

Change in IFN- γ -producing capacity in early life and exposure to environmental microbes

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Background: Exposure to environmental microbes in early life might lead to type 1-skewed T cell responses and therefore reduce the risk of allergic diseases.

Objective: To investigate whether the cytokine responses at birth and at age 3 months are associated with environmental factors, especially exposure to microbes. Living in a farm, level of house dust endotoxin, cleanliness of the home, and presence of cats and dogs in the household were studied as possible determinants of cytokine production.

Methods: Twelve farmers' and 17 nonfarmers' children were studied. Production of IL-4, IL-6, TNF- α , and IFN- γ in cord blood and in peripheral blood at 3 months was measured after 8-hour and 24-hour stimulation with phorbol ester plus concanavalin A.

Results: IFN- γ responses at age 3 months were associated with farming (median, 53 vs 17 pg/mL; $P = .019$) and cats and dogs (49 vs 14 pg/mL; $P = .014$) (8 hours). Change in IFN- γ -producing capacity from birth to 3 months was larger in children with higher than median endotoxin concentration in bed dust ($P = .038$) and in children with a cat or dog ($P = .005$) (8 hours). Increased IL-6 responses at birth were associated with cat or dog exposure ($P = .004$; 8 hours) and endotoxin level in settled dust ($P = .039$; 24 hours).

Conclusion: The development of IFN- γ -producing capacity during the first 3 months of life is associated with farming, endotoxin in house dust, and cat and dog exposure. These environmental characteristics may indicate some microbial exposure capable of driving developing immune system toward T_H1 responses. (*J Allergy Clin Immunol* 2005; 116:1048-52.)

Key words: Allergy, cord blood, cytokines, endotoxin, IFN- γ , IL, microbes, T_H cell

The role of host and environmental factors in regulating T_H cell responses has been increasingly discussed, particularly in context of pathogenesis of T_H2-mediated allergy

Abbreviations used

ConA: Concanavalin A

EU: Endotoxin unit

PMA: Phorbol 12-myristate 13-acetate

and asthma.¹⁻⁴ Farming environment,⁵⁻⁸ level of house dust endotoxin,^{9,10} and presence of cats and dogs in the household^{8,11} have been associated with decreased risk of allergic diseases, although the results are partly conflicting.^{12,13} This phenomenon may be related to the increased exposure to environmental microbes capable of altering immunological development of the child.

The underlying mechanisms by which microbial exposures can influence the development of T_H2 immune responses are under investigation. It is known that recognition of various microbes by Toll-like receptors initiates a pathway leading to induction of T_H1 responses.¹⁴⁻¹⁶ In contrast, T_H2 differentiation has been hypothesized to occur by default, resulting from the reduced T_H1 signaling¹⁷ or via currently unspecified receptors.¹⁶ There is some evidence that production of T_H1-type cytokine IFN- γ at birth is inversely associated with recurrent wheezing¹⁸ or atopy in the first year of life¹⁹ and atopy and asthma at the age of 6 years.²⁰ The reduced IFN- γ secretion has been also shown in atopic individuals.²¹⁻²³

In this study, we investigated whether the stimulated cytokine production in cord blood and in peripheral blood at age 3 months is associated with environmental factors, especially exposure to microbes. Living in a farm, concentration of endotoxin in house dust, cleanliness of the home, and presence of cats and dogs in the household were studied as possible determinants of cytokine production.

METHODS

Study subjects and blood sampling

Twelve farming and 17 nonfarming healthy women, who all gave birth to full-term, healthy newborns in the Department of Obstetrics at Kuopio University Hospital, were recruited to the study. Cord blood samples were collected after cord clamping by aspiration technique. Peripheral venous blood samples were drawn from the children 3 months after delivery at the Laboratory of Kuopio University Hospital. The samples were stored at 4°C until processed further within 24 hours. Informed consent was obtained from all subjects

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TABLE I. Stimulated production of IFN- γ at birth and at age 3 months, and the change in the IFN- γ -producing capacity in relation to environmental factors

