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Prenatal maternal stress and cord blood innate and adaptive cytokine responses in an inner-city cohort

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At a Glance Commentary

Scientific Knowledge on the Subject

Although there are animal data demonstrating the influence of maternal stress on fetal immune development, little is known about this relationship in humans. Still less is known about this relationship in high-risk, inner-city, ethnic minority populations.

What this Study Adds to the Field

We demonstrate that prenatal psychological stress is independently associated with alterations in both innate and adaptive immune responses as indexed by stimulated cord blood cytokine responses.

Abstract: *Rationale:* Stress-elicited disruption of immunity begins *in utero. Objectives:* Associations among prenatal maternal stress and cord blood mononuclear cell (CBMC) cytokine responses were prospectively examined in the Urban Environment and Childhood Asthma Study (N=557 families). *Methods:* Prenatal maternal stress included financial hardship, Difficult Life Circumstances, community violence, neighborhood/block and housing conditions. Factor analysis produced latent variables representing three contexts - individual stressors and ecological-level strains (housing and neighborhood problems) which were combined to create a composite cumulative stress indicator. CBMCs were incubated with innate [lipopolysaccharide, Poly I:C, CpG, peptidoglycan] and adaptive [tetanus, dust mite, cockroach] stimuli, RSV, phytohemagglutinin, or medium alone. Cytokines were measured using multiplex ELISAs. Using linear regression, associations among increasing cumulative stress and cytokine responses were examined adjusting for sociodemographic factors, parity, season of birth, maternal asthma and steroid use, and potential pathway variables (prenatal smoking, birth weight for gestational age). *Measurements and Main Results*: Mothers were primarily minorities [Black (71%), Latino (19%)] with an income <$15,000 (69%). Mothers with the highest cumulative stress were older and more likely to have asthma and deliver lower birth weight infants. Higher prenatal stress was related to increased IL-8 production after microbial (CpG, PIC, PG) stimuli and increased TNF-α to microbial stimuli (CpG, PIC). In the adaptive panel, higher stress was associated with increased IL-13 after dust mite stimulation and reduced PHA-induced IFN-γ. *Conclusions:* Prenatal stress was associated with altered innate and adaptive immune responses in CBMCs. Stress-induced perinatal immunomodulation may impact the expression of allergic disease in these children.

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Introduction

The increased prevalence and enormous costs in the management of atopic disorders motivate efforts to identify early risk factors(1). Moreover, atopy is associated with asthma, the most costly pediatric disease in the United States. The functional differentiation of immune cells plays a central role with immunological abnormalities preceding development of atopic disorders, even being evident at birth(2, 3). Early life environmental factors may organize physiological systems, including immune function. Although diverse mechanisms are likely involved, focus has been on the systemic propensity for the type 2 T-helper (Th2) allergic response relative to type 1 (Th1) pathways(4-7). While useful in understanding a large fraction of atopic subjects, Th2 biased polarization of adaptive immunity may be only one of several axes that alter susceptibility(8). Non-Th2 factors, e.g., interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and regulatory T cells play a role in allergic and non-allergic inflammation(9, 10). Specifically, antigen-independent innate responses may also modify early risk(11, 12).

Additionally, asthma remains a leading cause of health disparities not completely explained by physical factors. Ethnic minorities in urban disadvantaged communities are disproportionately burdened(13). This has led to the reconsideration of social determinants (e.g., psychological stress) in asthma epidemiology(14).

The role of stress in the ontogeny of atopic disorders remains poorly understood(15). Evidence from animal and human studies suggest that stress-elicited disruption of interrelated systems - autonomic, neuroendocrine, and immune systems - may increase vulnerability to atopy even beginning *in utero(15)*. For example, stress-induced alterations in maternal cortisol may influence fetal immunomodulation and Th2 cell predominance through direct influence on cytokine production(16) and prevent the development of regulatory T cells(17). This may induce selective suppression of Th1-mediated cellular immunity and potentiate Th2-mediated humoral immunity(18). In other studies, psychological stress is associated with increased proportions of natural killer T cells as well as altering their functional mechanisms(19, 20). Stress may also impact the maturation process of dendritic cells, further predisposing to a Th2 phenotype(21). Moreover, in animal studies, gestational exposure to maternal stress alters the development of immunocompetence in offspring. Evidence in rhesus monkeys links prenatal stress to antigen-induced responses at birth(22). Dysregulated immune response to antigen challenge has also been demonstrated in prenatally stressed adult mice, reflected by an enhanced Th2 adaptive response(23).

Although behavioral and neuroendocrine effects of prenatal stress have been more extensively studied(24), no human studies have examined effects of prenatal stress on immune responses as indexed by cytokine profiles at birth(25). We examined cord blood mononuclear cell (CBMC) cytokine profiles following stimulation of both innate and adaptive/mitogen activation related to differential stress exposure in an urban birth-cohort. We specifically examined whether increased prenatal stress may be associated with distinct patterns of immune modulation assessed at birth.

