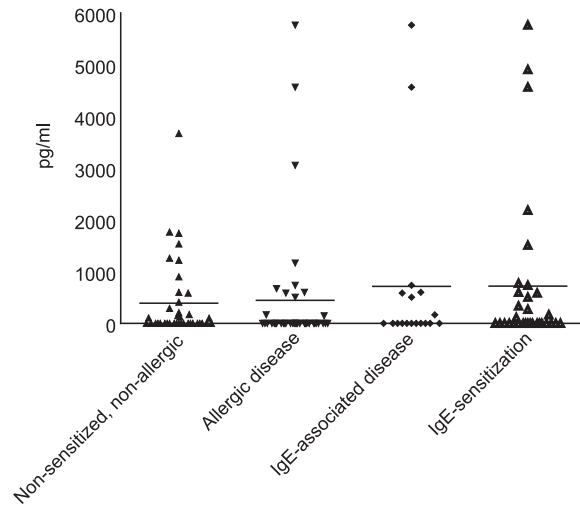


Fig. 3. Secretion of IFN- γ in β -lactoglobulin-stimulated cord blood mononuclear cells in children with allergic disease, IgE-associated disease, IgE sensitization and in non-sensitized, non-allergic children at an age of 2 yr. Horizontal lines show mean values.

IL-5 secretion in PHA-stimulated CBMC did correlate ($p = 0.000$, $r = 0.49$). Probiotic bacteria given to mothers had no effect on IL-2, IL-4, IL-5, IL-10, IL-13 or IFN- γ secretion of CBMC (data not shown).

In CBA measurements, concentrations of IFN- γ , IL-6 and TNF- α in undiluted samples were above the detection range, while IL-2, IL-4 and IL-10 were at detectable levels. Use of diluted samples is not recommended due to non-reliable results; and IFN- γ was thus studied by an ELISA.

Discussion

These results reveal that the pattern of immunological response of CBMC is an intrinsic feature of the newborn infant, and a pronounced Th2-biased responsiveness at birth predisposes for development of IgE sensitization to environmental antigens later in life. Our results are in agreement with those findings showing immuno-

Table 2. γ -interferon, IL-2, IL-13, IL-10, IL-5 and IL-4 secretion in PHA-stimulated cord blood mononuclear cells (PHA-stimulated–unstimulated CBMC)

	n	Allergic disease	n	IgE-associated disease	n	IgE sensitization	n	Non-sensitized, non-allergic children
IL-2	19/31	8 (0–62)	13/16	13 (0–62)	25/28	15 (0–517)*	25/32	10 (0–698)
IL-4	31/31	1506 (700–2884)	16/16	1598 (789–2427)	28/28	1503 (789–2427)	31/31	1654 (1119–2373)
IL-5	32/40	115 (0–3689)	15/18	117 (0–3689)	27/31	143 (0–3689)**	26/39	70 (0–915)
IL-10	31/31	267 (30–831)	15/15	270 (56–831)	27/27	294 (56–986)	32/32	348 (40–1072)
IL-13	40/40	4851 (174–26,584)	18/18	4677 (174–9352)	31/31	6757 (274–26,584)	39/39	4231 (115–19,430)
IFN- γ	39/40	7741 (0–318,345)	18/18	14,572 (111–299,284)	31/31	16,200 (644–299,284)	38/39	21,066 (0–630,509)

Values are represented as medians (ranges) pg/ml. n , number of samples above detection limit/total number of samples. * $p < 0.05$ in comparison with non-sensitized, non-allergic children. ** $p = 0.07$ in comparison with non-sensitized, non-allergic children.

logical events driving T helper development at cytokine level as being already initiated during the fetal period (20). Our results indicate, however, that the Th2 skewing which occurs *in utero* is mediated by an increased expression of the Th2-associated transcription factor GATA-3 as a response to stimulation, and the expression of GATA-3 further correlated with IL-5 secretion of stimulated CBMC.

In the course of pathologic conditions such as atopic diseases, polarized Th2 responses are found to be critical to the outcome of the disease (15–17). The transcription factor GATA-3 is selectively expressed in Th2 cells, is expressed at a high level in naive, freshly activated T cells, but subsides to a minimal level in Th1 lineage cells (12). Mice expressing a dominant-negative mutant of GATA-3 have reduced levels of IL-4, IL-5 and IL-13. Furthermore, the key features of asthma, such as airway eosinophilia, mucus production and IgE synthesis, were greatly attenuated in the transgenic mice (13).

