Published in final edited form as:
Pediatrics. 2007 January ; 119(1): e171–e178.

Cord Blood Cytokines and Acute Lower Respiratory Illnesses in the First Year of Life

Ngoc P. Ly, MD, MPH[a,b], **Sheryl L. Rifas-Shiman, MPH**[c], **Augusto A. Litonjua, MD, MPH**[a], **Arthur O. Tzianabos, PhD**[a], **Bianca Schaub, MD**[d], **Begoña Ruiz-Pérez, PhD**[a], **Kelan G. Tantisira, MD, MPH**[a], **Patricia W. Finn, MD**[e], **Matthew W. Gillman, MD, SM**[c], **Scott T. Weiss, MD, MSc**[a], and **Diane R. Gold, MD, MPH**[a]

a *Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts*

b *Division of Pediatric Pulmonary Medicine, Massachusetts General Hospital for Children and Harvard Medical School, Boston, Massachusetts*

c *Department of Ambulatory Care and Prevention, Harvard Pilgrim Health Care and Harvard Medical School, Boston, Massachusetts*

d *Department of Pulmonary Medicine, University Children's Hospital Munich, Munich, Germany*

e *Pulmonary and Critical Care Division, University of San Diego Medical Center, San Diego, California*

Abstract

OBJECTIVES—Little is known about the relation between cytokine profile at birth and acute lower respiratory illnesses in the first year of life. The purpose of this work was to examine the relation between cytokine secretions by cord blood mononuclear cells and acute lower respiratory illness in a birth cohort of 297 children.

METHODS—Cord blood mononuclear cells were isolated, and secretion of interferon-γ, interleukin-13, interleukin-10, and tumor necrosis factor-α at baseline and in response to allergens (*Blatella germanica 2* and *Dermatophagoides farinae 1*) and mitogen (phytohemagglutinin) were quantified using enzyme-linked immunosorbent assay. Acute lower respiratory illness was defined as a parental report of a diagnosis of bronchiolitis, pneumonia, bronchitis, and/or croup by a health care professional in the first year of life. Differences in the levels of cord blood cytokines between children with and without acute lower respiratory illness were examined using 2-sample Wilcoxon tests. Logistic regression models were used to examine the relation between various categories of cord blood cytokines and acute lower respiratory illness.

RESULTS—Median levels of interferon-γ secreted by cord blood mononuclear cells in response to *Blatella germanica 2* and *Dermatophagoides farinae 1* were higher among children without acute lower respiratory illness as compared with children with acute lower respiratory illness. After adjustment for other covariates, the odds of acute lower respiratory illness was reduced among children in the top category (at or more than the median of detectable values) of interferon-γ level, significantly so in response to *Blatella germanica 2*.

CONCLUSIONS—In a cohort of children from the general population, we found that upregulated interferon-γ secretion at birth is associated with reduced risk of acute lower respiratory illness in the first year of life.

Address correspondence to Ngoc P. Ly, MD, MPH, 181 Longwood Ave, 4th Floor, Boston, MA 02115. E-mail: ngoc.ly@channing.harvard.edu

The author have indicated they have no financial relationships relevant to this article to disclose.

Keywords

lower respiratory illnesses; cytokines; neonates; IFN-γ

Respiratory illnesses because of viral infections are common in children, especially in the first year of life. Low birth weight, premature birth, male gender, day care attendance, and having older siblings are some of the factors that are associated with respiratory illnesses.[1–4] Despite these risk factors, however, some children tend to have more frequent or severe respiratory illnesses than others. Prenatal and early life immune system development may influence a child's subsequent clinical and/or immunologic responses to viral pathogens.

Reduced interferon-*γ* (IFN-*γ*)[5–7] and increased interleukin (IL)-4[7,8] production by peripheral blood mononuclear cells (PBMCs) are associated with acute viral respiratory infections, including viruses such as respiratory syncytial virus (RSV), parainfluenza, and influenza. Moreover, defective IFN-*γ* secretion by PBMCs measured during acute infection has been associated with severe respiratory illnesses.[9–11] These data suggest that viruses influence immune responses by reducing T-helper 1 (Th1) responses and possibly increasing T-helper 2 (Th2) responses. However, most studies on cytokines and respiratory illnesses have been cross-sectional, with cytokines measured during the acute respiratory infection. Therefore, it is unclear whether viral infections influence subsequent immune responses or whether early immune responses influence the development of later respiratory infections. We hypothesized that reduced secretion of Th1 cytokine (IFN-*γ*) and/or increased secretion of Th2 cytokines (IL-4 or IL-13) represent a preexisting immune imbalance associated with increased risk for subsequent viral infections.

There are few studies on the relation between neonatal immune responses and acute lower respiratory illness (LRI) in the first year of life. In a Wisconsin study of infants selected on the basis of a parental history of allergies or asthma, increased phytohemagglutinin-stimulated IFN-*γ* secretion by cord blood mononuclear cells (CBMCs) was weakly associated with fewer symptomatic viral respiratory infections (ie, having a score ≥5 of 31 on a respiratory symptom scorecard).[12] To our knowledge, there are no previous prospective studies on neonatal cytokines and respiratory infection in the first year of life among children not selected on the basis of a parental history of asthma or allergies. Here we examine the relationship between neonatal secretions of IFN-*γ*, IL-13, IL-10, and tumor necrosis factor (TNF)-*α* at baseline (unstimulated) and in response to stimulation by allergens (*Blatella germanica 2* [*Bla g 2*] and *Dermatophagoides farinae 1* [*Der f 1*]) and mitogen (phytohemagglutinin) and parental reports of a diagnosis of acute LRI in the first year of life in a cohort of children not selected on the basis of a parental history of allergies or asthma.

