

Original article

T cell reactivity in neonates from an East and a West German city – results of the LISA study

Background: Within an ongoing birth cohort study (LISA) the cytokine production of cord blood T cells was compared between neonates from Leipzig (East Germany) and Munich (West Germany). The aim of this study was to analyse regional differences and influencing factors of the immune status.

Methods: Cytokine production was measured in a randomly selected subgroup of 158 children from the LISA (Life style – Immune system – Allergy) cohort by intracellular cytokine staining. Information on family “atopy” history (FAH) and home characteristics was obtained from questionnaires.

Results: Reduced numbers of interferon- γ (IFN- γ) and tumor necrosis factor- α (TNF- α) producing T cells were found in association with biparental FAH and housing renovation during pregnancy. In addition, cytokine production was influenced by season. In Munich, the frequency of biparental FAH and of renovation measures during pregnancy was significantly higher as compared to Leipzig. Neonates from Munich showed significantly decreased amounts of IFN- γ and TNF- α and elevated levels of interleukin-4 (IL-4) producing T cells. Differences in cytokine production between Munich and Leipzig were influenced by season (IL-4) and housing renovation (IFN- γ , TNF- α).

Conclusions: Since differences in the T cell cytokine production of neonates in Munich and Leipzig are independent from FAH our findings may provide evidence for the impact of environmental factors upon the fetal immune system.

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Higher prevalence of asthma and allergies has been reported among children (1, 2) and adults (3) in western Germany compared to eastern Germany. As a possible reason, a relationship between lifestyle and the development of allergic disease has been discussed (4, 5). During the 10 years since reunification living conditions in the east of Germany have changed greatly. A converging tendency of allergy prevalence in children in East and West Germany was found in some studies (6, 7) but not in all (8, 9).

Currently we are performing a prospective cohort study (“LISA”: Life style – Immune system – Allergy) in Munich (West Germany) and Leipzig (East Germany) to investigate the influence of lifestyle related

factors on the development of the immune system in early childhood (10). Furthermore, we will investigate whether or not East–West differences in the prevalence of allergies in children still exist 10 years after German reunification.

The balance between interleukin (IL)-4 producing type 2 T cells and interferon- γ (IFN- γ) producing type 1 T cells regulates IgE synthesis. IgE-mediated allergy is characterized by the predominance of type 2 cells. It has been shown that neonates with a familial risk of IgE-mediated allergy (30) have a significantly decreased IFN- γ production (11) and that reduced IFN- γ production at birth is correlated with the occurrence of allergic manifestations in later childhood

(12). Thus, a reduced secretion of IFN- γ at birth is discussed as a further risk factor for the development of IgE-mediated disorders in childhood (11–13).

Therefore, we investigated the production of cytokines involved in IgE synthesis by cord blood T cells. To characterize the balance of type 1 and type 2 T cells in neonates IL-4 and IFN- γ were analysed using the method of intracellular cytokine staining. TNF- α was measured as a supplemental type 1 cytokine. In addition to cord blood IgE and family atopy history (FAH) the cytokine secretion profile of cord blood T cells was used to describe the atopic constitution of the newborn children.

The aim of this study was to compare the immune status of neonates from Leipzig (East Germany) and Munich (West Germany), in particular the cytokine secretion profile of cord blood T cells, a possible predictor for allergic disease. Furthermore, we wanted to analyse possible influencing factors and regional differences between the atopic states of children at the time of birth.

Subjects and Methods

Subjects

For a prospective cohort study (LISA), 2443 neonates born between December 1997 and January 1999 were recruited in Leipzig ($n=976$) and Munich ($n=1467$). Only healthy term neonates of German descent were included. Newborn children whose mothers suffered from autoimmune disease or infectious disorders during pregnancy were excluded. The participation rate was 64% in Leipzig and 58% in Munich. It was only possible to obtain cord blood from 1553 children (64% of the cohort). Cord blood specimens were randomly selected for cytokine analysis. Due to logistic reasons, only 45 blood specimens of children in Munich were obtained for cytokine analysis in contrast to 113 in Leipzig. In Leipzig, cytokine measurements were performed in children born between December 1997 and January 1999, whereas in Munich, cytokines were measured in children born between May 1998 and January 1999. Participation in the study was voluntary. Informed consent was obtained from the parents of all children. The study was approved by the Ethics Committees of the University of Leipzig and the Bavarian Medical Association.