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Methods

Study population

The Urban Environment and Childhood Asthma (URECA) prospective birth cohort examines environmental and genetic factors that influence immunologic development and asthma risk in early childhood. The study was approved by Institutional Review Boards at participating institutions. Expectant families were recruited during the prenatal period in Baltimore, Boston, New York, and St. Louis; written informed consent was obtained. Selection criteria included living in an area with >20% residents below the poverty level, mother or father of the index child with a history of allergic rhinitis, eczema, and/or asthma, and gestational age ≥ 34 weeks. Between February 2005 and March 2007, 1853 families were screened, 779 met eligibility criteria, and 557 were enrolled(26). For further detail, see the online supplement.

Prenatal Maternal Stress Assessment

At the baseline prenatal visit, mothers completed the following measures.

Difficult Life Circumstances (DLC): The 26-item DLC assesses life events occurring in the past 6 months with partner (including domestic violence), household members, substance abuse, and child rearing(27) tapping into interpersonal difficulties likely to be experienced by low-income women(28). Respondents answer yes or no to items; scores were summed across items. Acceptable reliability and construct validity have been reported(29).

Economic Strain: Respondents were asked: “How difficult is it for you to live on your total household income right now?”, “In the next two months, how likely is it that you and your family will experience actual hardships, such as inadequate housing, food, or medical attention?”, and “How likely is it that you and your family will have to reduce your standard of living to the bare necessities in life?”. Items are scored on a 5-point scale and summed.

Neighborhood/Block Conditions: Using the Community Survey Questionnaire, a measure with previously demonstrated validity and reliability(30), subjects were asked to rate twelve urban problems as 0 = “no problem”, 1 = “a minor problem”, or 2 = “a serious problem” and items were summed: property damage, drugs, groups of young people hanging around, physical assaults on the street, organized gangs, gunshots, lack of supervised activities for youth, feeling unsafe while out alone, inadequate recreational facilities, and poor city services. Respondents were also asked, “On a scale of 1 to 10, how would you rate your neighborhood as a place to live? (10 is best, 1 is worst, reverse scored).”

Perceived Community Violence: Subjects reported whether five events had occurred in their neighborhood in the past 6 months (not including self-vicitmization) and items were summed: (1) fight in which a weapon was used, (2) violent argument between neighbors, (3) gang fight, (4) sexual assault or rape, and (5) robbery or mugging. This measure relates directly to police-reported homicides which are less vulnerable to reporting limitations(30) and has been linked to urban asthma morbidity(31).

Housing Worries: Adapting previous work linking worry about housing conditions to psychological distress(32, 33), subjects were asked “How much do the following things bother you about the home where you live?”: hard to heat, high rent, worry about eviction, rodents or cockroaches, water leaks, mold, street noise, others in the home, and landlord difficulties. These were scored on a 5-point scale from “never” to “all the time” summing across items. Respondents were also asked, “On a scale of 1 to 10, how would you rate your (house/apartment) as a place to live? (10 is best, 1 is worst, reverse scored).”

Covariates

Information on maternal asthma as well as inhaled corticosteroid (ICS) use, prenatal smoking, and sociodemographics was collected during a standardized interview. Gestational age and birth weight were abstracted from the medical record. The percentile (z-value) of birth weight adjusted for gestational age was calculated using national reference data(34).

Cord Blood Cytokine Response Outcomes

Procedures are detailed elsewhere(35). Briefly, cord blood was collected using sterile procedures. Blood was transferred from syringes to sterile 50 mL tubes, diluted 1:1 with RPMI 1640 heparinized medium, and kept at room temperature pending cell separation. Mononuclear cells were separated by density gradient using Accuspin tubes (Sigma) and incubated in the presence of medium and specific immune stimulants or medium alone(35). After incubation for 24 hrs (innate and polyclonal stimuli) or 5 days (antigens), supernatants were collected, divided into aliquots, frozen at -80°C, and shipped to a central laboratory. Supernatants were analyzed for cytokines with a bead-based multiplex assay (Beadlyte, Upstate Biotechnology, Lake Placid, NY). Cytokines (Table 1) were selected based on specific innate and adaptive immune responses previously related to allergic inflammation and viral immune responses.

Statistical analysis

As stress research suggests that individuals may be increasingly vulnerable when adverse events are experienced across multiple domains [i.e., cumulative stress(36, 37)], we used data reduction steps to derive a composite measure of cumulative stress. Principal components factor analysis (38) was employed to combine the items on the individual scales in the stress battery and extract a reduced number of factors interpreted to represent different domains of stress. The analysis produced factor loadings that are essentially correlation coefficients between the scales and the unmeasured underlying factor. Scores were derived by multiplying the factor loadings by the values for the variables included in the factor and summing those values. Resultant factors represented three contexts (Table 2) - individual-level stressors and ecological-level strains related to housing and neighborhood problems. Each subscale was then divided into tertiles and given values of 1= low, 2 = medium, and 3 = high exposure; an overall cumulative stress score was derived by summing the tertile values across the three factors. For example, if a subject was in tertile 1 for individual-level stressors, tertile 2 for housing problems, and tertile 3 for neighborhood problems the composite score would equal 6 [range 3 (low on all domains) to 9 (high on all domains)].

