doi: 10.1111/j.1365-2222.2007.02891.x
*Clinical and Experimental Allergy*, **38**, 298–304


ORIGINAL PAPER **Clinical Mechanisms in Allergic Disease**

# The influence of processing factors and non-atopy-related maternal and neonate characteristics on yield and cytokine responses of cord blood mononuclear cells

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Clinical and Experimental Allergy

## Summary

*Rationale* Several studies have evaluated the associations between cord blood cellular responses and atopic diseases in children, but the results of these studies are inconsistent. Variations in blood processing factors and maternal and infant characteristics are typically not accounted for and may contribute to these inconsistencies.

*Methods* Cord blood samples were obtained from 287 subjects participating in the Childhood Origins of ASThma project, a prospective study of children at high risk for the development of asthma/allergies. Mononuclear cells were stimulated with phytohaemagglutinin (PHA), phorbal myristate acetate/ionomycin or a suspension of killed staphylococcus, and IFN-γ, IL-10 and IL-13 were quantitated by ELISA. Cell yields and cytokine production were related to processing factors and maternal and infant characteristics.

*Results* The strongest relationships between independent variables and cell yield or cytokine responses occurred with the season of birth. The highest median cell yields were seen in fall, and the lowest in summer (difference of 47%, $P = 0.0027$). Furthermore, PHA-induced IL-5 and IL-13 responses were approximately 50% higher in spring and summer than in fall or winter ($P < 0.0001$). Clots in the cord blood samples were associated with a reduced median cell yield (42% reduction, $P < 0.0001$), and an increased PHA-induced IL-10 secretion (27% increase, $P = 0.004$).

*Conclusions* These data suggest that season of collection, and to a lesser extent clotting in samples, affect cord blood mononuclear cell yield and cytokine responses. Careful documentation and analysis of processing and environmental variables are important in understanding biological relationships with cytokine responses, and also lead to greater comparability among studies using these techniques.

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**Keywords** cord blood, environmental exposures, interleukins, mononuclear cells, season of year
*Submitted 14 December 2006; revised 19 September 2007; accepted 21 September 2007*

## Introduction

A significant body of research indicates that factors present during foetal development influence cellular responses in early life, and that certain patterns of response can be linked to the development of atopic disease in childhood. Observational studies have linked certain neonate characteristics to the development of atopic disease, while other studies have related exposures during pregnancy and/or parental history to cellular responses in offspring that predict the development of allergies and asthma.

However, despite a seemingly consistent overall message regarding the importance of *in utero* influences on immune system development, inconsistencies exist regarding the link between *specific* elements of history or exposures, cord blood cellular responses and atopy or asthma. A number of studies have demonstrated that a family history of atopy is associated with reduced IFN-γ

cord blood mononuclear cell (CBMC) responses in children who later develop atopic disease [1–7], but the pattern is less clear for other cytokines. A number of studies have linked increased IL-4, IL-5, IL-10 and IL-13 to later development of allergies, asthma and eczema in childhood [8–13], but similar studies have also found an association between decreased IL-4, IL-6, IL-12 and IL-13 cord blood responses and these outcomes [14–16]. A similar lack of consensus in results exists in studies of CBMC-proliferative responses. Some studies have linked an increase in responses to stimuli such as dust mite and ovalbumin to an increase in childhood atopy [17, 18], but these relationships are not universally accepted [19, 20].

In a 2002 editorial, Devereux and Barker [21] suggested that variation in unmeasured influences might affect the consistency of cellular responses between studies. Yet little research has been carried out to elucidate what these unmeasured influences might be and how they may or may not work together to influence cord blood responses. These factors may be related to the method by which cord blood samples are processed or to characteristics of the mother and/or child that are not directly related to atopic history or allergen exposure.