Factor	Birth*	3 mo*	Change†
Farming family			
No	0 (0-20) 15	17 (0-300) 14	7.9 (–16 to 300) 12
Yes	2.1 (0-54) 10	53 (12-540) 10	47 (–37 to 260) 8
<i>P</i> value	.397	.019	.069
Dog or cat in the household			
No	0.1 (0-54) 13	14 (0-67) 12	0.5 (–37 to 61) 10
Yes	2.1 (0-36) 12	49 (12-540) 12	47 (5-300) 10
<i>P</i> value	.936	.014	.005
Poor or moderate cleanliness of the home			
No	0 (0-54) 13	20 (0-540) 13	3 (–37 to 300) 10
Yes	2.1 (0-15) 12	31 (11-260) 11	38 (–4 to 260) 10
<i>P</i> value	.979	.361	.063

*Eight-hour stimulation with PMA (15 ng/mL) and ConA (10 μ g/mL). The data are presented as median (range) *n*.

†Cytokine concentration (3 months) – cytokine concentration (birth).

according to the protocol approved by the Ethical Committee for Human Research at the University of Kuopio.

Assessment of cytokine production

T_H1 (IFN- γ) and T_H2 (IL-4) cytokine production and general inflammatory cytokine responses (IL-6, TNF- α) were measured in whole blood cell culture supernatants collected after stimulation with phorbol 12-myristate 13-acetate (PMA) and concanavalin A (ConA). Heparinized blood (500 μ L) was added to 1.5 mL RPMI cell culture medium supplemented with 10% FBS, 1% L-glutamine, and 1% penicillin-streptomycin antibiotic mixture, all from Gibco (Paisley, United Kingdom). Cells were incubated with the combination PMA (15 ng/mL) and ConA (10 μ g/mL; both from Sigma, St Louis, Mo) for 8 and 24 hours in 5% carbon dioxide at 37°C. After incubation, the cell cultures were centrifuged at 380g for 10 minutes, and the supernatants were frozen at –80°C for later cytokine analysis. Concentrations of IFN- γ , IL-4, IL-6, and TNF- α in the supernatants were measured by using ELISA kits (R&D Systems, Minneapolis, Minn). Each sample was run in duplicate. Assays were performed according to the manufacturer's instructions, and the in-house assay sensitivities were as follows: IL-4, 8 pg/mL; IL-6, 4 pg/mL; TNF- α , 10 pg/mL; and IFN- γ , 5 pg/mL.

IgE measurements

At age 3 months, the specific IgE against food (codfish, peanut, cow's milk, egg, soy bean, wheat) and inhalant (birch pollen, timothy, mugwort, cat, horse, dog, *Dermatophagoides pteronyssinus*, *Cladosporium herbarum*) allergens was determined by fluorescence enzyme immunoassay in the Department of Clinical Chemistry, Kuopio University Hospital. The sensitivity for detection of specific IgE was 0.35 kU/L.

House dust collection and endotoxin analysis

Four different house dust samples were collected for determination of home characteristics affecting endotoxin levels: mother's mattress dust, floor dust from the rug of mother's bedroom, vacuum cleaner dust bag dust, and settled dust.²⁴ Endotoxin concentrations were determined with Limulus Amebocyte Lysate assay (Kinetic QCL; Biowhittaker, Walkersville, Md), and the results are expressed as endotoxin units (EU) per milligram of dust. Studied children were divided into groups of low endotoxin exposure and high endotoxin exposure by using median concentrations of endotoxin as cutoff

points (bed dust, 4 EU/mg; floor dust, 13 EU/mg; dust bag dust, 24 EU/mg; settled dust, 9 EU/mg).

Pet ownership and cleanliness of the home

Seven out of 12 farming families and 7 out of 17 nonfarming families had a cat and/or dog. The cleanliness of the home was estimated by the field worker with 3 level categories (poor, moderate, good). In analysis, the poor and moderate categories were combined because of the small number in the poor category. Poor or moderate cleanliness was in 8 out of 12 farming and in 5 out of 17 nonfarming homes.

Statistical analysis

Data are reported as medians (ranges). The change of cytokine-producing capacity was calculated as cytokine concentration (3 months) minus cytokine concentration (cord blood). Normal distribution of cytokine data was not obtained with standard transformations. Therefore, the nonparametric Mann-Whitney test was used when the differences in cytokine production in relation to environmental (farming/nonfarming family, high/low endotoxin exposure, yes/no cat and/or dog, poor or moderate/good cleanliness of home) and maternal (yes/no doctor-diagnosed asthma, self-reported atopic eczema, self-reported hay fever) characteristics were evaluated. Endotoxin concentrations were logarithmically transformed for statistical analysis. The differences in endotoxin concentrations in relation to environmental characteristics were analyzed with the *t* test. The statistical analyses were performed with the SPSS software package version 11.5 (SPSS Inc, Chicago, Ill).