METHODS

Study Population

Study subjects were a subgroup of participants from Project Viva, a prospective cohort study of women and their children in the Boston, Massachusetts, metropolitan area. Subjects were enrolled between April 1999 and July 2002. Detailed enrollment criteria were described previously.[13] Briefly, pregnant women were approached at their initial prenatal visit. Consent was obtained for their participation, including cord blood collection and longitudinal follow-up of their offspring. Exclusion criteria included multiple gestation, inability to answer questions in English, plans to move out of the area before delivery, and gestational age >22 weeks at initial prenatal clinic appointment. This study was approved by the institutional review boards of Brigham and Woman's Hospital and Harvard Pilgrim Health Care.

Demographic, Birth, and Parental Conditions and Other Variables

Parental demographic and health history information were collected by interview and self-administered questionnaires. Data on birth weights and NICU admissions were obtained from hospital records. Questionnaires about the infant health, day care, and the home environment were administered both when the child was 6 months and 1 year old.

Definition of 1-Year Outcome Variables

Acute LRI was defined as a parental report of a diagnosis of bronchiolitis, pneumonia, bronchitis, and/or croup by a health care professional (eg, a doctor, physician assistant, or nurse practitioner) in the first year of life. Wheeze was defined as a report of wheezing (or whistling in the chest) in the first year of life. Eczema was defined as a parental report of a diagnosis of eczema by a health care professional (eg, a doctor, physician assistant, or nurse practitioner) in the first year of life.

Cord Blood Samples

Cord blood samples were collected by needle/syringe from the umbilical vein after delivery. Samples were processed fresh, noncryopreserved within 24 hours, and CBMCs were isolated from umbilical cord blood by density gradient centrifugation with Ficoll-Hypaque Plus (Pharmacia, Uppsala, Sweden).

Cytokine Measurements

Aliquots of 5.0×10^5 CBMCs were incubated in triplicate in 96-well round-bottom tissue culture plates (Corning, New York, NY) at 37°C in 5% CO_2. At the start of the culture, cells were either unstimulated (media) or stimulated with each of the following antigens: cockroach (*Bla g 2*) at 30 μg/mL, house dust mite (*Der f 1*) at 30 μg/mL, and phytohemagglutinin at 5 μg/mL. Cell supernatant fluids were harvested 72 hours after stimulation and analyzed for cytokine (IFN-γ, IL-13, IL-10, and TNF-α) production by enzyme-linked immunosorbent assay (Endogen, Rockford, IL) according to the manufacturer's instructions. The sensitivities of the assays were <2 pg/mL for IFN-γ, <7 pg/mL for IL-13, <3 pg/mL for IL-10, and <2 pg/mL for TNF-α. Values that fell within the linear portion of the standard curve generated by a set of standards were considered to be detectable values. Values that fell below the linear portion of the standard curve were considered to be below the limits of detection.

Statistical Analyses

The χ^2 test was used to compare between-group proportions. An unpaired *t* test was used to compare between-group means. The distribution of cytokine levels was statistically skewed, with a significant number of undetectable values; therefore, median cytokine levels were presented, and differences in the levels of cord blood cytokines between children with and without acute LRI were examined using nonparametric 2-sample Wilcoxon tests. Wilcoxon testing is based on the ranks of the pairwise differences between the 2 samples and does not require the assumption of normal distribution of data.[14] In complementary analyses allowing for adjustment of potential confounders, we estimated the odds of acute LRI by categories of IFN-γ, comparing levels that are at or more than and less than the median of all detectable values to undetectable levels of IFN-γ, using stepwise logistic regression. In the final logistic models, we included variables that were significant at $P < .05$ or that satisfied a change in estimate criterion (≥10%) in the odds ratio (OR). All of the analyses were performed using SAS 9 (SAS Institute, Cary, NC).

The following variables were considered in multivariate models of the relationship between acute LRI and cytokine productions: race, gender, gestation age, birth weight, NICU admission, maternal smoking during pregnancy, breastfeeding in the first year of life (yes versus no), day

care attendance, numbers of children in household <12 years of age (≤1 vs >1), maternal age, and maternal/paternal history of allergies and asthma.

RESULTS

Study Cohort

Among the 446 subjects with any cytokine measurement at birth, 297 had follow-up at age 1 year (Table 1). Subjects with follow-up were more likely to be white and to live in households with an annual income more than $40 000 compared with those without follow-up. Fewer mothers smoked during pregnancy among subjects with follow-up than among subjects without follow-up. There were no significant differences between children with and without follow-up with respect to gender, parental history of asthma, eczema, hay fever, maternal age, and infant gestational age and birth weight. Furthermore, the median cytokine levels (IFN-γ, IL-13, TNF-α, and IL-10) did not differ between children who were included as compared with those who were not included in the analysis.