Using data from a high-risk birth cohort study involving cord blood samples, we sought to determine the influence of several non-atopy-related variables on cord blood cell and cytokine yields. Specifically, we assessed the effect of sample processing factors, such as method of collection and time to processing; neonate characteristics, including weight and gestational age; and maternal factors unrelated to atopic history and allergen exposure, like age and number of pregnancies. We developed multivariate regression models using cell yield and cytokine levels as dependent variables and incorporating factors that were both linked and not directly related to atopy as independent variables in an attempt to isolate both new and previously reported influences on CBMC yields and cytokine responses.

## Materials and methods

### *Subject characteristics/family history*

*Study subjects and experimental design*. Subjects for this study were a cohort of children who had at least one parent with respiratory allergies (defined as one or more positive aeroallergen skin tests) and/or asthma (defined by a positive questionnaire response to either a physician diagnosis of asthma and/or the prescribed use of asthma medications). Additional details regarding eligibility criteria have been published previously [22]. Two hundred and seventy-two subjects with complete questionnaire data were included in the analyses for this evaluation. Relatively similar numbers of subjects were recruited in spring (March–May, $N = 86$), summer (June–August, $N = 57$), fall (September–November, $N = 55$) and winter (December–February, $N = 74$).

*Parental history*. Asthma history was ascertained by questionnaire, administered on participating parents at the consenting visit (before the birth of the child/participant). A parent was considered to have a positive asthma history if he/she reported a physician diagnosis of asthma or the prescribed use of a β-agonist or asthma controller medications. Respiratory allergy was confirmed by skin prick testing. Participating fathers were tested for reaction to 12 common aeroallergens at the consenting visit; for safety reasons, maternal testing for reaction to the same allergens was completed during the year following the birth of the child. Additionally, mothers of study participants were retrospectively queried as to whether the severity of their asthma or allergies had increased during pregnancy through questions about the use of medications, frequency of MD/ER visits for asthma/allergies and personal assessment of asthma/allergy symptoms during pregnancy.

### *Blood sample collection and processing*

*Collection and handling of blood samples*. Cord blood samples were obtained from the umbilical cord vein using standard techniques. For vaginal deliveries, the umbilical cord was ligated before delivery of the placenta. The sample was then collected as blood was allowed to drip from the umbilical cord into sterile, heparinized tubes. Caesarean section collections were different in that the sample was collected by a sterile needle/syringe from the umbilical vein following the delivery of the child and the placenta.

After collection, tubes were labelled with the subject name and time of collection and were enclosed in a plastic bag with a data collection form containing information such as the time of birth, time of collection, time of placental delivery, length, weight and head circumference of the child. Simple hand mixing/rotation of the blood tubes was completed, and the tubes and data form were placed in a designated slot on the obstetrical unit where they were kept at room temperature. Obstetrical staff contacted study personnel to inform them of the presence of the cord blood sample so that the staff could pick up the sample from the hospital and transport it to the University of Wisconsin Medical School for processing. Cord blood was collected from six different hospitals within south-central Wisconsin using these methods.

Upon receipt at the laboratory, samples were processed the same day if they arrived before 4:00 P.M., and otherwise were placed on a rocker at room temperature until the following morning. Each sample was inspected for clots, and the presence of any amount of clotting was reported on the processing form. In the majority of these specimens, only a small amount of clotted material was present, and clots were extracted before density

centrifugation of samples (as described below). Processing time was calculated as the amount of time between the birth of the child and incubation of the cells. One person was primarily responsible for collecting cord blood samples throughout the entire collection period for the study, collecting >90% of the samples. Four persons were responsible for processing the samples, with the majority (approximately 75%) being processed by the same individual. The distribution of the percentage of samples processed by each of the four people did not vary throughout the collection period.