Logarithmic transformation of the cytokine outcomes was used given skewness of the data in order to symmetrize the residuals. Linear regression was used to model the relationship between cytokine responses and cumulative stress. All models were adjusted for media control background cytokine production. Variables previously identified as being related to psychological stress and cord blood immunomodulation were examined as potential confounders. Stepwise modeling was done initially adjusting for parity (primiparous vs. multiparous)(6), child gender and race; next adding income and maternal education. We next adjusted for season of birth, maternal history of asthma, and ICS use. Finally, we considered variables that are potentially in the pathway through which stress might contribute to altered immune function - birth weight for gestational age and maternal smoking(39). Statistical analyses were performed on SAS Version 9.1.3 (SAS Institute, Cary, NC) and the R system for statistical computing (version 2.9.1)(40). A test for linear trend was used to determine the relationships among the cumulative stress score on mean cytokine outcomes(41). Significance was set at $P < 0.05$.

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Results

Data from 560 newborns (n= 272 girls, n=288 boys, three sets of twins) and their mothers were analyzed. The distribution of covariates (Table 3) and cumulative stress scores across covariates (Table 4) are summarized. The majority of mothers self-identify as ethnic minorities [Black (71%), Latino (19%)], with a high school education or less (75%) and report an annual income <$15,000 (69%). Mothers in the highest cumulative stress group were older, more likely to have a history of asthma, and delivered babies with lower birth weight for gestational age.

We graphically examined associations among the cumulative stress indicator and the cytokine x stimulant outcomes. Increasing cumulative prenatal stress was related to patterns that were consistent across a number of stimuli on the innate panel (Figure 1): increased IFN-γ following incubation with PG and CpG; reduced IL-10 following incubation with PIC and LPS; higher levels of TNF-α following incubation with PIC and CpG; and higher levels of IL-8 following incubation with RSV, PIC, and CpG. In addition, there was some suggestion for the increased production of IL-8 with increasing levels of cumulative stress in the media control assay. In the adaptive panel (Figure 2), increased cumulative prenatal maternal stress was related to lower levels of IFN-γ when stimulated with PHA, cockroach, as well as media control, and increased IL-13 when stimulated with dust mite antigen.

Linear regression analyses were run for cumulative stress predicting the cytokine outcomes. Table 5 shows the geometric mean cytokine values (pg/ml) across levels of the composite cumulative stress score (p for trend). For the innate panel, increasing stress was significantly related to increased CpG-induced IL-8 production, even in the fully adjusted model($p=0.02$).

A significant association between higher stress and increased TNF-α production after incubation with PIC was also evident.

In the adaptive panel (Table 5), increased stress was significantly associated with greater dust-mite induced IL-13 when adjusted for potential confounders and pathway variables ($p=0.03$). The association between higher cumulative stress and reduced IFN-γ production to stimulation with PHA (a non-specific mitogen) was robust even in fully adjusted models ($p=0.004$).

Discussion

Research continues to delineate the relationships among early environmental influences, immune system alterations, and allergy and asthma(42-44). Children at risk for atopy, including asthma, are born with impairments of both adaptive and innate immune responses. Regulatory T cells have been found to be less effective in neonates born to atopic mothers as compared with children of nonatopic mothers(12). Also, neonates at risk for asthma later in life have been found to have impaired Th1-mediated responses at birth(4). We extend this literature with the first prospective data examining associations between prenatal maternal stress and cytokine profiles in cord blood mononuclear cells in urban infants at high risk for atopic diseases based on family history. These data suggest that prenatal psychological stress may alter both adaptive and innate immune axes assessed at birth.

When examining findings across innate stimuli, notable patterns emerge that were consistent across stimuli likely to operate through similar pathways. For example, higher stress was associated with increased IL-8 production following microbial (CpG, PIC) stimuli albeit the association was significant only for CpG. We also observed stress-induced increased TNF-α production to microbial stimuli (CpG, PIC); while notably in the same direction, only PIC was significantly related. These findings suggest that stress may modify the neonatal immune response through Toll-like receptor (TLR)-dependent pathways. TLRs, implicated in allergy and asthma, are an essential part of the innate and adaptive immune response (45). They operate through detection of a wide range of microbial- and viral-associated molecular patterns(46). TLRs 7 and 9 recognize pathogen-derived nucleic acid molecular patterns, specifically RNA or DNA, respectively. Bacterial DNA containing unmethylated CpG motifs are ligands for TLR9(47). TNF-α is an important regulator of TLR2 expression in response to diverse microbial stimuli. Consistent with these findings, evidence in murine models suggests that stress modulates the immune response in a TLR4-dependent manner. Powell and colleagues demonstrated that stress modulates Toll-like receptor-dependent cytokine secretion in response to CpG DNA and PIC in splenic dendritic cells(48). Zhang and others have linked stress and TLR4-mediated P13K/Akt signaling in mice(49). A number of TLRs have also been identified as candidates for playing a key role in the immune response to RSV including TLR2, TLR4, TLR6 and TLR7(50, 51). We did not find a relationship between increasing stress and RSV-induced IL-8 or TNF- α.