Measurement of T cell function

Cord blood T cell function was analysed by intracellular cytokine staining following an established method published elsewhere (14, 15). Briefly, cord blood cells were stimulated within 6 h after delivery with phorbol ester (PMA) 10 ng/ml (Sigma Aldrich, Deisenhofen, Germany) and ionomycin 1 μ M (Calbiochem, Bad Soden, Germany) in the presence of monensin 2.5 μ M (Sigma Aldrich, Deisenhofen, Germany) for 5 h at 37°C and 5% CO₂. Stimulated cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% saponin. Specific monoclonal antibodies (mAb) against the human cytokines IFN- γ , TNF- α , IL-2 and IL-4 and isotype controls were obtained from Beckmann-Coulter (Krefeld, Germany). The phenotypes of cytokine producing cells were simultaneously identified by mAb against T cell surface markers (CD3, CD4, CD8), obtained by Beckmann-Coulter. Fluorescence-labeled cells were analysed on a flowcytometer (FACScan, Becton Dickinson, Heidelberg, Germany) using CELLQUEST software; 10 000 cells were collected. Results are percentages of cytokine producing cells of the respective cell population.

To provide comparability all cytokine samples were finally analysed in the same laboratory (University of Leipzig). Blood samples of the Munich children were stimulated and fixed in Munich and then shipped to the cytokine detection laboratory overnight.

IgE detection

Serum samples were stored at -20°C until further processing for IgE measurements. Cord blood IgE levels were determined by the Pharmacia system (Pharmacia, Uppsala, Sweden) (neonate IgE kit) following the manufacturer's instructions.

Family atopy history (FAH)

The atopic constitution was defined on the basis of FAH and was classified according to parental report of IgE-mediated disease in self-administered questionnaires. In detail, at least one of the following criteria was used to determine the presence of IgE-mediated disease: ever asthma; hay fever or the atopic eczema/dermatitis syndrome (AEDS) of either mother or father. Single positive FAH resulted from IgE-mediated allergy of either mother or father, double positive of both parents.

Environmental factors

Information regarding home characteristics and traffic burden were obtained from questionnaires.

Statistical analysis

Statistical analysis of data was performed using the soft-ware STATISTICA (Version 5). Measurements were given as percentages of cytokine producing cord blood T cells. Due to their binomial nature, a logistic transformation was applied to the cytokine data. The linear regression model was based on the following covariates: age of mother; house renovation during pregnancy; $\sin(2\pi d/365)$ and $\cos(2\pi d/365)$ with d as the day of birth in order to model the annual cycle with possible phase shift; dummy variables were used to model the effect of the place of birth (study centre: 1 = Munich, 0 = Leipzig), as well as single positive and double positive FAH. Additionally, differences between groups were tested with the non-parametric Mann-Whitney Rank Sum Test with an entry criterion of $p < 0.05$. Homogeneity between groups was tested by χ^2 test, Mann-Whitney Test and Student's t -test, as appropriate. Correlation between cytokines was tested using the non-parametric Spearman Rank Correlation Test.

Results

Within the subgroup of 158 children with cytokine measurements no significant difference was found regarding elevated cord blood IgE levels equal to or above 0.9 kU/L ($P=0.966$) between Munich and Leipzig. However, the frequency of negative and double positive FAH differed significantly between the Munich and the Leipzig cytokine study populations (see Table 1). In Munich, the frequency of double positive FAH was significantly higher as compared to Leipzig. No correlation was found between cord blood IgE and FAH (data not shown). The median age of Munich mothers was significantly higher than that of Leipzig mothers. No correlation between cytokine producing T cells and age of mother was found in either group (data not shown).

Concerning gender, no differences were found between Leipzig and Munich (Table 1). The amount of IFN- γ , TNF- α or IL-4 producing T cells was not significantly influenced by gender (all $P > 0.05$).

T lymphocytes from neonates with a double positive FAH showed a significantly lower PMA/ionomycin induced IFN- γ production (medians: 2.1% vs. 3.9%) and TNF- α (medians: 9.1% vs. 13.2%) than T cells from infants without a familial risk of IgE-mediated allergies. There was also a significant difference in the type 1 cytokine production of T cells between neonates with a single and those with a double positive FAH. Comparison of the single positive and the negative family history groups did not reach significance (Fig. 1). Although IL-4 tended to be higher in the group with double positive FAH (double positive: 1.1%, negative: 0.8%), no significant differences were detectable in IL-4 production. There were no differences in the cytokine profiles when maternal and paternal single FAH were compared (all $P > 0.05$). However, when ratios of IFN- γ /IL-4 and TNF- α /IL-4 were calculated – due to the opposing trends of type 1 and type 2 cytokines – all differences between negative or single positive and double positive history were significant (all $P < 0.05$). A correlation between cord blood IgE and cytokine producing cells was not evident ($P = 0.22$ for IFN- γ , $P = 0.56$ for TNF- α , $P = 0.87$ for IL-4 producing CD3⁺ T cells).