*Mononuclear cell stimulation.* Mononuclear cells (MNC) were separated using density centrifugation (LSM® Lymphocyte Separation Medium, ICN Biomedicals Inc., Aurora, OH, USA), and plasma was removed and stored at −80 °C in labelled microcentrifuge tubes. The MNC were suspended ($10^6$ cells/mL) in RPMI-1640 supplemented with 10% foetal bovine serum (Hyclone, Logan, UT, USA), L-glutamine (2 mM), penicillin (100 U/mL) and streptomycin (100 μg/mL) and incubated (24 h, 37 °C, 5% $CO_2$) in a 24-well flat bottom cell culture plate (1 mL/well, Corning Inc., Corning, NY, USA) with phytohaemagglutinin (PHA, 5 μg/mL, Sigma, St Louis, MO, USA), *Staphylococcus aureus* cells (SAC, $10^{-4}$ dilution w/v, Calbiochem, La Jolla, CA, USA), phorbal myristate acetate/ionomycin (PMA, 100 ng/mL, Sigma) or medium alone. The PHA, SAC or PMA was aliquotted into single-use portions and frozen at −80 °C at the beginning of the study so that the same stimulant was used throughout the study.

*Cytokine enzyme-linked immunoassay.* The levels of IFN-γ, IL-5, IL-10 and IL-13 in culture supernates were evaluated by an ELISA (Pharmingen, San Diego, CA, USA). The manufacturer's protocol was followed, except that the sample volume was reduced to 50 μL. The sensitivities of the ELISAs in these analyses were as follows: IFN-γ = 3.1 pg/mL; IL-5 = 1.9 pg/mL; IL-10 = 7.8 pg/mL; and IL-13 = 3.1 pg/mL. Duplicate wells were run for each sample, and the mean values are reported.

## Statistical analyses

Univariate differences in cell yields, IFN-γ, IL-10 and IL-13 by maternal, neonatal and blood processing characteristics were conducted using Kruskal–Wallis tests. Owing to the fact that a large proportion of our samples had non-detectable levels of IL-5 in the cord blood, IL-5 was considered to be a dichotomous detectable vs. non-detectable variable and analysed using Pearson's $\chi^2$ test.

In multivariate analyses, linear regression models were developed that incorporated maternal, neonatal and processing characteristics as independent variables and used log-transformed cell yields and cytokine responses (IFN-γ, IL-10 and IL-13) to approximate a more normal distribution for these dependent variables. Multivariate logistic regression models were developed for IL-5 levels, once again considering the cytokine as a dichotomous detectable vs. undetectable dependent variable.

All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA). A nominal two-sided *P*-value of 0.05 was regarded as being statistically significant.

## Results

### *Cell yield*

Technicians routinely inspected blood specimens for any evidence of clots in the heparinized specimens. Clots were present in 59% of samples from vaginal deliveries, and 51% of samples from caesarean deliveries; the majority of these clots were quite small and did not impede the processing procedures. The presence of any clots was associated with lower median cell yields ($1.52 \times 10^5$ vs. $2.29 \times 10^5$ cells/mL, $P<0.0001$; Table 1), as was collection of the sample in the summer [$1.36 \times 10^5$ cells/mL (summer) vs. $2.14 \times 10^5$ cell/mL (fall), $2.13 \times 10^5$ cells/mL (winter) or $1.96 \times 10^5$ cells/mL (spring), $P = 0.0027$]. Dog ownership at the time of the subject's birth was associated with higher cell yields ($2.14 \times 10^5$ vs. $1.69 \times 10^5$ cells/mL, $P = 0.0088$).

In multivariate models, clotting ($P<0.0001$) and birth in the summer [vs. fall ($P = 0.013$), winter ($P = 0.069$) and spring ($P = 0.021$)] continued to be associated with lower cell yields, while dog ownership remained associated with higher cell yields ($P = 0.031$).

### *Cytokine responses*

The strongest relationships between independent variables and cytokine responses were seen with the season of birth (see Table 1). IL-13 responses were higher in the spring and summer than in the fall or winter for all stimulants. A similar seasonal pattern was observed with IL-5 responses. These relationships persisted in multivariate models of IL-13 and IL-5. Univariate data suggest a summer peak for PMA-induced IFN-γ and SAC-induced IL-10, but these relationships were non-significant in multivariate models.

In addition to associations with season, the presence of any clotting was associated with lower PHA- (93.9 vs. 110.6 pg/mL, $P = 0.0066$) and SAC-stimulated IL-10 responses (33.0 vs. 77.4 pg/mL, $P = 0.0022$), but did not affect those of other cytokines (see Table 1). The relationship between clotting and IL-10 remained significant in multivariate models ($P = 0.0005$).