In addition, the increased production of IL-8 with increasing levels of cumulative stress in the non-specific media control assay may point to stress effects on still another immune axis. Certain pollutants such as ozone, a potent oxidant, particulates, and endotoxin are thought to induce asthma through non-allergic mechanisms, perhaps related to NF-κB activation and IL-8 secretion(52). Notably, psychological stress is also an oxidant and may operate through these same pathways(14).

Risk of Th2 skewing may occur through pathways related to the interaction between stress, innate immune cells, adaptive immune cells, and their cytokine and chemokine mediators(4). For adaptive responses, there was evidence that increased stress was associated with lower levels of IFN-γ production, which has previously been linked to increased risk for later atopic disease(53). Macaubas and colleagues found that detectable levels of IFN-γ (constitutively) was associated with lower risk of asthma at age 6 years(44). Mitogen-induced IFN-γ tended to be lower in newborns whose mothers reported more stress. This may suggest that the immunomodulatory effect of higher stress as reflected by reduced expression of IFN-γ by stimulated CBMCs is a more generalized, nonspecific response rather than being allergen specific. A delayed maturation of Th1-immune responses may increase the risk of sensitization to aeroallergens(4) and susceptibility to viral illnesses(54) as these children grow older. Continued follow-up in this cohort with serial assessments of these cytokine responses will allow us to examine whether prenatal stress differentially influences the rate of maturation of the Th1 response and subsequent risk for asthma and allergies.

We also observed increasing production of IL-13 to allergen-specific stimulation with dust mite in association with increasing cumulative stress. Notably, the pattern of increased IL-13 response to house dust mite has been associated with allergic sensitization in older children(55). The production of IL-13 by CBMCs stimulated with cockroach extract was unchanged across the cumulative stress levels. These findings may reflect a differential likelihood of prenatal exposure to these inhalant allergens. Prior studies have demonstrated evidence for the transfer of house dust mite through the placenta or in amniotic fluid(56) as well as a dose-related association between prenatal dust mite and cord blood immune response (IgE)(57, 58). Data supporting maternal-fetal transfer of cockroach antigen or the role of cockroach in sensitization at birth are less clear(58). Alternatively, antigenic stimulation can cause low-level activation of recent thymic T cell emigrants in a nonspecific fashion(59). While the debate continues as to whether primary sensitization to allergens begins before birth, these findings suggest the possibility that prenatal stress may enhance the neonates response to inhalant antigens, specifically those antigens that the fetus is likely to encounter more directly *in utero* (e.g., house dust mite).

These data suggest that psychological stress is involved in perinatal programming (the concept that environmental factors acting early in life may permanently organize or imprint physiological systems) of the infant immune response. Evidence linking stress to asthma and other atopic disorders suggests that early disruption of neuroimmunoregulatory processes are involved [for a detailed discussion see Wright(15) and references therein]. Stress experienced *in utero* begins to shape stress neurobiology resulting in disturbed regulation of endocrine and autonomic processes (e.g., HPA axis, sympathetic-adrenal-medullary system). It is hypothesized that prenatal stress affects maternal stress physiology which, in turn, modulates the maternal immune system with enhanced polarization toward a Th2 phenotype. This may expose the fetus to neurohormonal factors (e.g., glucocorticoids, neurotrophins) and an even greater propensity towards a Th2 cytokine/chemokine milieu *in utero* that then modulates fetal immune development(15). Such changes may set the stage for the altered reactivity characteristic of asthma and related phenotypes. While these *in utero* responses may be adaptive in the short term, being geared toward coping with anticipated environmental challenges, they may exact a toll in contributing to an increased risk of atopic disorders as these children get older.

Stress may also influence immunomodulation indirectly through its effects on maternal behaviors such as smoking as well as the relationship seen here between increased stress exposure in mothers and lower birthweight adjusted for gestational age. However, including these potential pathway variables in the regression models did not substantially alter our findings.

This study has a number of strengths including the prospective design, the large sample size, and the broad assessment of immunophenotypes at birth related to both innate and adaptive stimuli in a high-risk urban sample. Another strength is the assessment of psychological stress across a number of domains using standardized measures. While there is no consensus on how best to conceptualize and measure the role of socioeconomic status-related stressors on health, a number of concepts grounded in stress theory justify the *a priori* approach taken in the current analyses. Stressors typically predict outcomes similarly across stressor domains rather than specific types of stressors impacting outcomes differently(36). Moreover, beyond experiencing discrete types of stressors, individuals may be increasingly vulnerable when exposed to cumulative effects of multiple stressors(37). This may be particularly relevant in urban poor communities where exposure to multiple stressors is more prevalent. We found qualitatively similar influences of increasing maternal prenatal stress on the cytokine profiles in cord blood mononuclear cells across the independent stress factors (i.e., individual stressors, housing problems, and neighborhood problems) (data not shown) and the composite cumulative stress indicators which enhances confidence that findings are not spurious. Notably, we found no significant differences in the distribution across cumulative stress categories based on race/ethnicity, income or mother's educational status. This may reflect that we are considering stress experienced within the context of urban disadvantage where all subjects experience a relatively high "threshold". Given multiple comparisons, we cannot rule out the possibility that some relationships were observed by chance. However, we also note that despite the reasonably large sample size, the cytokine assays may have enough variability and noise in them to reduce our ability to find a significant association even when one exists. We also note that exploration of these data for nonlinear relationships (e.g., U-shape, threshold effects) between the cumulative stress measure and the stimulant by cytokine outcomes did not yield significant relationships (data not shown).

