Parental characteristics, somatic fetal growth, and season of birth influence innate and adaptive cord blood cytokine responses

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Background: Immunologic responses at birth likely relate to subsequent risks for allergic diseases and wheezing in infancy; however, the influences of parental characteristics and prenatal factors on neonatal immune responses are incompletely understood.

Objective: This study investigates potential correlations between urban parental, prenatal, and perinatal factors on innate and adaptive stimuli-induced cytokine responses.

Methods: Five hundred sixty and 49 children of parents with and without allergic disease or asthma, respectively, were enrolled into a prospective birth cohort study (Urban Environment and Childhood Asthma). Cord blood mononuclear cells were incubated with innate and adaptive immune stimuli, and cytokine responses (ELISA) were compared with season of birth, parental characteristics, *in utero* stressors, and fetal growth.

Results: Many cytokine responses varied by season of birth, including 2-fold to 3-fold fluctuations with specific IFN-α and IFN-γ responses. Birth weight was inversely associated with IFN-γ responses to respiratory syncytial virus ($R = -0.16$), but positively associated with IL-8 responses to a variety of innate stimuli ($R = 0.08$-0.12). Respiratory syncytial virus–induced cytokine responses were 21% to 54% lower in children of mothers with asthma. Cytokine responses were generally lower in babies born to parents with allergy/asthma.

Conclusions: Innate cytokine responses are associated with parental allergic or airway disease, somatic fetal growth, ethnicity, and season of birth. Collectively, these findings suggest that urban prenatal exposures and familial factors affect the development of the fetal immune system. (J Allergy Clin Immunol 2009;124:1078-87.)

Key words: *Atopy, immune response, cord blood, birth cohort, gestational age, cytokines*

Immune development is an active process in the prenatal and perinatal periods, and is potentially influenced by genetic factors as well as the intrauterine and perinatal environment. Consequently, at birth the cytokine responses of cord blood mononuclear cells (CBMCs) to innate and adaptive stimuli may reflect these influences. These relationships are of interest because there is evidence that neonatal cord blood cytokine responses not only reflect perinatal exposures but also may predict later risk of wheezing with viral illnesses,[1-3] atopic dermatitis, allergy,[4] and possibly asthma.[5]

Several characteristics related to parents, prenatal exposures, and birth are also related to the subsequent risk of allergic diseases and asthma. For example, maternal asthma[6] and African American or Puerto Rican ethnicity[7] predict increased risk of early childhood asthma. In addition, preterm birth,[8,9] low birth weight for gestational age (somatic growth),[10] and infant adiposity[11] have all been linked to increased early life wheeze and asthma risk, and in separate studies, to skewed neonatal cytokine responses.[12-14]

Moreover, season of birth has been linked to allergy and asthma risk.[15] Each of these factors could represent a complex of *in utero* environmental exposures, epigenetic effects, and for parental influences, genetics. The timing during pregnancy of intrauterine

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Abbreviations used
CBMC: Cord blood mononuclear cell
CpG: Cytosine-phosphate-guanosine
PIC: Polyinosinic-polycytidylic acid
RSV: Respiratory syncytial virus
TLR: Toll-like receptor
URECA: Urban Environment and Childhood Asthma

influences, which may be linked to seasonality of viral and other prenatal exposures,[16] may be important in determining the effects of these influences on immune development.[17]

Collectively, these findings support the hypothesis that certain prenatal exposures modify immune development *in utero* to influence immune responses in the newborn, and ultimately the risk of atopic outcomes in early childhood. Prenatal and perinatal exposures may have their greatest influence on innate (rather than adaptive) immune responses. These responses, which are present at birth and continue to develop in childhood, are likely to be major influences on the subsequent establishment of adaptive immune responses.[18,19] Factors that influence the prenatal development of innate immune responses are incompletely understood.

Prenatal modification of developmental immunity may be especially relevant to children who grow up in urban environments, where there are a host of unfavorable environmental exposures (eg, maternal smoking) and health conditions (eg, maternal asthma, prematurity, poor growth) that could increase the risk of subsequent asthma. Furthermore, most studies on neonatal cytokine patterns and their relation to hereditary and environmental influences have included only white or predominantly white and Mexican American populations.[5,17,20,21] To address these gaps in knowledge, we evaluated an urban cohort of newborns of predominantly African American and Hispanic ethnicity to define the relationship of innate and adaptive CBMC cytokine responses to parental allergic disease and ethnicity, *in utero* stressors, birth measures of fetal growth, and season of birth. We conceived of these rather broad categories of exposures as representing larger groups of potential *in utero* or perinatal stressors that could ultimately skew immune function toward an allergic or asthmatic phenotype.