The cytokine production of cord blood T cells differed significantly between Leipzig and Munich

regarding IFN- γ , TNF- α and IL-4 producing T cells (Table 2). Neonates from Munich showed decreased amounts of IFN- γ and TNF- α producing T cells and elevated levels of IL-4 producing T cells. This effect was seen in the CD3⁺ total T cell population as well as in the CD4⁺ (helper) and the CD8⁺ (cytotoxic) T cell subpopulations. With regard to ratios of IFN- γ /IL-4 and TNF- α /IL-4 all P -values were found to be < 0.001 (data not shown).

There was a strong positive correlation between the type 1 cytokines IFN- γ and TNF- α ($r = 0.47$, $P < 0.0001$), but neither a positive nor a negative correlation between IFN- γ or TNF- α and IL-4.

Apart from the influence of FAH on the neonatal T cell function, additional seasonal variations in cord blood T cell cytokine production levels were observed. As shown in Fig. 2, IL-4 producing T cells reached highest levels in the summer (July/August). The IFN- γ and TNF- α production was lower during this period. This effect was observed in the Leipzig as well as in the Munich study population.

As shown before, the cytokine production of cord blood T cells was affected by season, place of birth and by FAH. After adjustment for season, mother's age and FAH, the differences in cytokine production between Leipzig and Munich remained significant for CD3⁺/CD4⁺ IFN- γ producing cells and CD3⁺, CD3⁺/CD4⁺ and CD3⁺/CD8⁺ TNF- α producing cells. Even though the Munich children were relatively over-represented in the positive FAH group the

Table 1. Characterization of the subgroup of 158 children with cytokine measurements: Comparison between Leipzig and Munich concerning homogeneity of the groups

	Leipzig (East Germany)	Munich (West Germany)	<i>P</i> -value
Family atopy history	<i>n</i> = 113	<i>n</i> = 45	$< 0.001^*$
Negative	71 (62.8%)	18 (40.0%)	
Single positive	37 (32.7%)	17 (37.8%)	
Double positive	5 (4.4%)	10 (22.2%)	
Cord blood-IgE [kU/l]	<i>n</i> = 110	<i>n</i> = 33	0.97*
Normal (< 0.9)	93 (84.5%)	28 (84.8%)	
Elevated (≥ 0.9)	17 (15.5%)	5 (15.2%)	
Gender	<i>n</i> = 113	<i>n</i> = 45	0.78*
Male	60 (53.1%)	25 (55.6%)	
Female	53 (46.9%)	20 (44.4%)	
Birth weight [g]	<i>n</i> = 113	<i>n</i> = 45	0.89***
Mean	3493	3482	
Gestational age [weeks]	<i>n</i> = 111	<i>n</i> = 44	0.19**
Median	40.0	40.0	
Apgar score (10 min)	<i>n</i> = 108	<i>n</i> = 45	0.06**
Median	10.0	10.0	
Cord blood pH	<i>n</i> = 105	<i>n</i> = 44	0.98***
Mean	7.29	7.29	
Mode of birth	<i>n</i> = 109	<i>n</i> = 44	0.07*
Caesarian section	18 (16.5%)	13 (29.5%)	
Other	91 (83.5%)	31 (70.5%)	
Age of mother [years]	<i>n</i> = 113	<i>n</i> = 45	$< 0.001^{**}$
Median	29.0	33.0	

Homogeneity was tested by appropriate tests: * χ -square; **Mann–Whitney U-test and ***Student's t-test as appropriate. *P*-values are given.