Of the 293 subjects who have information on acute LRI in the first year of life, 87 (29.7%) had a parental report of acute LRI diagnosed by a health care professional (Table 1). Children with acute LRI were more likely to wheeze ($P < .01$). For example, among the 87 children with a report of having acute LRI, 50 (57.5%) had ≥1 episode of wheeze by age 1 year. Increasing gestational age was associated with reduced risks of acute LRI (OR: 0.82; 95% confidence interval [CI]: 0.71–0.95). Children living with >1 sibling under the age of 12 years were more likely to have acute LRI (OR: 1.95; 95% CI: 1.17–3.25). Black children were less likely to have acute LRI than white children ($P < .05$). This is most likely because of the fact that study participants were less likely to be black. Maternal smoking during pregnancy; maternal age at pregnancy; maternal/paternal history of asthma, eczema, or allergies; birth weight; admission to the NICU during the neonatal period; breastfeeding in the first year of life; day care attendance in the first year of life; and a diagnosis of eczema in the child were not associated with acute LRI.

Cord Blood Cytokines and Acute LRI

Information on the number of cord blood IFN-γ, IL-13, IL-10, and TNF-α values below and above the limit of detection, as well as the median levels and ranges of detectable cytokines, are provided in Table 2. The percentage of children with acute LRI in the first year of life by categories of IFN-γ is depicted in Fig 1. The number of children with diagnosed acute LRI decreased from undetectable to at or more than the median of all detectable levels of cord blood IFN-γ (at baseline [media] and after stimulation with *Bla g 2*, *Der f 1*, and phytohemagglutinin).

We found that the median levels of IFN-γ secreted by CBMCs in response to *Der f 1* were significantly higher among children who did not have acute LRI as compared with children who had acute LRI in the first year of life (Table 3). The median levels of IFN-γ secreted by CBMCs at baseline and in response to *Bla g 2* and phytohemagglutinin were higher among the children without acute LRI in the first year of life, although such differences were not statistically significant. We did not find significant differences among IL-13, IL-10, and TNF-α between children with and without acute LRI (Table 3).

In Table 4, we summarize the results of the univariate and multivariate analyses of the relation between categories of IFN-γ level and the odds of having acute LRI in the first year of life. Univariate and multivariate analyses suggested a trend for reduced odds of acute LRI in the first year of life with increasing IFN-γ, although the P value for trend was not always<.05. In univariate models, the odds of having acute LRI were significantly lower among children in the top category (at or more than the median detectable level) compared with children in the

lowest category (undetectable level) of IFN-γ secretion by CBMCs at birth in response to *Bla g 2* (OR: 0.4; 95% CI: 0.2−0.9) and to *Der f 1* (OR: 0.4; 95% CI: 0.2−0.9). In multivariate models adjusted for race, gender, gestation age (weeks), and having >1 sibling under the age of 12 years in the home, the odds of having acute LRI in the first year of life was reduced among children in the top category of IFN-γ, significantly so in response to *Bla g 2* (OR: 0.4; 95% CI: 0.2−0.9). There were no differences between the different categories of IL-13, IL-10, and TNF-α and their relations to acute LRI in the first year of life (data not shown).

In multivariate models, increasing gestational age was independently associate with reduced risk for acute LRI ($P < .05$), and having >1 sibling under the age of 12 years in the household was associated with an approximately threefold increase in the odds for acute LRI in the first year of life ($P < .05$). Although we found a significant inverse association between cord blood IFN-γ secretion and acute LRI, we found no association between cord blood IFN-γ secretions and wheeze or eczema in the first year of life (data not shown).

DISCUSSION

In a cohort of children not selected on the basis of a parental history of allergies or asthma, we found an inverse relationship between cord blood IFN-γ levels and acute LRI in the first year of life. Children with high levels of IFN-γ secreted by CBMCs at birth, particularly in response to *Bla g 2* stimulation, had significantly reduced risk for acute LRI compared with children with low or undetectable levels. Th1 immune responses with the elaboration of cytokines, such as IFN-γ, are important in host defense again viral pathogens. Previously, it has been shown that severe bronchiolitis because of RSV infection was associated cross-sectionally with defective IFN-γ secretion by PBMCs measured during the acute infection.[10,11] However, when and how this defect in Th1 immune responses occurs is still not clear.