RESULTS

Endotoxin concentrations in home dust

The endotoxin concentrations in bed dust and in settled dust were higher in farming homes than in nonfarming homes (bed dust, median, 8.0 vs 3.0 EU/mg, *P* = .076; settled dust, 17 vs 8.9 EU/mg, *P* = .048), with the results of floor dust and dust bag dust quite similar.²⁴ The concentration of endotoxin in bed dust and settled dust was also higher in homes with lower cleanliness level (bed dust, 9.0 vs 2.0 EU/mg, *P* = .037; settled dust, 19 vs 7.0 EU/mg, *P* = .024). Pet ownership did not significantly affect endotoxin levels.²⁴

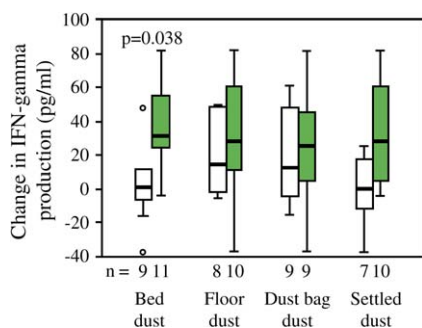


FIG 1. Change in the IFN- γ -producing capacity from birth to age 3 months in children with low (white boxes) and high (green boxes) endotoxin exposure. Box plots show the interquartile range, median (horizontal line), adjacent values (whiskers), and outliers (circles). Adjacent values are observations not more than 1.5 times the interquartile range beyond either quartile. Two extreme values (260 and 300 pg/mL) are not shown for graphic reasons.

Stimulated production of cytokines in relation to environmental factors

After 8-hour stimulation with PMA/ConA, the production of IFN- γ at the age of 3 months was increased in children from farming families compared with children from nonfarming homes (Table I). Also, the increase in IFN- γ producing capacity during the first 3 months of life tended to be larger in farmers' children (Table I). Change in the production of IFN- γ from birth to age 3 months was larger in children with higher than median endotoxin concentration in any of the home dust samples (Fig 1). Statistically significant difference was detected only for mother's bed dust. Also, the presence of a cat or dog in the household was associated with higher concentrations of IFN- γ at age 3 months and with increase in IFN- γ producing capacity from birth to 3 months (Table I). General cleanliness of the home tended to have an effect only on change in the IFN- γ -producing capacity (Table I). Statistically significant increase was detected in the 8-hour stimulated production of IL-6 at birth in children with cat or dog in their homes (median, 610 pg/mL; range, 560-730 pg/mL) compared with children without such exposure (median, 570 pg/mL; range, 210-650 pg/mL; $P = .004$).

After 24-hour stimulation with PMA/ConA, detected concentrations of IFN- γ at birth were 3.4 to 890 pg/mL, and at age 3 months, 3.5 to 1100 pg/mL. Respectively, IL-6 ranged at birth from 220 to 680 pg/mL and at 3 months from 120 to 650 pg/mL, and TNF- α ranged at birth from 800 to 6000 pg/mL and at 3 months from 430 to 6000 pg/mL. Production of IL-6 was significantly increased in cord blood samples of children with high endotoxin concentration in settled dust (610 vs 590 pg/mL; $P = .039$). Otherwise, no statistically significant determinants were found for 24-hour stimulated production of IL-6, IFN- γ , and TNF- α at birth or at 3 months. IL-4 responses were very weak both at 8 hours and 24 hours, and their determinants could not be studied (data not shown).

Cytokine responses in relation to allergic diseases of the mother and child

Change in the IFN- γ -producing capacity tended to be smaller in children with maternal history of hay fever (median, 5.5 vs 29; $P = .069$; 8-hour stimulation). No differences were found in other cytokines, in 24-hour stimulation, or in relation to maternal asthma or atopic eczema. However, maternal history of atopy did not differ between farmers and nonfarmers: prevalence for hay fever was 4 of 12 in farmers and 6 of 17 in nonfarmers. Respective numbers for atopic eczema were 4 of 12 and 5 of 17, and for doctor-diagnosed asthma, 0 of 12 and 1 of 17. Pet ownership was also not associated with maternal atopy: 7 of 14 mothers with history of asthma, eczema, and/or hay fever and 8 of 15 mothers with no such history had a cat and/or dog in their household.