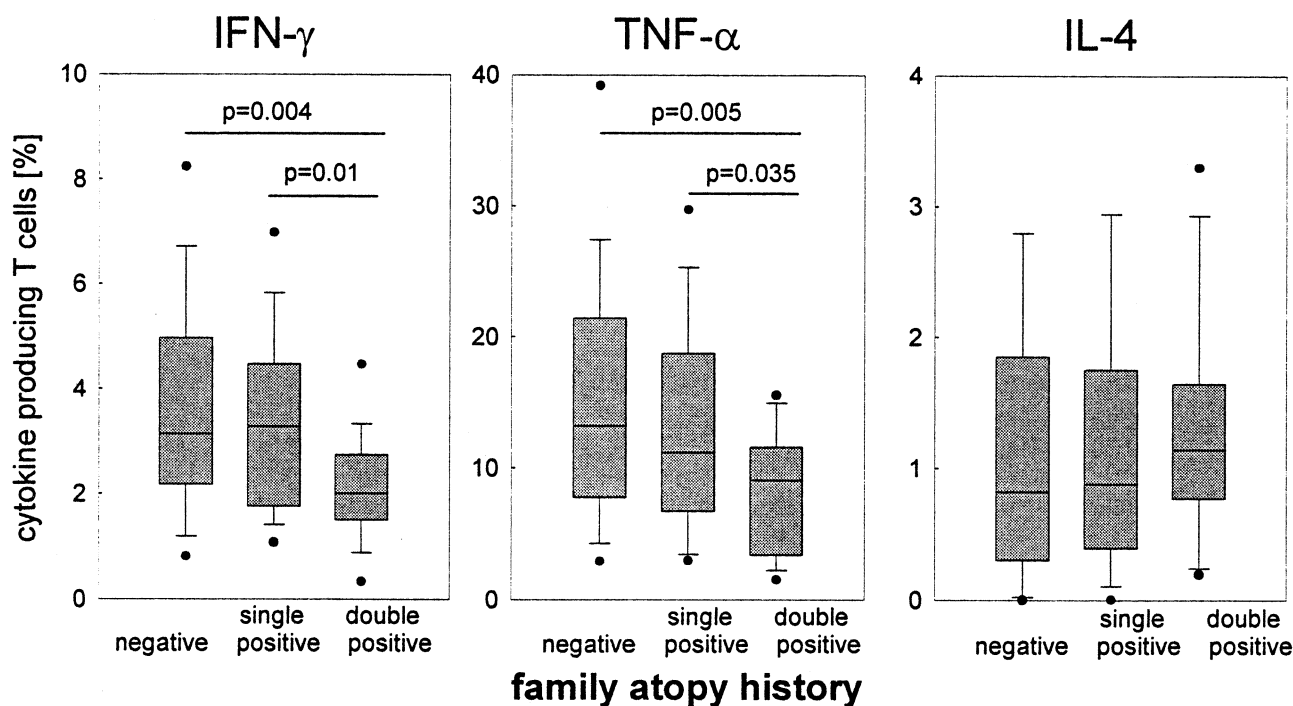


Figure 1. Cytokine production of cord blood T cells regarding FAH. Box plots represent medians, interquartile ranges and min/max deviation of percentages of cytokine producing cells for the respective cell population. Significant differences between infants with double positive FAH and those with single positive and negative FAH, respectively, are marked by indicating the P-values.

decreased amounts of IFN- γ and TNF- α producing T cells in children with biparental heredity were still significant after adjustment for place of birth and the other factors mentioned above within a linear regression model (Table 2).

In addition, it was investigated whether environmental factors may explain the differences in the cytokine secretion profile between Munich and Leipzig children. Detailed questionnaires were used to obtain information on home characteristics. As shown in Table 3 indoor conditions differed between Munich and Leipzig only concerning housing renovation activities during pregnancy. Moreover, children whose parents reported about renovation activities during their pregnancy had significantly reduced amounts of CD4⁺ IFN- γ producing and CD3⁺, CD4⁺, and CD8⁺ TNF- α producing cord blood T cells. CD3⁺ and CD8⁺ IFN- γ producing T cells tended to be reduced also (Fig. 3). IL-4 producing T cells were not significantly affected. When renovation during the pregnancy was introduced into the multiple regression analysis the IFN- γ and TNF- α producing T cells (with the exception of CD8⁺ T cells) were no longer associated with the study area (Table 2). Renovation during pregnancy is associated with reduced amounts of type 1 cytokine producing T cells, and the higher frequency of renovation activities during pregnancy in Munich contributed substantially to the reduced amounts of type 1 cytokine producing cells in this study area.

Discussion

In accordance with other authors, we found that IFN- γ production was markedly reduced in neonates with biparental atopic predisposition (11, 16). Similarly, the number of TNF- α producing T cells was decreased in these infants. Thus neonates at high risk of IgE sensitization are deficient in their overall capacity to produce type 1 cytokines. Interestingly, IL-4 production was not increased. However, decreased numbers of type 1 cytokine (IFN- γ and TNF- α) producing T cells