A processing time >12 h was associated with significantly lower levels of PHA-stimulated IL-5 in uni- (2.0 vs. 3.2 pg/mL, $P = 0.0009$) and multivariate models

 

**Table 1.** Influence of non-atopy-related variables on median cell and cytokine yields

| | Cell yield | IFN-γ (pg/mL) | | | IL-5 (pg/mL) | | | IL-13 (pg/mL) | | | IL-10 (pg/mL) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ($10^5$ cells/mL) | PHA | SAC | PMA | PHA | SAC | PMA | PHA | SAC | PMA | PHA | SAC | PMA* |
| Neonate factors | | | | | | | | | | | | | |
| Birthweight | | | | | | | | | | | | | |
| <7.5 lbs ($N$ = 113) | 1.90 | 55.2 | 13.2 | 800.0 | 1.9 | 1.8 | 16.8 | 255.3 | 7.0 | 160.6 | 96.1 | 62.1 | |
| 7.5+ lbs ($N$ = 159) | 1.83 | 58.4 | 12.2 | 868.5 | 2.5 | 2.6 | 16.9 | 290.7 | 6.8 | 143.6 | 105.6 | 37.1 | |
| $P$-value | 0.89 | 0.49 | 0.42 | 0.80 | 0.58 | 0.55 | 0.99 | 0.42 | 0.81 | 0.49 | 0.10 | 0.96 | |
| Gestational age | | | | | | | | | | | | | |
| <40 weeks ($N$ = 200) | 1.89 | 55.0 | 12.5 | 786.4 | 2.2 | 2.3 | 17.9 | 287.0 | 6.7 | 147.6 | 98.0 | 39.6 | |
| 40+ weeks ($N$ = 72) | 1.80 | 63.7 | 12.9 | 1121.0 | 1.8 | 2.3 | 15.9 | 274.7 | 8.3 | 151.1 | 103.4 | 53.2 | |
| $P$-value | 0.85 | 0.24 | 0.62 | **0.016** | 0.66 | 0.81 | 0.38 | 0.32 | 0.57 | 0.63 | 0.78 | 0.19 | |
| Season of birth | | | | | | | | | | | | | |
| Spring ($N$ = 86) | 1.96 | 45.9 | 12.3 | 717.4 | 4.2 | 4.2 | 12.5 | 391.8 | 7.9 | 140.3 | 106.6 | 39.6 | |
| Summer ($N$ = 57) | 1.36 | 69.7 | 15.0 | 1184.1 | 4.9 | 4.4 | 27.8 | 343.0 | 8.1 | 227.1 | 110.3 | 68.2 | |
| Fall ($N$ = 55) | 2.14 | 63.9 | 15.8 | 785.3 | 1.8 | 1.8 | 16.3 | 223.9 | 4.9 | 159.4 | 87.1 | 44.5 | |
| Winter ($N$ = 74) | 2.13 | 55.3 | 8.9 | 570.3 | 1.8 | 1.8 | 12.8 | 211.4 | 7.8 | 123.4 | 97.7 | 30.6 | |
| $P$-value | **0.0027** | 0.26 | 0.34 | **0.0010** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | 0.19 | < 0.0001 | 0.79 | **0.046** | |
| Gender | | | | | | | | | | | | | |