It is generally believed that Th2 immune responses, which are important in maintaining pregnancy, predominate at birth with less well-developed accompanying Th1 immune responses.[15,16] Reduced Th1 immune responses at birth may explain the overall increased risk for infections in the first year of life and the need for maternal antibodies to viruses. Moreover, our data add to the evidence that even at birth, some children may have less well-developed Th1 immune responses than others, and this heightened reduction in IFN-γ may influence the clinical response to exposure to acute LRI in the first year of life. In a previous prospective study of children with ≥ 1 parent with allergy or asthma (Wisconsin study),[12] investigators found an inverse correlation between phytohemagglutinin-stimulated IFN-γ production by CBMCs and the number of moderate-to-severe viral infections in the first year of life ($r_s = -0.11$; $P = .05$). In contrast to the Wisconsin study, 41.1% of families in our cohort had neither parent with hay fever or asthma. However, despite the fact that our cohort was not preselected for a family history of allergy, we found similar inverse associations of IFN-γ production with risk for acute LRI. In contrast to the Wisconsin study, our strongest associations were for IFN-γ production by CBMCs in response to allergen stimulation. After controlling for potential confounders, the odds for acute LRI in the first year of life were reduced by 60% in children who had the highest levels compared with children who had undetectable levels of IFN-γ secretion at birth in response to *Bla g 2*. Similar to the Wisconsin study,[12] we found no association between IL-13 or IL-10 levels at birth and acute LRI in the first year of life. We also found no relationship between levels of TNF-α secreted by CBMCs and risk for acute LRI in the first year of life. Despite differences between our study and that of the Wisconsin group, including differences in subject selection, assessment of acute LRI in the first year of life, and choice of antigens used to stimulate CBMCs, the consistent conclusion from our study and theirs is that upregulated IFN-γ (Th1 cytokine) secretion at birth, before any infections of the child, is protective against acute LRI in the first year of life. If reduced neonatal IFN-γ is in the pathway to increased risk of respiratory infection in early life, the

relative contribution of heredity versus the environment toward reduced IFN-γ at birth is not well understood.

The reason for frequently finding the strongest and/or most statistically significant associations between cytokine secretion by CBMCs after stimulation with dust mite (*Der f 1*) and cockroach (*Bla g 1*) allergens but not after stimulation with mitogen (phytohemagglutinin) is not clear. It is plausible that these 2 inhalant allergens are more widely distributed in the environment, and, therefore, maternal exposure to these allergens during pregnancy results in transplacental transfer of these allergens to the fetus leading to allergen-specific T-cell priming in utero.[17,18] Alternatively, it is possible that the mother's specific immune response to allergens, which is known to be transferred across the placenta, influences the CBMC response to these specific allergens. We have demonstrated in another cohort study that maternal IgG antibodies to specific allergens, such as cat,[19] can be passively transferred from the mother to the child. Finally, it is possible that naïve cord blood T-cells, perhaps in interaction with monocytes/dendritic cells, have different responses to newly encountered allergens/antigens than they would to a mitogen and that these responses are more predictive of future encounters with infectious agents.

In keeping with findings by other investigators,[2,12,20] we found that most children who had a diagnosis of acute LRI were more likely to have a report of wheeze in the first year of life. However, we, like others, found no association between cord blood cytokine levels and wheeze in the first year of life.[12,21] We also found no association between cord blood cytokine levels and eczema in the first year of life. The lack of association between neonatal cord blood cytokines and wheeze/eczema suggests either that the immune mechanisms involved in defining atopy/wheeze are more complex than those defined by the Th1/Th2 immune responses at birth or that the immune mechanisms involved in defining atopy/wheeze are influenced by factors during the postnatal rather than prenatal or perinatal periods. For example, in 1 birth cohort study, investigators found no association between IFN-γ levels measured at birth and wheeze in the first year of life. They did, however, find an association between reduced IFN-γ measured subsequently at 3 months of age and elevated risk of wheeze in the first year of life.[21] Another study found no association between IL-13 and IL-5 measured at birth and atopic markers, such as absolute eosinophil count and total immunoglobulin E at age 1 year, but did find a cross-sectional association between increased levels of IL-5 and IL-13 at age 1 year and atopic markers of allergy at age 1 year.[22] Moreover, in studies that have demonstrated an association between reduced cord blood Th1 cytokines[23,24] and/or increased Th2 cytokines[25,26] and atopy or asthma, the diagnoses of atopy and asthma were made in early childhood and not in the first year of life, when transient wheeze is related to airway size[27–29] and does not necessarily represent a tendency to asthma. In our own study, we examined the relations between cytokines and acute LRI with and without wheeze in the first year of life and found no significant differences between any of the cytokines and acute LRI with either wheeze or no wheeze, but the results were limited by reduced statistical power because of sample size (data not shown).

Similar to findings from previous studies, we found an association between having ≥ 1 young sibling in the home and acute LRI in early life.[30,31] Although previous studies have found an association between very premature birth (<33 weeks' gestation) and risk for acute LRI in early childhood,[1,32] we found that increasing gestational age was inversely associated with acute LRI in the first year of life even among a cohort of children with mean gestational age of 39.6 $\pm$ 1.7 weeks.

We recognize several limitations to our findings. First, we assessed acute LRI in the first year of life using parental reports of a clinical diagnosis of respiratory infection. This is a standard epidemiologic method[33,34] for evaluating clinically significant LRIs, almost all of which are

likely to have been initiated by an infection. Although we were conservative in our assumptions, some investigators label the reporting of doctor-diagnosed croup, bronchiolitis, bronchitis or pneumonia as "respiratory infection" because of the high likelihood that this is what they represent.[35,36] Because we did not assess asymptomatic infections, the inverse association between IFN-γ levels and acute LRI could either be related to reduced events or reduced severity of acute LRI. However, children who had a clinical diagnosis of acute LRI by a health care professional in our study were probably more likely to have severe infections that required medical attention. A second related limitation is the fact that we did not isolate specific viruses associated with the diagnosis of acute LRI; therefore, we could not determine whether the association between IFN-γ and acute LRI is related to specific viruses. However, data have shown that clinically diagnosed acute LRI before age 1 year are most commonly caused by/initiated by RSV,[37,38] rhinoviruses,[39–41] influenza,[6,8] and parainfluenza.[8] Moreover, as additional viruses are identified, an increasing proportion of clinical LRIs are being found to be initiated by viruses expressed as clinical illness in susceptible children.

