DR. NIKO PAALANNE (Orcid ID : 0000-0001-7563-6466)

DR. MARJO RENKO (Orcid ID : 0000-0003-0507-4773)

Article type : Brief Report

CORD BLOOD CYTOKINE PROFILE IS ASSOCIATED WITH THE RISK OF ASTHMA AT THE AGE OF EIGHT YEARS

Niko Paalanne MD, PhD^{1,2}, Marjo Renko, MD, PhD^{1,2}, Hannele Pruikkonen, MD, PhD^{1,2}, Hanna Juntti, MD, PhD^{1,2}, Virpi Glumoff, PhD³, Teija Dunder, MD, PhD^{1,2}, Minna Honkila, MD, PhD^{1,2}, Tytti Pokka, MSc^{1,2}, Matti Uhari, MD, PhD^{1,2}, Terhi Tapiainen MD, PhD^{1,2,4}

¹ PEDEGO Research Unit, and Medical Research Centre Oulu, University of Oulu, Oulu, Finland

² Department of Pediatrics and Adolescence, Oulu University Hospital, Oulu, Finland

³ Research Unit of Biomedicine, Medical Microbiology and Immunology, University of Oulu, Oulu, Finland

⁴ Biocenter Oulu, University of Oulu, Oulu, Finland

Correspondence to: Niko Paalanne, MD
Department of Children and Adolescents
University of Oulu
BOX 5000
FIN-90014 University of Oulu, Finland
email:niko.paalanne@oulu.fi
Tel +358-8-315-8427, Fax +358-8-315-5559

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/APA.15141](#)

This article is protected by copyright. All rights reserved

Antenatal epidemiological risk factors such as parental asthma, maternal smoking and the number of older siblings have been shown to be associated with the risks of atopy and asthma in children.¹ Using cord blood mononuclear cells (CBMCs) obtained at birth, a decreased production of Th1-associated cytokines, such as IFN- γ and IL-12, has been reported in newborn infants who subsequently develop allergy and atopy.^{2,3} Th1/Th2 balance may thus be biased towards Th2 already at birth in children later developing allergic diseases.⁴ Most studies investigating the subsequent health and the cytokines of CBMCs, however, have focused on the development of wheezing in infancy with short follow-up periods, up to 2 years of age. We set out to investigate whether the cytokine profile found in the cord blood predicts asthma in school-aged children. We invited participants of an earlier prospective cohort study⁵, with cytokine concentrations and responses of CBMCs at birth, for a follow up study at the age of eight years.

This study was carried out at the Department of Pediatrics and Adolescent Medicine, Oulu University Hospital, Finland. From August to November 2001 we had collected a cord blood sample from each newborn of at least 37 gestational weeks (n=1084) whose parents provided their written informed consent. After earlier study investigating the risk of bronchiolitis⁵, we had cord blood samples of 88 newborn infants available for the follow up study. The protocol for the follow up study was approved by the Ethical Committee of the Northern Ostrobothnia Hospital District, Oulu, Finland

Altogether 60 children (68% of the original cohort with cord blood samples) participated in the follow up study at the age of eight years. Children underwent a clinical examination including spirometry during the study visit. Study physician reviewed the medical records of the children. Parents completed a standardized questionnaire regarding possible asthma or allergic symptoms. Current asthma was defined as the presence of continuous maintenance medication for asthma at the time of the follow-up visit. In addition to the available cytokine assays of cord blood,⁵ samples of peripheral blood mononuclear cells (PBMCs) at the age of eight years were analysed for cytokine levels and cytokine responses.

After thawing, the PBMCs were plated on 24-well cell culture plates at 2×10^6 viable cells/mL in RPMI1640 medium supplemented with 0.6 mg/mL penicillin, 60 mg/mL streptomycin, 2 mmol/L L-glutamine, 20 mmol/L HEPES and 5% FCS (Integro BV, Dieren, The Netherlands) and

stimulated with LPS from *Escherichia coli* (100 ng/mL; HB101; Sigma, St Louis, Mo) and RSV cultured in a HEP-2 cell line (ATCC CCL-23) and used at dilutions with a 50% infection rate. After 24 hours of stimulation of the mononuclear cells, the culture supernatants were harvested and stored at -20°C for later cytokine determination. Cytokine levels in the cell-culture supernatants were determined by means of flow cytometry with the human TH1/TH2 10plex Kit II (Bender MedSystems, Vienna, Austria), the detection limits being as follows: TNF- α , 7.9 pg/mL; IFN- γ , 7.0 pg/mL; IL-1b, 4.5 pg/mL; IL-2, 8.9 pg/mL; IL-4, 6.4 pg/mL; IL-5, 5.3 pg/mL; IL-6, 4.7 pg/mL; IL-8, 6.4 pg/mL; IL-10, 6.9 pg/mL; and IL-12p70, 9.7 pg/mL. The data were analysed with IBM SPSS statistics for Windows, version 25 (SPSS Inc., Chicago, Ill). The Mann-Whitney U test was used to assess differences in cytokine responses. P-values were adjusted for multiple comparisons with false discovery rate 0.10 using Benjami-Hochberg correction.