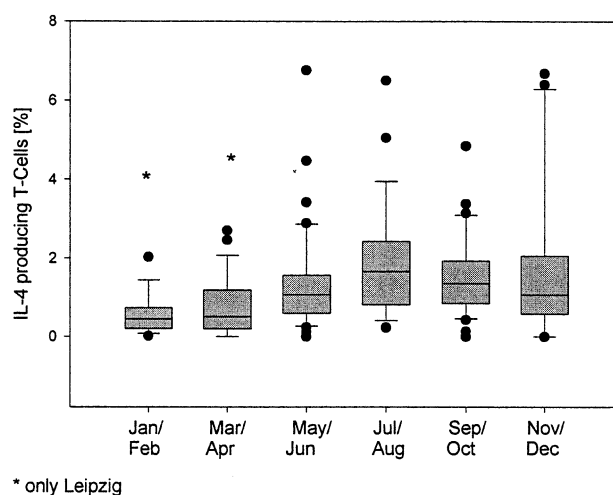


Figure 2. IL-4 producing cord blood T cells subject to season. The raw data of the Leipzig and the Munich newborn children are included.

Table 2. Influence of place of birth and double positive family atopy history (FAH) on proportions of cytokine producing cord blood T cell populations. The raw cytokine data are shown as medians and interquartile ranges (IQR) of cytokine producing proportions of the respective cell population. In addition, results of linear regression models are shown

Cytokine [T cell population]	Place of birth					Family atopy history				
	Leipzig n=113	Munich n=45	P*	P**	P***	Negative or single n=143	Double n=15	P*	P**	P***
Interferon-γ [CD3 ⁺]	3.27 (2.11–4.96)	2.78 (1.74–3.36)	0.01	0.48	0.87	3.16 (2.04–4.81)	2.01 (1.45–2.78)	0.004	0.02	0.01
[CD3 ⁺ /CD4 ⁺]	1.34 (0.66–2.07)	0.82 (0.22–1.38)	0.005	0.02	0.08	1.12 (0.59–1.87)	0.87 (0.40–1.38)	0.09	0.57	0.46
[CD3 ⁺ /CD8 ⁺]	7.33 (4.23–11.1)	3.74 (1.81–6.64)	<0.001	0.05	0.26	6.71 (3.68–10.7)	3.35 (1.81–3.77)	0.003	0.02	0.01
Tumor necrosis factor-α [CD3 ⁺]	13.35 (8.76–21.88)	7.95 (4.36–12.95)	<0.001	0.006	0.14	12.79 (7.55–20.37)	9.09 (3.04–12.07)	0.008	0.04	0.01
[CD3 ⁺ /CD4 ⁺]	15.90 (9.07–24.67)	8.45 (5.14–12.65)	<0.001	0.005	0.09	13.79 (7.73–23.06)	8.88 (3.44–11.04)	0.009	0.1	0.04
[CD3 ⁺ /CD8 ⁺]	8.64 (4.49–12.46)	3.83 (1.20–9.95)	<0.001	<0.001	0.01	7.70 (3.87–11.63)	3.79 (1.97–7.69)	0.07	0.92	0.92
Interleukin-4 [CD3 ⁺]	0.77 (0.28–1.69)	1.33 (0.69–1.95)	0.01	0.54	0.48	0.84 (0.33–1.81)	1.14 (0.71–1.71)	0.41	0.71	0.71
[CD3 ⁺ /CD4 ⁺]	0.71 (0.36–1.53)	1.41 (0.72–2.41)	0.003	0.29	0.34	0.76 (0.39–1.80)	1.12 (0.66–2.39)	0.31	0.71	0.67
[CD3 ⁺ /CD8 ⁺]	0.83 (0.21–2.26)	1.52 (0.61–2.43)	0.03	0.14	0.19	1.01 (0.33–2.32)	1.26 (0.13–2.14)	0.86	0.43	0.52

*P-values resulting from the Rank Sum Test (raw data).

**P-values resulting from the linear regression model adjusted for place of birth, FAH, season and age of mother.

*** P-values resulting from the linear regression model adjusted for renovation measures during pregnancy, place of birth, FAH, season and age of mother.

point to a shifted immune response towards the type 2 phenotype. It is possible that this shift might contribute to a higher susceptibility to subsequent allergic disease. In fact, it has been demonstrated that reduced IFN- γ secretion at birth is associated with the development of allergic symptoms in early childhood (12, 17).

In 1992 prevalence rates of allergic sensitization, allergic rhinitis and asthma in subjects aged 20–44 years were found to be almost twice as high in West Germany

than in East Germany (3, 18). Since IgE-mediated disease in parents thus can be expected to be more prevalent in Munich we found positive FAH more frequently in Munich than in Leipzig.