An additional limitation of this study is that whereas all of the cytokine levels that we treat as measurable fell within the linear portion of the standard curve, we recognize that the lowest (and highest) values may be measured with less precision than those in the midrange. A final potential limitation considered was the potential that contamination with lipopolysaccharide may influence the interpretation of cytokine responses. We tested the allergens *Der f 1* and *Bla g 2*, as well as the mitogen phytohemagglutinin for endotoxin contents by Limulus assay and found low concentrations of endotoxin (<0.01 EU/mL = 0.002 ng/mL). These concentrations did not significantly change cytokine secretion in CBMCs. In addition, we also assessed cytokine secretion of TNF-α and IL-6, 2 cytokines that typically increased after stimulation with lipid A (an active component of lipopolysaccharide). These cytokines were not increased with lipid A as low as 0.002 ng/mL, as detected in our reagents. Thus, our data suggest that endotoxin content had a minimal influence on the outcomes of cytokine secretion.

CONCLUSIONS

Our findings suggest that upregulated IFN-γ (Th1 immune response) secretion at birth is associated with reduced risk of acute LRI in the first year of life. Factors that influence immune mechanisms in the prenatal and/or perinatal periods, specifically with the upregulation of Th1 immune responses before exposure with viral pathogens, may have significant impact on the development of acute LRI in early life. With evidence suggesting an association between early life acute LRI and subsequent development of asthma,[36,42] further study is needed to determine prenatal and/or perinatal factors that can influence immune system development.

Acknowledgements

This study was supported by National Institutes of Health grants HD 034568, HL 61907, HL 64925, and HL07427.

References

1. Greenough A, Giffin FJ, Yuksel B, Dimitriou G. Respiratory morbidity in young school children born prematurely—chronic lung disease is not a risk factor? Eur J Pediatr 1996;155:823–826. [PubMed: 8874121]
2. Wright AL, Taussig LM, Ray CG, Harrison HR, Holberg CJ. The Tucson Children's Respiratory Study. II. Lower respiratory tract illness in the first year of life. Am J Epidemiol 1989;129:1232–1246. [PubMed: 2729259]
3. Celedon JC, Litonjua AA, Weiss ST, Gold DR. Day care attendance in the first year of life and illnesses of the upper and lower respiratory tract in children with a familial history of atopy. Pediatrics 1999;104:495–500. [PubMed: 10469775]

4. Rylander E, Eriksson M, Pershagen G, Nordvall L, Ehrnst A, Ziegler T. Wheezing bronchitis in children. Incidence, viral infections, and other risk factors in a defined population. Pediatr Allergy Immunol 1996;7:6–11. [PubMed: 8792378]
5. Renzi PM, Turgeon JP, Marcotte JE, et al. Reduced interferon-gamma production in infants with bronchiolitis and asthma. Am J Respir Crit Care Med 1999;159:1417–1422. [PubMed: 10228104]
6. Joshi P, Shaw A, Kakakios A, Isaacs D. Interferon-gamma levels in nasopharyngeal secretions of infants with respiratory syncytial virus and other respiratory viral infections. Clin Exp Immunol 2003;131:143–147. [PubMed: 12519398]
7. Legg JP, Hussain IR, Warner JA, Johnston SL, Warner JO. Type 1 and type 2 cytokine imbalance in acute respiratory syncytial virus bronchiolitis. Am J Respir Crit Care Med 2003;168:633–639. [PubMed: 12773328]
8. Kristjansson S, Bjarnarson SP, Wennergren G, et al. Respiratory syncytial virus and other respiratory viruses during the first 3 months of life promote a local T(h)2-like response. J Allergy Clin Immunol 2005;116:805–811. [PubMed: 16210054]
9. Schauer U, Hoffjan S, Rothoeft T, et al. Severe respiratory syncytial virus infections and reduced interferon-gamma generation in vitro. Clin Exp Immunol 2004;138:102–109. [PubMed: 15373911]
10. Bont L, Heijnen CJ, Kavelaars A, et al. Local interferon-gamma levels during respiratory syncytial virus lower respiratory tract infection are associated with disease severity. J Infect Dis 2001;184:355–358. [PubMed: 11443563]
11. Aberle JH, Aberle SW, Dworzak MN, et al. Reduced interferon-gamma expression in peripheral blood mononuclear cells of infants with severe respiratory syncytial virus disease. Am J Respir Crit Care Med 1999;160:1263–1268. [PubMed: 10508817]
12. Copenhaver CC, Gern JE, Li Z, et al. Cytokine response patterns, exposure to viruses, and respiratory infections in the first year of life. Am J Respir Crit Care Med 2004;170:175–180. [PubMed: 15087299]
13. Gillman MW, Rich-Edwards JW, Rifas-Shiman SL, Lieberman ES, Kleinman KP, Lipshultz SE. Maternal age and other predictors of newborn blood pressure. J Pediatr 2004;144:240–245. [PubMed: 14760269]
14. Hollander, M.; Wolfe, DA. Nonparametric Statistical Methods. New York, NY: Wiley; 1999.
15. Bjorksten B. The intrauterine and postnatal environments. J Allergy Clin Immunol 1999;104:1119–1127. [PubMed: 10588990]
16. Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol Today 1993;14:353–356. [PubMed: 8363725]
17. Jones AC, Miles EA, Warner JO, Colwell BM, Bryant TN, Warner JA. Fetal peripheral blood mononuclear cell proliferative responses to mitogenic and allergenic stimuli during gestation. Pediatr Allergy Immunol 1996;7:109–116. [PubMed: 9116874]
18. Holt PG, Macaubas C. Development of long-term tolerance versus sensitisation to environmental allergens during the perinatal period. Curr Opin Immunol 1997;9:782–787. [PubMed: 9492978]
19. Jenmalm MC, Holt PG, Bjorksten B. Maternal influence on IgG subclass antibodies to Bet v 1 during the first 18 months of life as detected with a sensitive ELISA. Int Arch Allergy Immunol 1997;114:175–184. [PubMed: 9338612]
20. Sly PD, Hibbert ME. Childhood asthma following hospitalization with acute viral bronchiolitis in infancy. Pediatr Pulmonol 1989;7:153–158. [PubMed: 2797929]
21. 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;169:70–76. [PubMed: 14525803]
22. Neaville WA, Tisler C, Bhattacharya A, 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;112:740–746. [PubMed: 14564354]
23. Prescott SL, King B, Strong TL, Holt PG. The value of perinatal immune responses in predicting allergic disease at 6 years of age. Allergy 2003;58:1187–1194. [PubMed: 14616132]
24. Macaubas C, de Klerk NH, Holt BJ, et al. Association between antenatal cytokine production and the development of atopy and asthma at age 6 years. Lancet 2003;362:1192–1197. [PubMed: 14568741]