| Male ($N$ = 152) | 1.83 | 65.6 | 15.3 | 803.6 | 2.5 | 3.0 | 18.3 | 271.5 | 7.7 | 158.2 | 103.8 | 47.6 | |
| Female ($N$ = 120) | 1.88 | 53.5 | 11.7 | 847.4 | 1.8 | 1.8 | 16.3 | 298.9 | 4.8 | 141.8 | 98.0 | 40.3 | |
| $P$-value | 0.88 | 0.15 | 0.68 | 0.58 | 0.23 | 0.11 | 0.43 | 0.39 | 0.81 | 0.060 | 0.85 | 0.60 | |
| Maternal factors | | | | | | | | | | | | | |
| Maternal age at birth | | | | | | | | | | | | | |
| <30 ($N$ = 103) | 1.84 | 53.1 | 13.9 | 833.4 | 1.9 | 2.5 | 16.9 | 255.3 | 7.4 | 141.1 | 90.5 | 40.3 | |
| 30+ ($N$ = 169) | 1.83 | 63.0 | 11.9 | 831.7 | 2.4 | 1.9 | 17.1 | 292.4 | 6.7 | 157.0 | 107.3 | 43.9 | |
| $P$-value | 0.63 | 0.49 | 0.42 | 0.80 | 0.58 | 0.56 | 0.99 | 0.36 | 0.81 | 0.49 | 0.10 | 0.96 | |
| Processing factors | | | | | | | | | | | | | |
| Clotting in sample | | | | | | | | | | | | | |
| Yes ($N$ = 142) | 1.52 | 51.5 | 13.1 | 897.5 | 2.9 | 2.1 | 18.1 | 310.9 | 5.8 | 162.1 | 93.9 | 33.0 | |
| No ($N$ = 130) | 2.29 | 66.5 | 11.9 | 784.4 | 1.8 | 2.7 | 16.3 | 265.4 | 7.9 | 143.4 | 110.6 | 77.4 | |
| $P$-value | **<0.0001** | 0.12 | 0.87 | 0.53 | 0.07 | 0.83 | 0.33 | 0.42 | 0.093 | 0.34 | **0.0066** | **0.0022** | |
| Processing time | | | | | | | | | | | | | |
| <1 h ($N$ = 139) | 1.77 | 65.8 | 11.7 | 719.5 | 3.2 | 1.8 | 24.9 | 385.8 | 4.2 | 167.0 | 83.9 | 24.7 | |
| 12+ h ($N$ = 133) | 1.91 | 54.8 | 12.9 | 843.3 | 2.0 | 2.4 | 16.6 | 274.7 | 7.4 | 149.6 | 104.7 | 46.0 | |
| $P$-value | 0.35 | 0.10 | 0.28 | 0.55 | **0.0009** | 0.91 | 0.19 | 0.21 | 0.74 | 0.18 | 0.46 | **0.0004** | |
| Caesarean delivery | | | | | | | | | | | | | |
| Yes ($N$ = 37) | 1.73 | 65.8 | 11.7 | 719.5 | 3.2 | 1.8 | 24.0 | 385.8 | 4.2 | 167.0 | 83.9 | 24.7 | |
| No ($N$ = 235) | 1.91 | 54.8 | 12.9 | 843.3 | 2.0 | 2.4 | 16.6 | 274.7 | 7.4 | 149.6 | 104.7 | 46.0 | |
| $P$-value | 0.091 | 0.57 | 0.58 | 0.60 | 0.29 | 0.59 | **0.029** | 0.11 | 0.69 | 0.25 | 0.30 | 0.22 | |