Among children, the concentrations of specific IgE for all tested allergens at age 3 months were below 0.35 kU/L and considered negative. Only 1 child from a nonfarming family had doctor-diagnosed atopic eczema, and 1 child from a farming family had symptoms of milk allergy. Thus, the associations between cytokines and child's allergic diseases could not be investigated.

DISCUSSION

In this study, we found associations between increased T_H1 -like immune responses at the age of 3 months, measured as PMA/ConA-stimulated production of IFN- γ in whole blood cell culture, and living on a farm, concentration of endotoxin in house dust samples, and presence of cats and dogs in a household. Our results provide evidence that exposure to these environmental factors at birth and during early infancy may have an important role in the maturation of the developing immune system.

To our knowledge, this is the first study investigating the change in the cytokine-producing capacity during the first months of life and exposure to farming. Epidemiological studies have shown a lower prevalence of allergen sensitization in farmers' children,^{5,7,8,25} but the underlying protective agents and mechanisms are not clear. It has been hypothesized that exposure to high levels of microbial compounds present in farming environment may enhance T_H1 -type immune responses that repress atopy-related T_H2 reactions. Our findings support this view by showing increased T_H1 responses at the age of 3 months in relation to prenatal and neonatal exposure to farming environment. Change in the cytokine-producing capacity tended to be smaller in children with maternal history of hay fever. Maternal atopy, however, did not differ between children from farming and nonfarming families and thus did not explain the observed difference in T_H1 responses.

In search of protective microbial compounds, the main focus has been on endotoxin because the level of house dust endotoxin has been reported to be inversely associated with risk of allergen sensitization.^{9,10,26} We found higher endotoxin concentrations in home dust of farming

families than in that of nonfarming families, as shown earlier.^{9,27} Furthermore, the capacity of a child's blood cells to produce IFN- γ was associated with endotoxin concentration in the mother's bed dust sample. This finding is in line with the previous study by Gereda et al²⁶ showing a correlation between the proportion of IFN- γ -producing cells in infants and endotoxin concentration in house dust. Unfortunately, because of the small number of studied subjects, we could not analyze further with multivariate models whether our findings were dependent on exposure to farming or endotoxin. In the study by Braun-Fahrlander et al,⁹ endotoxin alone did not explain decreased risk of allergic diseases among farmers' children, suggesting that the possible protective effect of microbial exposure is more likely to arise from a wider spectrum of microbial compounds capable of influencing development of the immune system.

Stimulated production of IFN- γ was higher in children who had a cat or dog in their homes, indicating some pet-related exposure capable of provoking T_H1-type immune responses. Gem et al²⁸ reported increased levels of IL-10 and IL-13 in stimulated blood cells and reduced risk of allergen sensitization and atopic dermatitis in 1-year-old children exposed to dogs. Genetic variation of CD14, a molecule required for endotoxin-induced cellular activation, together with dog exposure further influenced the risk of atopic dermatitis. In our study, cats or dogs had no significant effect on the level of endotoxin in house dust²⁴ even though it has been a significant determinant of endotoxin levels in many earlier studies.²⁹⁻³¹ Thus, pets may be a source of other microbiological or biological compounds with T_H1-inducing potency. Pet ownership in relation to immune development is of special interest because exposure to cats and dogs during infancy has been reported to both increase^{12,32} and inhibit^{8,11,33} subsequent allergic diseases.

It has been shown that gestational and labor factors have a strong effect on cytokine production,^{34,35} and therefore, the connection between perinatal exposures and cytokines in cord blood is difficult to demonstrate. In our study, the production of IL-6 in cord blood was associated with the presence of cat or dog in the household and the level of endotoxin in a settled dust sample. In other cytokines, no consistent differences in relation to studied environmental factors were found. Responses of T_H2-type IL-4 were very weak both at birth and at 3 months, and its determinants could not be studied. This may be related to the lack of allergies among studied children or to the low capacity of combination of PMA and ConA to stimulate production of this cytokine. Low numbers of IL-4-producing T cells have also been reported after PMA/ionomycin stimulation,³⁶ with the peak production at 8 hours.³⁷

We used the nonspecific stimulant PMA, a potent activator of protein kinase C, together with mitogen ConA to measure the general T-cell effector function of the studied children by the time of investigation. Inclusion of a specific microbial stimulant LPS would have enabled us to evaluate whether the cytokine production in response to

this stimulation is associated with the child's previous endotoxin exposure. Further investigation of the association between immune responses and microbial exposure would also require the expansion of the measured cytokine panel (eg, IL-10 and IL-12 as important immune regulatory cytokines).