25. Rowe J, Heaton T, Kusel M, et al. High IFN-gamma production by CD8+ T cells and early sensitization among infants at high risk of atopy. J Allergy Clin Immunol 2004;113:710–716. [PubMed: 15100678]
26. Lange J, Ngoumou G, Berkenheide S, et al. High interleukin-13 production by phytohaemagglutinin- and Der p 1-stimulated cord blood mononuclear cells is associated with the subsequent development of atopic dermatitis at the age of 3 years. Clin Exp Allergy 2003;33:1537–1543. [PubMed: 14616866]
27. Young S, O'Keeffe PT, Arnott J, Landau LI. Lung function, airway responsiveness, and respiratory symptoms before and after bronchiolitis. Arch Dis Child 1995;72:16–24. [PubMed: 7717730]
28. Hanrahan JP, Brown RW, Carey VJ, Castile RG, Speizer FE, Tager IB. Passive respiratory mechanics in healthy infants. Effects of growth, gender, and smoking. Am J Respir Crit Care Med 1996;154:670–680. [PubMed: 8810604]
29. Martinez FD, Wright AL, Taussig LM, et al. Asthma and wheezing in the first six years of life. N Engl J Med 1995;332:133–138. [PubMed: 7800004]
30. Ball TM, Castro-Rodriguez JA, Griffith KA, Holberg CJ, Martinez FD, Wright AL. Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. N Engl J Med 2000;343:538–543. [PubMed: 10954761]
31. Svanes C, Jarvis D, Chinn S, Omenaas E, Gulsvik A, Burney P. Early exposure to children in family and day care as related to adult asthma and hay fever: results from the European Community Respiratory Health Survey. Thorax 2002;57:945–950. [PubMed: 12403876]
32. Elder DE, Hagan R, Evans SF, Benninger HR, French NP. Recurrent wheezing in very preterm infants. Arch Dis Child Fetal Neonatal Ed 1996;74:F165–F171. [PubMed: 8777678]
33. Monto AS, Napier JA, Metzner HL. The Tecumseh study of respiratory illness. I. Plan of study and observations on syndromes of acute respiratory disease. Am J Epidemiol 1971;94:269–279. [PubMed: 5093655]
34. Gold DR, Weiss ST, Tager IB, Segal MR, Speizer FE. Comparison of questionnaire and diary methods in acute childhood respiratory illness surveillance. Am Rev Respir Dis 1989;139:847–849. [PubMed: 2923381]
35. von Mutius E, Illi S, Hirsch T, Leupold W, Keil U, Weiland SK. Frequency of infections and risk of asthma, atopy and airway hyperresponsiveness in children. Eur Respir J 1999;14:4–11. [PubMed: 10489821][see comments]
36. Illi S, von Mutius E, Lau S, et al. Early childhood infectious diseases and the development of asthma up to school age: a birth cohort study. BMJ 2001;322:390–395. [PubMed: 11179155]
37. Glezen WP, Taber LH, Frank AL, Kasel JA. Risk of primary infection and reinfection with respiratory syncytial virus. Am J Dis Child 1986;140:543–546. [PubMed: 3706232]
38. Hall CB. Respiratory syncytial virus: a continuing culprit and conundrum. J Pediatr 1999;135:2–7. [PubMed: 10431132]
39. Lemanske RF Jr, Jackson DJ, Gangnon RE, et al. Rhinovirus illnesses during infancy predict subsequent childhood wheezing. J Allergy Clin Immunol 2005;116:571–577. [PubMed: 16159626]
40. Heymann PW, Carper HT, Murphy DD, et al. Viral infections in relation to age, atopy, and season of admission among children hospitalized for wheezing. J Allergy Clin Immunol 2004;114:239–247. [PubMed: 15316497]
41. Brooks GD, Buchta KA, Swenson CA, Gern JE, Busse WW. Rhinovirus-induced interferon-gamma and airway responsiveness in asthma. Am J Respir Crit Care Med 2003;168:1091–1094. [PubMed: 12928311]
42. Ponsonby AL, Couper D, Dwyer T, Carmichael A, Kemp A. Relationship between early life respiratory illness, family size over time, and the development of asthma and hay fever: a seven year follow up study. Thorax 1999;54:664–669. [PubMed: 10413716]