Previous studies have suggested cord blood IgE as a useful predictor for IgE-mediated disorders in infancy (19–21). During the last few years, however, the predictive potential of cord blood IgE has been questioned (22–25). In agreement with reports from other groups, we were unable to show that cord blood IgE is associated with FAH or with cytokine secretion of neonatal T cells (11, 12). These findings suggest that in addition to FAH the type 1 cytokine secretion of T cells, which can be performed at birth, might be a better indicator of an atopic constitution than cord blood IgE.

The differences in type 1 T cells between the Munich and Leipzig study populations remained significant after adjustment for FAH and seasonal variations. Therefore, it can be assumed that antenatal environmental factors must have affected the neonatal T cell function.

An association between reduced amounts of type 1 cytokine producing T cells and housing renovation during pregnancy was observed. As shown in previous studies, renovation activities are associated with elevated indoor concentrations of volatile organic compounds (VOC) (26–28). Recently we were able to show changes in the cytokine secretion profile of

Table 3. Comparison between Leipzig and Munich concerning home characteristics, traffic burden, and house renovation activities. Only children with cytokine measurements are included

	Leipzig East Germany	Munich West Germany	P – value (χ^2 -test)
Traffic burden	n=102	n=44	0.70
Apartments on main road	27(26.5%)	13(29.5%)	
Dampness and/or visible mould	n=104	n=44	0.99
	33(31.7%)	14(31.8%)	
Cooking with gas	n=104	n=44	0.085
	15(14.4%)	2(4.5%)	
Restoration since 1990			
House	n=103	n=42	0.23
	53(51.5%)	17(40.5%)	
Apartment	n=101	n=43	0.08
	56(55.4%)	17(39.5%)	
Renovation during pregnancy			
Painting, flooring, new furniture	n=95	n=43	0.014
	52(54.7%)	33(76.7%)	