In conclusion, even though the number of studied children was small, the development of IFN- γ -producing capacity during the first 3 months of life was associated with farming environment, level of endotoxin in house dust, and the presence of cat or dog in the child's home. These environmental characteristics may indicate some microbial exposure capable of driving the developing immune system toward T_H1 responses. The causative compounds and the mechanism by which they can influence the maturation and initiation of immunological function need further investigation.

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REFERENCES

1. Strachan D. Family size, infection and atopy: the first decade of the "hygiene hypothesis." *Thorax* 2000;55(suppl 1):S2-10.
2. Braun-Fahrlander C, Lauener R. Farming and protective agents against allergy and asthma. *Clin Exp Allergy* 2003;33:409-11.
3. Holt PG. The role of genetic and environmental factors in the development of T-cell mediated allergic disease in early life. *Paediatr Respir Rev* 2004;5:S27-30.
4. Romagnani S. The increased prevalence of allergy and the hygiene hypothesis: missing immune deviation, reduced immune suppression, or both? *Immunology* 2004;112:352-63.
5. Braun-Fahrlander C, Gassner M, Grize L, Neu U, Sennhauser FH, Varonier HS, et al. Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community. SCARPOL Team. Swiss Study on Childhood Allergy and Respiratory Symptoms With Respect to Air Pollution. *Clin Exp Allergy* 1999;29:28-34.
6. Kilpeläinen M, Terho EO, Helenius H, Koskenvuo M. Farm environment in childhood prevents the development of allergies. *Clin Exp Allergy* 2000;30:201-8.
7. Riedler J, Braun-Fahrlander C, Eder W, Schreuer M, Waser M, Maisch S, et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 2001;356:1129-33.
8. Remes ST, Iivanainen K, Koskela H, Pekkanen J. Which factors explain the lower prevalence of atopy amongst farmers' children? *Clin Exp Allergy* 2003;33:427-34.
9. Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002;347:869-77.
10. Gehring U, Bischof W, Fahlbusch B, Wichmann HE, Heinrich J. House dust endotoxin and allergic sensitization in children. *Am J Respir Crit Care Med* 2002;166:939-44.
11. Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. *JAMA* 2002;288:963-72.
12. Lindfors A, van Hage-Hamsten M, Rietz H, Wickman M, Nordvall SL. Influence of interaction of environmental risk factors and sensitization in young asthmatic children. *J Allergy Clin Immunol* 1999;104:755-62.
13. Bolte G, Bischof W, Borte M, Lehmann I, Wichmann HE, Heinrich J, et al. Early endotoxin exposure and atopy development in infants: results of a birth cohort study. *Clin Exp Allergy* 2003;33:770-6.