Abbreviations

IFN-γ

interferon-γ

IL

	interleukin
PBMC	peripheral blood mononuclear cell
RSV	respiratory syncytial virus
Th1	T-helper 1
Th2	T-helper 2
LRI	lower respiratory illness
CBMC	cord blood mononuclear cell
TNF	tumor necrosis factor
Bla g 2	Blatella germanica 2
Der f 1	Dermatophagoides farinae 1
OR	odds ratio
CI	confidence interval

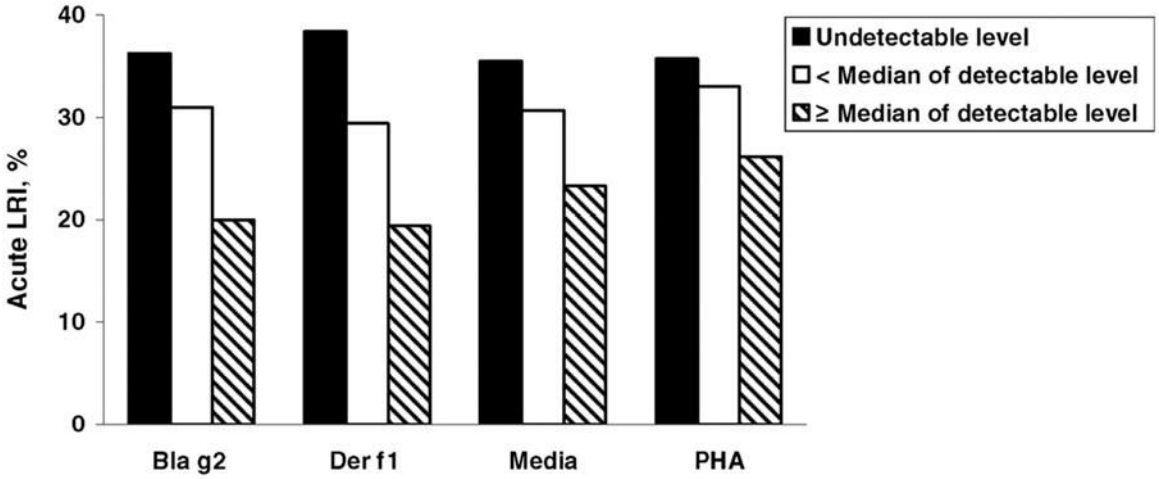

FIGURE 1.

Percent of children with acute LRIs in the first year of life in relation to levels of stimulant-specific cord blood IFN-*γ*. Children with cord blood IFN-*γ* at or above the median of all detectable levels have the lowest percentage of acute LRI in the first year of life compared with those with cord blood IFN-*γ* below the median of all detectable levels and those with undetectable cord blood IFN-*γ*. Note that the categories of IFN-*γ* are stimulant specific, and levels of IFN-*γ* for media are much lower with a significant number of undetectable values as compared with levels for allergens and mitogen stimulants (refer to Table 2 for the median and ranges of stimulant-specific IFN-*γ* levels).

TABLE 1

Characteristics of Study Participants in Relation to Acute LRIs in the First Year of Life

Variable	Total (N = 297), n (%) [a]	Acute LRI (N = 87), n (%)	No Acute LRI (N = 206), n (%)
Gender			
Male	162 (54.5)	52 (59.8)	108 (52.4)
Female	135 (45.5)	35 (40.2)	98 (47.6)
Race			
White	224 (75.4)	72 (82.8)	152 (73.8)
Black	28 (9.4)	3 (3.4)	23 (11.2)[b]
Hispanic	17 (5.7)	4 (4.6)	13 (6.3)
Other	28 (9.4)	8 (9.2)	18 (8.7)
Reports of wheeze in the first year of life	84 (28.6)	50 (57.5)	31 (15.0)[c]
Physician-diagnosed eczema in the first year of life	63 (21.4)	16 (18.4)	45 (21.8)
Maternal history			
Eczema	53 (17.9)	15 (17.2)	36 (17.5)
Asthma	42 (14.1)	11 (12.6)	29 (14.1)
Hay fever	95 (32.0)	31 (35.6)	63 (30.6)
Paternal history			
Eczema	33 (11.1)	11 (12.6)	22 (10.7)
Asthma	43 (14.5)	12 (13.8)	31 (15.0)
Hay fever	104 (35.0)	33 (37.9)	71 (34.5)
Maternal smoking during pregnancy	16 (5.4)	5 (5.7)	11 (5.3)
Ever breastfed in the first year of life	256 (86.2)	77 (88.5)	175 (85.0)
Daycare attendance in the first year of life	146 (49.2)	48 (55.2)	95 (46.1)
Having >1 sibling <12 y of age in the household	150 (50.5)	54 (62.1)	94 (45.6)[c]
Household income, $			
≤40 000	28 (9.4)	6 (6.9)	22 (10.7)
>40 000	248 (83.5)	76 (87.4)	170 (82.5)
Unknown	21 (7.1)	5 (5.7)	14 (6.8)
Neonatal gestational age, wk[d]	39.6 (1.7)	39.2 (2.2)	39.8 (1.36)[b]
Neonatal birth weight, kg[d]	3.53 (0.5)	3.47 (0.57)	3.56 (0.51)
Maternal age, y[d]	32.5 (4.7)	32.9 (4.6)	32.3 (4.8)