*Insufficient number of samples with detectable levels of IL-10 for analysis. Bold indicates statistically significant at $P$<0.05.
PHA, phytohaemagglutinin; SAC, *Staphylococcus aureus* cells; PMA, phorbal myristate acetate/ionomycin.

($P$<0.0001). Conversely, a longer processing time predicted higher levels of SAC-induced IL-10 (46.0 vs. 24.7 pg/mL, $P$ = 0.0004) in univariate models, and this relationship remained significant in multivariate analyses ($P$ = 0.0047).

Additional significant relationships were found with pet ownership, although the magnitudes of the differences were small (see Table 2). Cat ownership at birth was associated with lower levels of PHA-induced IL-5 (1.8 vs. 2.5 pg/mL, $P$ = 0.039 for the univariate model, $P$ = 0.0061 in the multivariate model). Having a dog in the home at birth was predictive of higher levels of SAC-induced IFN-γ (19.5 vs. 10.2 pg/mL, $P$ = 0.019 for the univariate model, $P$ = 0.017 for the multivariate model).

Some previously reported associations between maternal or perinatal factors and cord blood immune responses were not present in our study population (see Table 2). Specifically, we found no significant link between a maternal history of asthma or allergies [11] or maternal smoking status during pregnancy [23] and any stimulated cytokine levels in uni- or multivariate models. We also did not find any relationship between method of delivery [24], or neonate characteristics such as gender or gestational age [25], and cord blood responses (see Table 1).

**Table 2.** Influence of atopy-related variables on median cell and cytokine yields

| | Cell yield ($10^5$ cells/mL) | IFN-γ (pg/mL) | | | IL-5 (pg/mL) | | | IL-13 (pg/mL) | | | IL-10 (pg/mL) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PHA | SAC | PMA | PHA | SAC | PMA | PHA | SAC | PMA | PHA | SAC | PMA* |
| Family history | | | | | | | | | | | | | |
| Maternal asthma | | | | | | | | | | | | | |
| Yes ($N = 114$) | 1.84 | 63.1 | 12.1 | 787.4 | 2.2 | 2.4 | 17.3 | 268.5 | 6.8 | 145.8 | 99.0 | 46.0 | |
| No ($N = 158$) | 1.83 | 54.0 | 14.7 | 1050.6 | 1.8 | 1.8 | 16.3 | 345.8 | 7.3 | 158.2 | 107.7 | 32.6 | |
| *P*-value | 0.96 | 0.83 | 0.39 | 0.43 | 0.87 | 0.19 | 0.92 | 0.90 | 0.070 | 0.77 | 0.78 | 0.067 | |
| Maternal allergy | | | | | | | | | | | | | |
| Yes ($N = 224$) | 1.85 | 64.5 | 13.2 | 697.0 | 2.3 | 3.0 | 17.2 | 265.9 | 9.3 | 141.2 | 103.6 | 62.1 | |
| No ($N = 48$) | 1.81 | 55.2 | 12.1 | 874.4 | 2.1 | 1.8 | 16.9 | 291.6 | 6.2 | 156.9 | 96.9 | 35.9 | |
| *P*-value | 0.70 | 0.73 | 0.51 | 0.11 | 0.26 | 0.94 | 0.72 | 0.073 | 0.40 | 0.85 | 0.79 | 0.24 | |
| Prenatal exposures | | | | | | | | | | | | | |
| Dog in the home | | | | | | | | | | | | | |
| Yes ($N = 94$) | 2.14 | 63.9 | 19.5 | 787.4 | 2.0 | 2.5 | 17.3 | 305.3 | 8.5 | 159.4 | 99.5 | 42.1 | |
| No ($N = 178$) | 1.69 | 55.0 | 10.2 | 841.5 | 2.1 | 2.1 | 16.9 | 271.5 | 6.2 | 144.8 | 102.4 | 42.1 | |
| *P*-value | **0.0088** | 0.25 | **0.019** | 0.95 | 0.65 | 0.67 | 0.92 | 0.36 | 0.19 | 0.85 | 0.61 | 0.61 | |
| Cat in the home | | | | | | | | | | | | | |
| Yes ($N = 79$) | 1.78 | 66.2 | 12.1 | 926.4 | 1.8 | 1.8 | 16.3 | 251.8 | 5.6 | 129.5 | 98.0 | 53.6 | |
| No ($N = 193$) | 2.13 | 58.4 | 12.9 | 821.8 | 2.5 | 3.0 | 17.6 | 300.2 | 7.0 | 159.5 | 104.0 | 40.3 | |
| *P*-value | 0.13 | 0.82 | 0.92 | 0.80 | **0.039** | 0.15 | 0.54 | 0.21 | 0.62 | 0.083 | 0.59 | 0.81 | |
| Maternal smoking | | | | | | | | | | | | | |
| Yes ($N = 67$) | 1.70 | 48.5 | 14.2 | 835.6 | 2.6 | 2.5 | 15.8 | 399.5 | 13.5 | 187.6 | 118.8 | 106.7 | |
| No ($N = 205$) | 1.92 | 63.5 | 12.2 | 831.7 | 2.0 | 2.1 | 17.6 | 297.8 | 7.9 | 154.0 | 105.0 | 65.2 | |
| *P*-value | 0.22 | 0.36 | 0.54 | 0.49 | 0.48 | 0.81 | 0.97 | 0.45 | 0.56 | 0.72 | 0.92 | 0.14 | |

*Insufficient number of samples with detectable levels of IL-10 for analysis. Bold indicates statistically significant at $P<0.05$.
PHA, phytohaemagglutinin; SAC, *Staphylococcus aureus* cells; PMA, phorbal myristate acetate/ionomycin.