In conclusion, prenatal stress appears to affect immune responses to both innate and adaptive stimuli at the time of birth, effects that may result in enhanced susceptibility to asthma or other atopic disorders. Continued follow-up of this prospective birth cohort will allow us to examine whether the stress-related disruptions in these early innate and adaptive immunophenotypes influence the expression of subsequent allergic sensitization and asthma expression in these children. Moreover, the URECA study design includes repeated measures of stress in these families during critical windows of development (pregnancy and annually during the first three years of the child's life), repeated assessment of immune response to the environmental stimuli included here, and ultimately clinical outcomes up to age three years. This will allow for a prospective cohort analysis to explore independent effects of prenatal and post-natal stress on infant immune development and ultimately the expression of clinical disease. Exploring the links between maternal prenatal stress and atopic risk may be particularly relevant in urban, high-risk U.S. populations that are disproportionately burdened by both phenomena.

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Legends

Figure 1. Relationship of cytokine responses (average log-transformed values) with composite stress score: Innate panel.

Figure 2. Relationship of cytokine responses (average log-transformed values) composite stress score: Adaptive panel.

Table 1. Stimulants used and cytokines measured in the cytokine secretion assays

Innate Responses			Adaptive and Polyclonal Responses		
Stimulants*	Final Concentration	Cytokines measured with all stimulants	Stimulants*	Final Concentration	Cytokines measured with all stimulants
Lipopolysaccharide[1]	0.1 µg/mL	IFN-α	Phytohemagglutinin[6]	15 µg/mL	IFN-γ
Polyinosinic-polycytidylic acid[2]	25 µg/mL	IFN-γ	Cockroach extract[7]	10 µg/mL	IL-10
Peptidoglycan[3]	1.25 µg/mL	IL-10	Dust mite (*D. pteronyssinus*) extract[8]	10 µg/mL	IL-13
CpG-C[4]	1 µg/mL	IL-12p40	Tetanus toxoid[9]	10 µg/mL	IL-4
Respiratory syncytial virus[5]	500 sfu/mL[†]	TNF-α	Medium alone	--	
Medium alone	--	IL-8			

* Sources for reagents: [1]Associates of Cape Cod (Falmouth, MA), [2]Amersham Biosciences (Piscataway, NJ), [3]InvivoGen (San Diego, CA), [4]Coley Pharmaceuticals (Wellesley, MA), [5]Ann Mosser (University of Wisconsin-Madison), [6]Sigma (St. Louis, MO), [7,8]Greer Inc. (Lenoir NC), [9]Massachusetts Biologics (Jamaica Plain, MA)
[†] sfu = syncytium-forming units

Table 2. Individual- and ecological-level stressor domains derived from factor analysis

	Stressor Domains (factor loadings)		
Stress Scales	**Interpersonal Problems** Cronbach's α = 0.85	**Housing Problems** Cronbach's α = 0.90	**Neighborhood Problems** Cronbach's α = 0.90
Difficult Life Circumstances	0.46 *	0.08	0.25
Neighborhood/block conditions	0.20	0.32	0.72 *
Perceived community violence	0.25	0.14	0.65 *
Rating of home	0.26	0.65 *	0.18
Rating of neighborhood	0.13	0.55	0.49 *
Housing conditions	0.46	0.48 *	0.33
Economic strain	0.48 *	0.24	0.08

* Denotes scales included in each factor (within each column) based on factor loadings. A scale was included in a particular factor if the loading was $\geq$ 0.45. Two scales loaded highly on more than one factor [i.e., Rating of Neighborhood loading with both the Neighborhood Problems factor and Housing Problems factor; Housing Score loading with the Interpersonal Problems and Housing Problems factors] in which case scales were kept in the factor with which they were most conceptually consistent. A standard extraction strategy supported a three-factor solution accounting for more than 75% of the total variance.