METHODS

Study population

The Urban Environment and Childhood Asthma (URECA) birth cohort study is designed to identify environmental, lifestyle, and genetic factors that influence immunologic development in early childhood and consequently modify the risk of developing recurrent wheezing and ultimately asthma.[22] The study protocol was approved by Institutional Review Boards at each of the participating institutions, and written, informed consent was obtained before enrollment.

Expectant families were recruited during the prenatal period in 4 large cities: Baltimore, Boston, New York, and St Louis. Selection criteria include residence in an area with >20% residents below the poverty level; mother or father with allergic rhinitis, eczema, and/or asthma; and birth at $\geq$34 weeks gestation. Exclusion criteria included conditions and congenital anomalies that could potentially affect lung or immune system development or function. Recruitment occurred between February 2005 and March 2007: 1853 families were screened, 779 met the eligibility criteria, and 560 were enrolled (Table I).

A separate group of families without allergies or asthma was recruited into the nonatopic comparison group. The eligibility and exclusion criteria were the same except for the absence of parental allergic diseases or asthma. Two hundred forty were screened, 70 met the eligibility criteria and consented to the study, and 49 families were enrolled (Table I).

Measurement of cytokine responses

Collection of cord blood. Cord blood samples were collected in the delivery room by using sterile needles and syringes, and all personnel were trained to use identical collection techniques as previously described.[23] After collection, the blood was transferred from syringes to sterile 50-mL tubes, diluted 1:1 with RPMI 1640 medium containing heparin, and kept at room temperature pending cell separation.

Cell stimulation and cytokine assays. At each research center, mononuclear cells were separated by density gradient centrifugation using Accuspin tubes (Sigma, St Louis, Mo) within 16 hours of collection, and cells (1×10^6 cells/1 mL) were incubated in the presence of medium and specific immune stimulants or medium alone (Table II), as previously reported.[23] After incubation for 24 hours (innate and polyclonal stimuli) or 5 days (antigens), cell supernatant fluids were collected, divided into aliquots, frozen at –80°C, and shipped to a central laboratory for analysis. Supernatants were analyzed for cytokines with a bead-based multiplex assay (Beadlyte; Upstate Biotechnology, Lake Placid, NY). Cytokines (Table II) were selected on the basis of involvement with specific innate and adaptive immune responses that have been related to allergic inflammation and the immune response to respiratory viruses. Detection limits are shown in Table II. For analysis purposes, results below detection were assigned a value just below the limit (eg, 6.8 if the detection limit was 6.9 pg/mL).

Several procedures were followed to minimize assay variability and monitor quality control.[23] All tissue culture reagents were purchased from a single source, and stimulants were purchased in bulk and then divided into single-use aliquots that were stored at –80°C. Technicians from all 4 clinical sites attended a centralized training session. The reproducibility of the assays was monitored approximately yearly by sending aliquots of a single sample of fresh blood to each of the clinical sites, and the detailed procedures and reproducibility results of these quality control tests have been previously published.[23]

Maternal characteristics and obstetric data

Information on allergic history, smoking and alcohol use before and during pregnancy, household and personal stress during the prenatal period, and basic demographic information was collected from the mothers during a prenatal interview (Table I).

Information regarding the pregnancy and delivery were abstracted from the medical record. This information included parity, pregnancy complications and comorbidities, medication use, duration of labor, and type of delivery. The child's gestational age, birth weight, and Apgar score were also recorded. In addition, to estimate fetal growth, the percentile (z value) of birth weight adjusted for gestational age was calculated for each infant by using national reference data.[24] Information about systemic infections during pregnancy was obtained from the maternal obstetrical charts. Included in this definition were pneumonia (n = 2), pyelonephritis (n = 14), chorioamnionitis (n = 22), and other respiratory and systemic infections (n = 20).

Statistical analysis

Pearson correlations between predictors of interest and log-transformed (base 10) cytokine levels were examined. Spearman correlations were examined to verify that the correlations were not severely affected by the distribution of the responses. These results were not markedly different, so Pearson correlations are presented. Because of the large number of cytokine outcomes and predictors, these correlations are presented visually by using matrix plots with colors indicating the direction and intensity of correlation between a given predictor and cytokine. The structure of the data contained high measures of association within the set of predictors as well as within the cytokine response values. A false discovery rate with $\alpha = 0.10$ was used to investigate the impact of multiple comparisons for