## Discussion

The purpose of these analyses was to identify the familial, environmental and technical factors that influence the yield and cytokine responses of CBMCs. Interestingly, the strongest and most consistent finding was that the season of the year in which cord blood samples were collected had significant associations with both these outcomes. Cell yields were the highest in the fall and winter months, while IL-5 and IL-13 responses were the highest in spring and summer. The seasonal effect on cytokines was stronger in multivariate analysis after controlling for clotting and seasonal effects on cell yields.

Other factors also seemed to influence cell yields and cytokine responses. Pet ownership was significantly related to cell yield and IFN-γ and IL-5 levels, although the absolute differences in levels between pet owners and non-owners were small in magnitude and not consistent across stimulants. Certain characteristics of the blood specimen and processing appeared to affect cord blood results as well. Specifically, the presence of any degree of clotting was inversely related to cell yields. Clotting also appeared to result in lower IL-5 and IL-10 responses, although this may be a consequence of the lower cell yields in samples where clotting was present. Furthermore, longer (>12 h) intervals between collection and processing were associated with reductions in IL-5 and increased IL-10 responses to some stimuli. Collectively, these associations indicate the importance of considering the influence of technical factors in the interpretation of cord blood responses.

The question of why season of birth is associated with cytokine yields requires further research. The seasonal nature of the IL-5 and IL-13 responses suggests that maternal exposures might influence cord blood cytokine response profiles. Exposure to outdoor aeroallergens, particularly grass and tree pollens, is high in the spring and early summer, the time period during which our cord blood samples exhibited the highest IL-5 and IL-13 responses. At the same time, the seasonal trend could be influenced by maternal immune response to viral or bacterial pathogens that occur in the fall and winter, perhaps suppressing T-helper type 2 (Th2) responses during the same timeframe. The fact that both these Th2 cytokine responses peaked at the same time, in conjunction with previous studies that have found a similar peak in cord blood IgE during the late spring and early summer months, suggests that cord blood cytokine yields may be sensitive to factors in the maternal environment [20–23].

Some previously reported relationships between parental/environmental factors and cord blood responses were not present in our study [24–28]. For example, we found

 

no association between maternal smoking, method of delivery or parental history and cell yields/cytokine responses. However, several factors may account for these differing results. First, in spite of our large sample size, the small group size for some of our independent variables may have limited the power to detect a difference between groups. For example, only a few of the study subjects were delivered by caesarean section (13%) or were born to mothers who smoked during pregnancy (25%). Technical factors may also have played a role. Currently, there are no standardized methods for processing and testing cord blood samples, and hence differences in the way in which the samples were handled may have led to different results. Finally, the cohort under study in these analyses consisted solely of children at high risk for the development of allergies and/or asthma due to a parental history of atopic disease. Some of the associations between environmental or genetic factors and cell yield or cytokine responses may only manifest themselves when a non-atopic cohort is analysed as a comparator group.

In summary, season of collection, and time from collection to processing may account for some variability in cord blood samples and their response to stimuli. Accounting for these factors in analyses that attempt to link cord blood proliferative or cytokine responses to a family history of atopy and/or exposures to allergens may allow for clearer characterization of these relationships and may provide a new insight into the predictive value of cord blood samples.

## Acknowledgements

This work was supported by National Institutes of Health grants, R01 HL61879-01, P01 HL70831-01, and General Clinical Research Center grant National Institutes of Health/National Center for Research Resources, M01 RR03186-16.

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