The mean age of the 60 study participants was 8.1 years (SD 0.1), and 35 (58%) were male. Eight of them (13%) has asthma at the study visit. The mean age at the time of asthma diagnosis had been 2.7 years (SD 1.9). Cytokines and cytokine responses of cord blood samples were skewed towards the Th2 phenotype in children with subsequent asthma at the study visit (Table 1). There were no significant differences in cytokine responses at the age of eight years between the asthma patients and the healthy controls.

In this follow up study, cytokine profile skewed towards the Th2 phenotype at birth was associated with subsequent asthma at the age of eight years. Majority of previous studies have reported the associations of cord blood cytokines and later health with short follow-up periods, up to one to two years of age, for viral wheezing and atopic eczema in infancy. Our study extends the results of earlier studies for asthma in school-aged children. Actual mechanism of Th2 lymphocyte differentiation from naive T-cells remains poorly understood. As immunological differences were present at birth, understanding the role of intrauterine environment in the pathogenesis of asthma would be important in the future.

References

1. Rasanen M, Kaprio J, Laitinen T, Winter T, Koskenvuo M, Laitinen LA. Perinatal risk factors for asthma in finnish adolescent twins. *Thorax*. 2000;55(1):25-31.

2. 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(11):1340-1344.
3. Wood RA, Bloomberg GR, Kattan M, et al. Relationships among environmental exposures, cord blood cytokine responses, allergy, and wheeze at 1 year of age in an inner-city birth cohort (urban environment and childhood asthma study). *J Allergy Clin Immunol*. 2011;127(4):913-9.e1-6.
4. Sandberg M, Frykman A, Ernerudh J, et al. Cord blood cytokines and chemokines and development of allergic disease. *Pediatr Allergy Immunol*. 2009;20(6):519-527.
5. Juntti H, Osterlund P, Kokkonen J, et al. Cytokine responses in cord blood predict the severity of later respiratory syncytial virus infection. *J Allergy Clin Immunol*. 2009;124(1):52-58.e1-2.

Table 1. Cytokine profile in cord blood and at the age of eight years in relation to asthma at eight years

	At birth			At eight years		
	Asthma	Healthy	P-value ^a	Asthma	Healthy	P-value ^a
	Cytokine level, pg/mL mean (SD)			Cytokine level, pg/mL mean (SD)		
Th1-associated						
TNF α	3.22 (6.65)	10.1 (18.2)	0.224	7.95 (12.5)	19.9 (71.8)	0.730
IL-2	17.5 (21.3)	13.97 (22.1)	0.3	21.2 (28.2)	42.0 (150)	0.539
IL-12	1.21 (2.36)	10.9 (19.0)	0.032 / 0.040 ^b	-	7.30 (43.6)	NA ^c
IFN - γ	16.8 (31.9)	1.41 (10.1)	NA ^d	5.81 (9.96)	15.0 (76.4)	0.368
Th2-associated						
IL-4	12.9 (10.1)	6.72 (17.2)	0.006 / 0.020 ^b	4.45 (8.04)	18.9 (82.3)	0.233
IL-5	10.6 (14.8)	4.43 (7.29)	0.671	0.30 (0.80)	14.7 (93.0)	0.881
Th17-associated						
IL-1 β	6.39 (11.7)	5.55 (22.5)	0.412	26.5 (49.0)	42.8 (137)	0.489
IL-6	4.19 (6.87)	7.48 (14.6)	0.543	60.3 (97.6)	300 (1585)	0.507
IL-8	170 (150)	77.3 (91.8)	0.022 / 0.030 ^b	3348 (2146)	3481 (1534)	0.576
Treg-associated						

IL-10	5.86 (15.3)	0.80 (1.78)	0.285	6.28 (9.87)	14.4 (73.3)	0.622
-------	-------------	-------------	-------	-------------	-------------	-------

^a Results of Mann-Whitney U-test

^b P-values were adjusted for multiple (ie. ten) comparisons with false discovery rate 0.10 using Benjami-Hochberg correction.

^c No detected values in the asthma group, no analyses performed

^d Detected values only in four subjects, no analyses performed