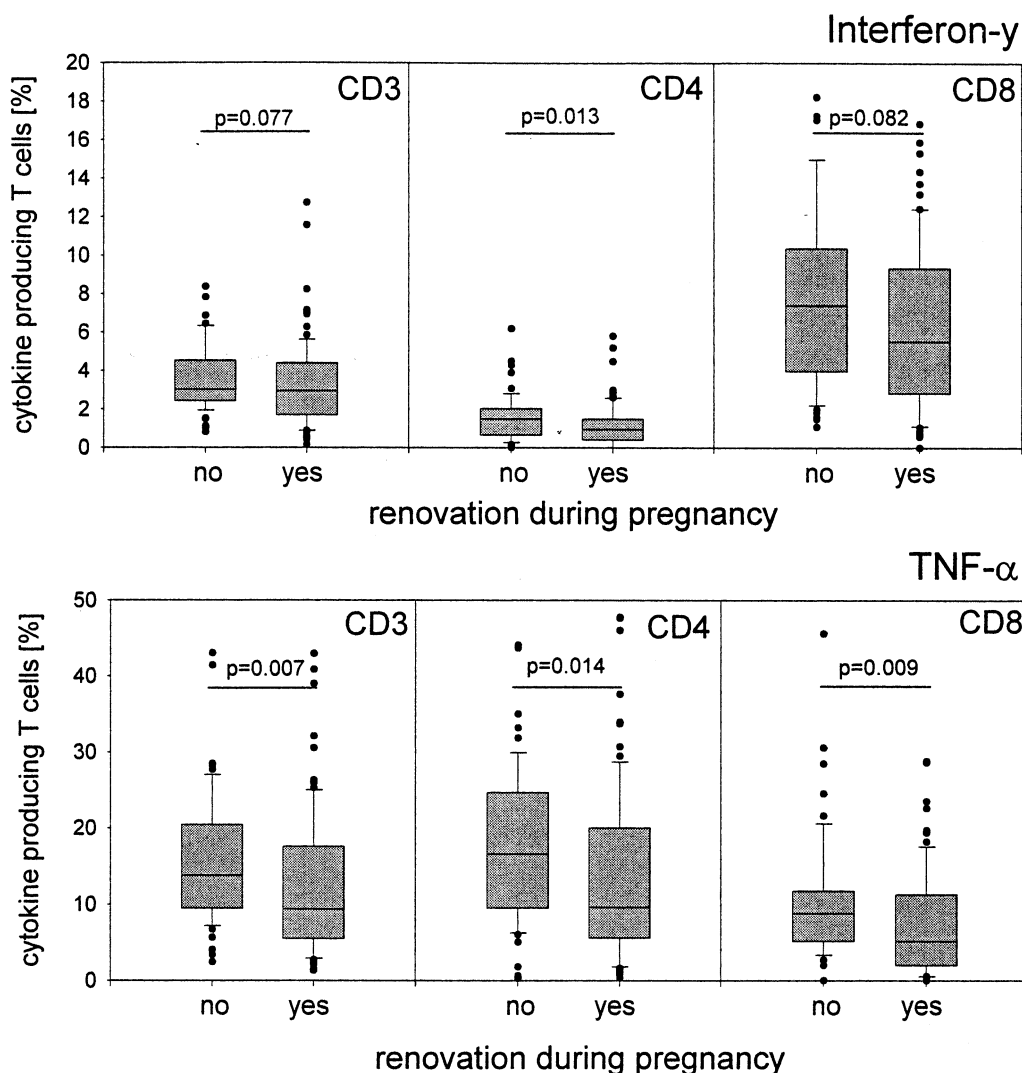


Figure 3. Influence of housing renovation during pregnancy on cytokine producing cord blood T cells ($n=158$). Renovation includes painting, flooring and new furniture. Box plots represent medians, interquartile ranges and min/max deviation of percentages of cytokine producing cells for the respective cell population. P -values from the U -test are given.

peripheral T cells toward a type 2 phenotype associated with exposure to VOC, leading to IgE sensitization in children aged 3 years (29). We suggest that maternal exposure to VOC in relation to renovation may influence the immune system of the fetus. Interestingly, the frequency of renovation activities was higher in the Munich study population. When renovation was introduced into the multiple regression model the influence of the study area on type 1 cytokine producing T cells was no longer significant (with exception for $CD8^+$ TNF- α producing T cells). Thus, apart from genetic influences, environmental factors have contributed to the observed differences between children from Munich and Leipzig in their capacity to produce cytokines.

In addition, environmental factors such as chemical indoor air pollutants could be partly responsible for

seasonal variations in cytokine production. Within the LISA study, we observed associations between elevated amounts of VOC in indoor air and altered amounts of cytokine producing cord blood T cells (manuscript submitted). Moreover, indoor VOC concentrations and cytokine producing cord blood cells show comparable seasonal variations.

At present, we do not know whether the impaired capability of the Munich neonates to produce IFN- γ and TNF- α and their slightly elevated IL-4 production are of clinical relevance. With regard to the reported association between double positive FAH or reduced IFN- γ at birth and the development of allergic manifestations in infancy our findings may point to a higher prevalence of IgE-mediated allergy in Munich children.

Within the LISA study the development of IgE

sensitization and clinical manifestations of allergy during the first two years of the children's lives will be investigated. Therefore, the LISA study will try to determine whether or not the observed differences in the cytokine profile between the Munich and Leipzig neonates indicate an increased susceptibility to allergic disorders in the infants from Munich.