14. Barton GM, Medzhitov R. Control of adaptive immune responses by Toll-like receptors. *Curr Opin Immunol* 2002;14:380-3.
15. Dabbagh K, Lewis DB. Toll-like receptors and T-helper-1/T-helper-2 responses. *Curr Opin Infect Dis* 2003;16:199-204.
16. Eisenbarth SC, Piggott DA, Bottomly K. The master regulators of allergic inflammation: dendritic cells in Th2 sensitization. *Curr Opin Immunol* 2003;15:620-6.
17. Medzhitov R. Toll-like receptors and innate immunity. *Nat Rev Immunol* 2001;1:135-45.
18. Guerra S, Lohman IC, Halonen M, Martinez FD, Wright AL. Reduced interferon gamma production and soluble CD14 levels in early life predict recurrent wheezing by 1 year of age. *Am J Respir Crit Care Med* 2004;169:70-6.
19. Neaville WA, Tisler C, Bhattacharya A, Anklam K, Gilbertson-White S, Hamilton R, et al. Developmental cytokine response profiles and the clinical and immunologic expression of atopy during the first year of life. *J Allergy Clin Immunol* 2003;112:740-6.
20. Macaubas C, de Klerk NH, Holt BJ, Wee C, Kehdall G, Firth M, et al. Association between antenatal cytokine production and the development of atopy and asthma at age of 6 years. *Lancet* 2003;362:1192-7.
21. Tang ML, Varigos G, Kemp AS. Reduced interferon-gamma (IFN-gamma) secretion with increased IFN-gamma mRNA expression in atopic dermatitis: evidence for a post-transcriptional defect. *Clin Exp Immunol* 1994;97:483-90.
22. Hoekstra MO, Hoekstra Y, De Reus D, Rutgers B, Gerritsen J, Kauffman HF. Interleukin-4, interferon-gamma and interleukin-5 in peripheral blood of children with moderate atopic asthma. *Clin Exp Allergy* 1997;27:1254-60.
23. Benson M, Strannegard IL, Wennergren G, Strannegard O. Low levels of interferon-gamma in nasal fluid accompany raised levels of T-helper 2 cytokines in children with ongoing allergic rhinitis. *Pediatr Allergy Immunol* 2000;11:20-8.
24. Hyvärinen A, Roponen M, Tiittanen P, Laitinen S, Nevalainen A, Pekkanen J. Endotoxin levels in urban and farming homes: Comparison of different sampling methods and importance of environmental factors. *Indoor Air*. In press.
25. Downs SH, Marks GB, Mitakakis TZ, Leuppi JD, Car NG, Peat JK. Having lived on a farm and protection against allergic diseases in Australia. *Clin Exp Allergy* 2001;31:570-5.
26. Gereda JE, Leung DY, Thatayatikom A, Streib JE, Price MR, Klennert MD, et al. Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet* 2000;355:1680-3.
27. von Mutius E, Braun-Fahrlander C, Schierl R, Riedler J, Ehlermann S, Maisch S, et al. Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin Exp Allergy* 2000;30:1230-4.
28. Gem JE, Reardon CL, Hoffjan S, Nicolae D, Li Z, Roberg KA, et al. Effects of dog ownership and genotype on immune development and atopy in infancy. *J Allergy Clin Immunol* 2004;113:307-14.
29. Gereda JE, Klennert MD, Price MR, Leung DY, Liu AH. Metropolitan home living conditions associated with indoor endotoxin levels. *J Allergy Clin Immunol* 2001;107:790-6.
30. Park J-H, Spiegelman DL, Gold DR, Burge HA, Milton DK. Predictors of airborne endotoxin in the home. *Environ Health Perspect* 2001;109:859-64.
31. Bischof W, Koch A, Gehring U, Fahlbusch B, Wichmann HE, Heinrich J, et al. Predictors of high endotoxin concentrations in the settled dust of German homes. *Indoor Air* 2002;12:2-19.
32. Suoniemi I, Björkstén F, Haahtela T. Dependence of immediate hypersensitivity in the adolescent period on factors encountered in infancy. *Allergy* 1981;36:263-8.
33. Perzanowski MS, Ronmark E, Platts-Mills TA, Lundback B. Effect of cat and dog ownership on sensitization and development of asthma among preteenage children. *Am J Respir Crit Care Med* 2002;166:696-702.
34. Jokic M, Guillois B, Cauquelin B, Giroux JD, Bessis JL, Morello R, et al. Fetal distress increases interleukin-6 and interleukin-8 and decreases tumour necrosis factor-alpha cord blood levels in noninfected full-term neonates. *BJOG* 2000;107:420-5.
35. Keski-Nisula L, Hirvonen MR, Roponen M, Heinonen S, Pekkanen J. Spontaneous and stimulated interleukin-6 and tumor necrosis factor-alpha production at delivery and three months after birth. *Eur Cytokine Netw* 2004;15:67-72.
36. Hagendorens MM, Van Bever HP, Schuerwegh AJ, De Clerck LS, Bridts CH, Stevens WJ. Determination of T-cell subpopulations and intracellular cytokine production (interleukin-2, interleukin-4, and interferon-gamma) by cord blood T-lymphocytes of neonates from atopic and non-atopic parents. *Pediatr Allergy Immunol* 2000;11:12-9.
37. Chalmers IM, Janossy G, Contreras M, Navarrete C. Intracellular cytokine profile of cord and adult blood lymphocytes. *Blood* 1998;92:11-8.