Table 3. Demographic characteristics and distribution of other covariates

	n	%
Total Mothers	557	100
Mother's age in years at child's birth (median, range)	23	13-42
Race or ethnicity of mother		
Hispanic of any race	107	19
Black alone	390	71
White alone	22	4
More than one race	20	4
All others	11	2
Missing	7	--
Household income < $15,000 per year	355	69
Mother's education		
< High school	231	42
High school or GED	183	33
> High school	136	25
Smoked during pregnancy	97	18
Maternal asthma	307	55
Inhaled corticosteroid use during pregnancy	54	10
Total Babies	560	100
Sex		
Male	288	51
Female	272	49
Firstborn	221	39
Season of Birth		
January-March	142	25
April-June	139	25
July-September	160	29
October-December	121	22
Birthweight, grams (median, range)	3220	1815-4850
Gestational age, weeks (median, range)	39	34-42

Table 4. Relationship of composite stress score to socio-demographic characteristics

	Composite Stress Score			
	3-5 N=222 (41%)	6-7 N=191 (35%)	8-9 N=126 (23%)	p-value
Mother's age in years at child's birth (median)	22	23	25	0.002
Primiparous (percent)	45	38	31	0.04
African-American (percent)	80	75	75	0.38
Household income < $15,000 per year (percent)	68	65	75	0.15
Mother's education (percent)				
< High school	46	41	36	0.11
High school or GED	35	31	36	
> High school	19	28	29	
Smoked during pregnancy (percent)	15	18	22	0.22
Maternal asthma (percent)	50	56	65	0.022
Inhaled steroid use during pregnancy (percent)	8	9	14	0.12
Season of Birth (percent)				
January-March	29	24	19	0.30
April-June	25	24	25	
July-September	26	28	35	
October-December	20	25	21	
Male child	50	53	48	0.65
Birthweight, z-value for gestational age (median)	-0.22	-0.29	-0.55	0.05

Table 5. Selected Geometric Mean Cord Blood Cytokine Levels (pg/ml) by Composite Stress Score.

Stimulant	Cytokine	n	% Detectable	Composite Stress Score 3-5	6-7	8-9	p-value* Model 1†	p-value Model 2†	p-value Model 3§	p-value Model 4‖
Innate Panel										
CpG	IFN-γ	500	64.0	16.6	18.9	18.1	0.33	0.33	0.44	0.60
	IL-10	500	93.4	68.1	61.4	66.2	0.61	0.58	0.61	0.65
	TNF-α	500	82.0	25.9	29.5	27.9	0.12	0.09	0.10	0.10
	IL-8	505	41.0	22817.9	24800.9	27323.9	0.05	0.03	0.02	0.02
LPS	IFN-γ	508	45.5	14	14.2	15.1	0.30	0.38	0.37	0.41
	IL-10	508	99.0	227.7	224.4	207.2	0.16	0.25	0.27	0.36
	TNF-α	508	99.6	1072.3	1053.8	985.8	0.70	0.86	0.87	0.97
	IL-8	513	96.7	232968.5	208460.8	214626.2	0.63	0.77	0.98	0.61
PG	IFN-γ	509	61.9	19.8	23.3	25.5	0.03	0.04	0.06	0.11
	IL-10	509	98.6	333	338.2	371.2	0.77	0.66	0.98	0.93
	TNF-α	508	98.6	1704	1817.6	1729	0.42	0.34	0.35	0.35
	IL-8	512	96.7	195505.4	195303	181181	0.70	0.82	0.94	0.64
PIC	IFN-γ	510	82.4	66.5	77.7	69.5	0.39	0.39	0.63	0.75
	IL-10	510	56.7	13.3	12.9	11.8	0.17	0.17	0.28	0.26
	TNF-α	510	78.2	22.5	24.2	26.8	0.03	0.02	0.02	0.02
	IL-8	513	34.3	21270.9	24075.6	23661.7	0.16	0.12	0.12	0.08
RSV	IFN-γ	482	70.7	41.7	57	41.6	0.99	1.00	0.63	0.96
	IL-10	482	78.2	24.8	24.4	25.8	0.61	0.47	0.52	0.47
	TNF-α	482	74.9	26.1	33.3	31.3	0.26	0.33	0.12	0.11
	IL-8	466	92.9	96067.1	113518.8	107420.6	0.30	0.24	0.21	0.13
Adaptive Panel										
CR	IL-10	440	8.0	7.4	7.3	7	0.36	0.30	0.35	0.43
	IL-13	440	15.2	8.2	8.1	7.9	0.80	0.78	0.52	0.61
	IFN-γ	440	25.5	10.8	9.5	8.7	0.46	0.47	0.53	0.53
DM	IL-10	488	33.8	8.9	8.8	8.8	0.83	0.96	0.79	0.66

	IL-13	488	44.7	13.3	15.4	17.1	0.03	0.03	0.01	0.03
	IFN-γ	488	35.0	13.1	11.1	13	0.11	0.12	0.05	0.09
PHA	IL-10	503	97.6	139.1	140.8	139.9	0.93	1.00	1.00	0.80
	IL-13	503	98.4	284.8	264.8	246.2	0.13	0.12	0.16	0.11
	IFN-γ	503	97.2	790.1	595.2	369	0.004	0.002	0.006	0.004

Each line represents a separate linear regression model
*Test for linear trend.
†Adjusted for media control value and race, sex, parity, and birth order of child.
‡Adjusted for media control, race, sex, parity, and birth order of child, household income, and education of mother.
§Adjusted for media control, race, sex, parity, and birth order of child, household income, education of mother, season of birth, whether the mother has asthma, and ICS use during pregnancy.
‖Adjusted for media control, race, sex, parity, and birth order of child, household income, education of mother, season of birth, whether the mother has asthma, ICS use during pregnancy, maternal smoking and birthweight for gestational age.