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References

- VON MUTIUS E, MARTINEZ FD, FRITZSCH C, NICOLAI T, ROELL G, THIEMANN HH. Prevalence of asthma and atopy in two areas of West and East Germany. *Am J Respir Crit Care Med* 1994;**149**:358–364.
- TREPKA MJ, HEINRICH J, WICHMANN HE. The epidemiology of atopic disease in Germany. An East-West Comparison. *Rev Environ Health* 1996;**11**:119–131.
- NOWAK D, HEINRICH J, JORRES R, et al. Prevalence of respiratory symptoms, bronchial hyperresponsiveness and atopy among adults: west and east. Germany *Eur Respir J* 1996;**9**:2541–2552.
- WICHMANN HE. Possible explanation for the different trends of asthma and allergy in East and West Germany. *Clin Exp Allergy* 1996;**26**:621–623.
- NOWAK D, WICHMANN HE, MAGNUSSEN H. Asthma and atopy in Western and Eastern communities - current status and open questions. *Clin Exp Allergy* 1998;**28**:1043–1046.
- VON MUTIUS E, WEILAND SK, FRITZSCH C, DUHME H, KEIL U. Increasing prevalence of hay fever and atopy among children in Leipzig, East Germany. *Lancet* 1998;**351**:862–866.
- WEILAND SK, VON MUTIUS E, HIRSCH T et al. Prevalence of respiratory and atopic disorders among children in the East and West of Germany five years after unification. *Eur Respir J* 1999;**14**:862–870.
- DUHME H, WEILAND SK, RUDOLPH P, WIENKE A, KRAMER A, KEIL U. Asthma and allergies among children in West and East Germany: a comparison between Munster and Greifswald using the ISAAC phase I protocol. *Int Study Asthma Allergies Childhood Eur Respir J* 1998;**11**:840–847.
- HEINRICH J, WJST M. Increase of allergy in East Germany. *Lancet* 1998;**351**:1813.
- GEHRING U, BOLTE G, BORTE M, et al. Exposure to endotoxin decreases the risk of atopic eczema in infancy: A cohort study. *J Allergy Clin Immunol* 2001;**108**(5):847–854.
- RINAS U, HORNEFF G, WAHN V. Interferon-gamma production by cord-blood mononuclear cells is reduced in newborns with a family history of atopic disease and is independent from cord blood IgE-levels. *Pediatr Allergy Immunol* 1993;**4**:60–64.
- TANG ML, KEMP AS, THORBURN J, HILL DJ. Reduced interferon-gamma secretion in neonates and subsequent atopy. *Lancet* 1994;**344**:983–985.
- 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**:423–430.
- SANDER B, ANDERSSON J, ANDERSSON U. Assessment of cytokines by immunofluorescence and the paraformaldehyde-saponin procedure. *Immunol Rev* 1991;**119**:65–93.
- JUNG T, SCHAUER U, HEUSSER C, NEUMANN C, RIEGER C. Detection of intracellular cytokines by flow cytometry. *J Immunol Meth* 1993;**159**:197–207.
- HOLT PG, CLOUGH JB, HOLT BJ et al. Genetic 'risk' for atopy is associated with delayed postnatal maturation of T-cell competence. *Clin Exp Allergy* 1992;**22**:1093–1099.
- KONDO N, KOBAYASHI Y, SHINODA S et al. Reduced interferon gamma production by antigen-stimulated cord blood mononuclear cells is a risk factor of allergic disorders – 6-year follow-up study. *Clin Exp Allergy* 1998;**28**:1340–1344.
- HEINRICH J, NOWAK D, WASSMER G, et al. Age-dependent differences in the prevalence of allergic rhinitis and atopic sensitization between an eastern and a western German city. *Allergy* 1998;**53**:89–93.
- KJELLMAN NI, CRONER S. Cord blood IgE determination for allergy prediction – a follow-up to seven years of age in 1,651 children. *Ann Allergy* 1984;**53**:167–171.
- MICHEL FB, BOUSQUET J, GREILLIER P, ROBINET-LEVY M, COULOMB Y. Comparison of cord blood immunoglobulin E concentrations and maternal allergy for the prediction of atopic diseases in infancy. *J Allergy Clin Immunol* 1980;**65**:422–430.
- BOUSQUET J, KJELLMAN NI. Predictive value of tests in childhood allergy. *J Allergy Clin Immunol* 1986;**78**:1019–1022.
- EDENHARTER G, BERGMANN RL, BERGMANN KE et al. Cord blood-IgE as risk factor and predictor for atopic diseases. *Clin Exp Allergy* 1998;**28**:671–678.
- BERGMANN RL, EDENHARTER G, BERGMANN KE et al. Predictability of early atopy by cord blood-IgE and parental history. *Clin Exp Allergy* 1997;**27**:752–760.
- HANSEN LG, HOST A, HALKEN S et al. Cord blood IgE. II. Prediction of atopic disease. A follow-up at the age of 18 months. *Allergy* 1992;**47**:397–403.
- KJELLMAN NI. IgE in neonates is not suitable for general allergy risk screening. *Pediatr Allergy Immunol* 1994;**5**:1–4.
- WIESLANDER G, NORBACK D, BJORNSSON E, JANSON C, BOMAN G. Asthma and the indoor environment: the significance of emission of formaldehyde and volatile organic compounds from newly painted indoor surfaces. *Int Arch Occup Environ Health* 1997;**69**:115–124.
- DIEZ U, KROESSNER T, REHWAGEN M, et al. Effect of indoor chemicals exposure on the development of infections of upper respiratory tract and atopy in the first weeks of life. *Pediatrics Related Topics* 1999;**37**:469–485.
- DIEZ U, KROESSNER T, REHWAGEN M, et al. Effects of indoor painting and smoking on airway symptoms in atopy risk children in the first year of life results of the LARS-study. Leipzig Allergy High-Risk Children Study. *Int J Hyg Environ Health* 2000;**203**:23–28.

29. LEHMANN I, REHWAGEN M, DIEZ U, et al. Enhanced in vivo IgE production and T cell polarization to the type 2 phenotype in association with indoor exposure to VOC. results of the LARS study. Intern J Hyg Environ Health 2001;**204**:1–11.
30. JOHANSSON SGO, HOURIHANE J, O'B, et al. Position paper. A revised nomenclature for allergy. An EAACI position. Statement from the EAACI Nomenclature Task Force. Allergy 2001; **56(9)**:813–824.