GATA-3 activates and stabilizes the expression of IL-4, IL-5 and IL-13 in T cells (11, 12, 21, 22). IL-4 and IL-13 induce B-cell switching to IgE production (15, 22). Interestingly, ectopic expression of GATA-3 is sufficient to drive IL-5 but not IL-4 gene expression (21, 23). GATA-3 regulates directly IL-5 and IL-13 expression, but the regulation for IL-4 is an indirect increase in the transactivation of the promoter region of IL-4 (24). GATA-3 deletion limits the growth of Th2 cells but not Th1 cells, and deletion of GATA-3 from established Th2 cells abolishes IL-5 and IL-13 but not IL-4 production (25). Our results of increased GATA-3 and IL-5 but not IL-4 activation in mitogen-induced CBMC in infants with IgE sensitization at an age of 2 yr are in good agreement with these experimental findings.

Interleukin-5 is a cytokine closely associated with eosinophil biology. It promotes eosinophilic inflammation in allergies by regulating the proliferation, differentiation and activation of eosinophils (15, 16). In the present study, IL-5 secretion in addition to mitogen-stimulated CBMC was increased in β -lactoglobulin-stimulated CBMC in infants with IgE sensitization at an age of 2 yr. Secretion of IL-5 and IL-2, cytokine induced by T-cell activation in CBMC cultures correlated positively. IL-5 secretion of stimulated CBMC did not differ between allergic or non-sensitized, non-allergic infants at an age of 2 yr. Results suggest that the primary defect in T cells favors Th2 skewing and activates mechanisms predisposing IgE sensitization, but does not necessarily have an effect on the development of allergic disease. We also found a positive

correlation of GATA-3 expression with IL-5 and IL-13, but not IL-4 secretion of the PHA-stimulated CBMC. That we found no positive correlation of secreted IL-4 in PHA-stimulated CBMC with other secreted Th2-type cytokines (IL-5, IL-10 or IL-13) is a further indication that regulation of IL-4, IL-5 and IL-13 activation is directed by different pathways. Our findings of the priming of GATA-3 and IL-5 pathway *in utero* suggests a primary role for eosinophils in the IgE sensitization.

The Th-2-type transcription factor GATA-3 expression of CBMC and Th2-type cytokine IL-5 secretion were higher in children who developed IgE-associated immune responses when compared with non-sensitized, non-allergic children, but expression of GATA-3 or secretion of IL-5 in CBMC did not differ between children with allergic diseases by age 2 yr when compared with non-sensitized, non-allergic children. This suggests that environmental factors such as colonization with microbes, infections and other environmental factors may have a great impact on the development of allergic symptoms (26). Probiotic bacteria, defined as live microorganisms administered in adequate amounts that confer a health effect on the host (27), seem to have a beneficial effect in diseases with failure of tolerance development (28, 29). In an environment highly infested with parasites causing high IgE levels, sensitization to allergens is common, but allergic symptoms rarely develop, possibly due to the development of regulatory mechanisms (26). Nematode infestation in mice induces such cells and suppresses allergic inflammation (30). In our study, a short bacterial supplementation during the last month of pregnancy did not affect the immune responses of infants. It seems likely that probiotic bacteria affect the immune system of the infant after delivery, when they are administered directly to infants, and intestinal colonization takes place.

T-bet, a member of the T-box family of transcription factors that is a master regulator of Th1 lineage commitment (14), controls the expression of the hallmark Th1 cytokine, IFN- γ (14). IL-12, a cytokine produced by dendritic cells, monocyte-macrophages, and other antigen-presenting cells, is a strong inducer of Th1 responses such as IFN- γ -mediated reactions. It acts by binding to its receptor (IL-12R) that is expressed in activated T cells and NK cells. In the present study, the Th1-type markers, the transcription factor T-bet, IL-12R or secreted IFN- γ in PHA-stimulated CBMC did not differ significantly between the groups. There are several

reports on the predictive value of decreased IFN- γ and IL-12 secretion by CBMC for atopy (20), but we could not confirm these findings although increased GATA-3 activation may in turn down-regulate Th1 responses. Children with IgE sensitization at an age of 2 yr showed an increased IL-2 response by PHA-stimulated CBMC compared to non-sensitized, non-allergic children. IL-2 is secreted by all activated T-cells. Higher IL-2 secretion of mitogen-stimulated CBMC reflected more pronounced Th2-type responses to the PHA stimulation, T-cell activation demonstrated as in children with IgE sensitization at an age of 2 yr.

We conclude that priming of the immune system towards a Th2-type response occurs *in utero*. Those children who developed IgE sensitization to environmental antigens during the first years of life, already showed increased up-regulation of transcription factor GATA-3 and IL-5 secretion at birth as a response to stimulation, suggesting that the primary defect in T cells predisposing to atopic phenotype directly favors Th2 skewing.

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