Figure 1. Relationship of cytokine responses (average log-transformed values) with composite stress score: Innate panel.

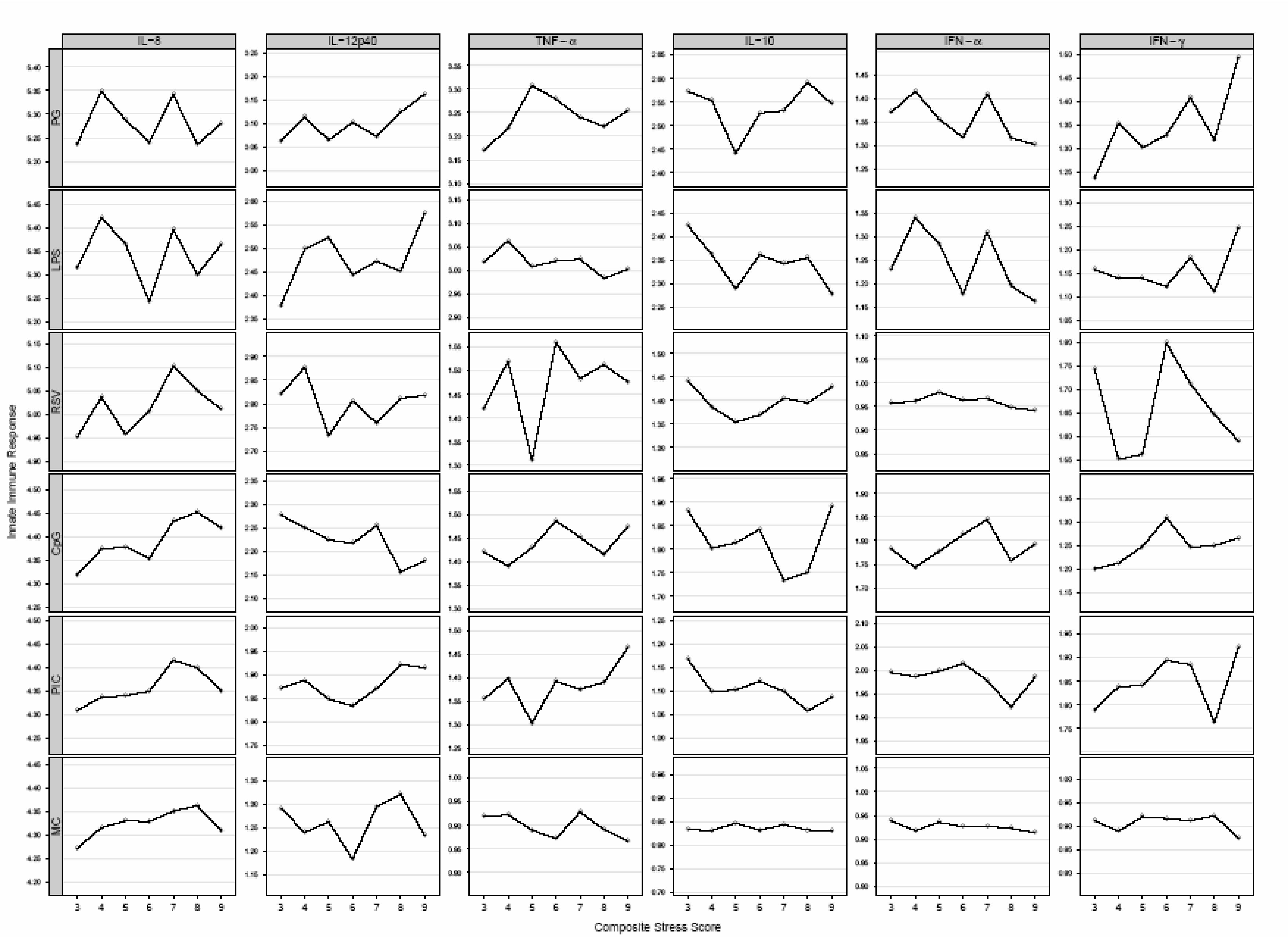
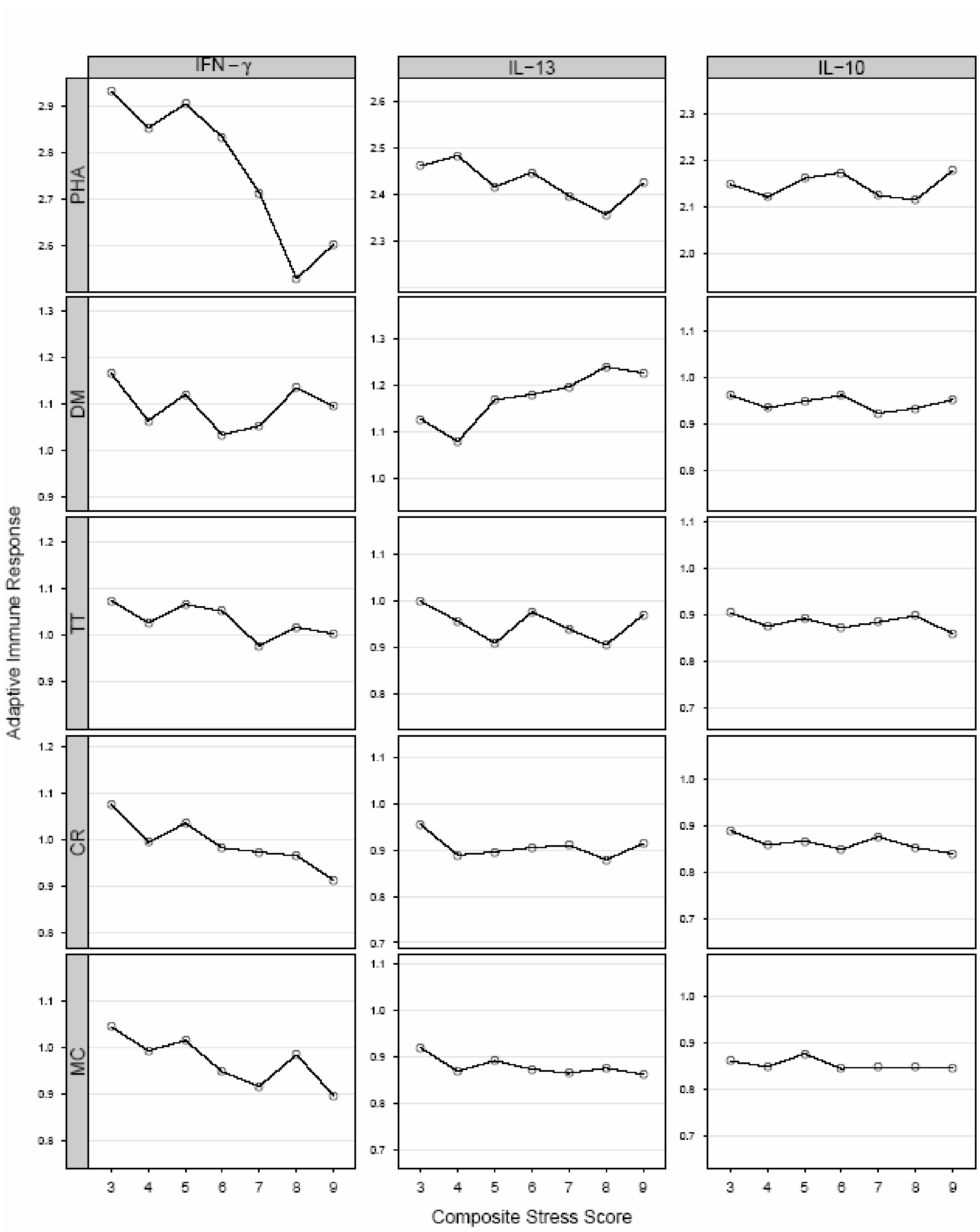