[a] Among the 297 study participants with follow-up at age 1 year, 293 have information on lower respiratory.

[b] Associated with lowered acute LRI in the first year of life, $P < .05$.

[c] Associated with increased acute LRI in the first year of life, $P < .05$.

[d] Value are mean (SD).

TABLE 2

Cytokine Values Below and Above the Limits of Detection

Cytokine	Stimulant	Below Limit of Detection (*n*)	Above Limit of Detection			
			Median Level	Less Than Median (*n*)	At or More Than Median (*n*)	Range
IFN-γ	*Bla g 2*	71	5.64	71	71	0.10–2262.32
	Der f 1	75	10.90	68	68	0.21–2924.00
	Media	112	3.44	75	75	0.10–196.20
	PHA	44	14.11	109	109	0.13–3459.00
IL-13	*Bla g 2*	156	17.05	29	29	0.18–422.80
	Der f 1	124	22.75	44	44	0.02–2298–00
	Media	193	9.74	34	35	0.12–210.88
	PHA	17	1310.00	123	124	0.16–8634.36
IL-10	*Bla g 2*	0	22.61	121	121	0.34–1269.00
	Der f 1	1	21.87	118	119	0.34–798.60
	Media	45	2.74	124	125	0.02–1225.00
	PHA	10	29.79	142	143	0.07–1025.00
TNF-α	*Bla g 2*	0	709.70	121	121	0.86–3550.00
	Der f 1	1	727.00	118	119	0.58–3996.00
	Media	5	27.34	144	145	0.23–2435.00
	PHA	0	1046.00	147	148	2.50–3943.00

TABLE 3

Cytokine Secretion by CBMCs in Relation to Acute Lower Median Cytokine Levels

Cytokine	Stimulant	Acute LRI (*n* = 87)		No Acute LRI (*n* = 206)		Wilcoxon *P*
		Cytokine Level, pg/mL	*n*	Cytokine Level, pg/mL	*n*	
IFN-γ	*Bla g 2*	1.25	61	2.89	149	.06
	Der f 1	0.73	61	4.67	147	.02
	Media	0.10	79	1.51	179	.06
	PHA	6.48	79	11.54	179	.14
IL-13	*Bla g 2*	[a]	62	[a]	149	.84
	Der f 1	[a]	62	[a]	147	.31
	Media	[a]	80	[a]	178	.40
	PHA	1498.61	80	1127.00	180	.35
IL-10	*Bla g 2*	24.26	68	19.86	171	.43
	Der f 1	22.01	67	21.73	168	.91
	Media	1.67	87	2.23	203	.27
	PHA	32.91	87	23.89	204	.29
TNF-α	*Bla g 2*	709.10	68	693.30	171	.18
	Der f 1	852.40	67	690.50	168	.30
	Media	25.36	87	26.90	203	.47
	PHA	1124.00	87	1036.00	204	.50

[a] Undetectable cytokine levels (below the limit of detection).

TABLE 4

Univariate and Multivariate Association Between Cord Blood IFN-γ and Acute LRIs in the First Year of Life

Model	Stimulant	OR (95% CI) for Acute LRI			
		IFN-γ Levels, pg/mL			*P* for Trend
		Below Limit of Detection	Less Than Median	At or More Than Median	
Univariate model	*Bla g 2*	Reference	0.8 (0.4–1.6)	0.4 (0.2–0.9)	.04
	Der f 1	Reference	0.7 (0.3–1.4)	0.4 (0.2–0.8)	.02
	Media	Reference	0.8 (0.4–1.5)	0.6 (0.3–1.1)	.08
	PHA	Reference	0.9 (0.4–1.9)	0.6 (0.3–1.4)	.19
Multivariate model[a]	*Bla g 2*	Reference	0.8 (0.4–1.8)	0.4 (0.2–0.9)	.03
	Der f 1	Reference	0.7 (0.3–1.5)	0.5 (0.2–1.1)	.07
	Media	Reference	0.7 (0.4–1.4)	0.5 (0.3–1.1)	.08
	PHA	Reference	0.9 (0.4–1.9)	0.7 (0.3–1.5)	.25

[a] Models were adjusted for race, gender, gestational age, and having >1 sibling <12 years of age living at home.