Figure 2. Relationship of cytokine responses (average log-transformed values) composite stress score: Adaptive panel.



Online Data Supplement

Prenatal maternal stress and cord blood innate and adaptive cytokine responses: The Urban Environment and Childhood Asthma Study (URECA)

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Study Population

Exclusion criteria included factors that potentially affect lung or immune system development (e.g., maternal human immunodeficiency virus at delivery and newborn factors including significant congenital anomalies, pneumonia requiring antibiotic treatment for 1 week or more, and respiratory distress requiring either intubation and ventilation for 4 or more hours or supplemental oxygen or continuous positive airway pressure for 4 or more days). Between February 2005 and March 2007, 1853 families were screened, 779 met eligibility criteria, and 557 were enrolled(26). Table E1 shows maternal characteristics at screening by enrollment status; no significant differences were seen between the two groups based on sociodemographic factors, maternal asthma, or stress indices minimizing concern for selection bias.

Table E1. Maternal characteristics at screening by enrollment status

	Enrolled		Not Enrolled		
	n	%	n	%	p-value
Total Mothers	557	100.0	218	100.0	
Mother's age in years at recruitment (mean, range)	24.5	13-42	24.2	14-47	0.55
Race or ethnicity of mother					
Hispanic of any race	107	19.4	25	12.6	0.29
Black alone	390	70.9	152	76.4	
White alone	22	4.0	9	4.5	
More than one race	20	3.6	9	4.5	
All others	11	2.0	4	2.0	
Missing	7	--	19	--	
Household income < $15,000 per year	314	69.0	110	71.9	0.50
Mother's education					
< High school	231	42.0	88	44.4	0.60
High school or GED	183	33.3	68	34.3	
> High school	136	24.7	42	21.2	
Maternal asthma	307	55.3	123	56.4	0.78
Difficult Life Circumstances (mean, range)	4.1	0-16	3.9	0-12	0.33
Neighborhood/block conditions (mean, range)	7.4	0-24	6.7	0-24	0.17
Perceived community violence (mean, range)	1.1	0-5	1.0	0-4	0.34
Rating of home (mean, range)	7.0	1-10	6.8	1-10	0.57