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Prenatal, perinatal, and heritable influences on cord blood immune responses

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Abstract

Background—Maternal and perinatal environmental exposures, as well as inherited factors, may influence neonatal immune responses.

Objective—To determine relations of maternal and perinatal exposures to antigen-specific cord blood lymphoproliferative responses.

Methods—In 427 newborns from a Boston pregnancy/birth cohort, lymphoproliferative responses in cord blood mononuclear cells to stimulation with cockroach (Bla g 2), house dust mite (Der f 1), ovalbumin, and mitogen phytohemagglutinin were measured as stimulation index (SI). We used the Wilcoxon rank sum and χ^2 tests to evaluate predictors of ovalbumin SI as a continuous ranked or dichotomous outcome. We used *t* test and Spearman correlation for univariate testing and linear regression to evaluate predictors of natural log-transformed Bla g 2, Der f 1, and phytohemagglutinin SI. Logistic multivariate regression was applied to evaluate predictors of Bla g 2, Der f 1, and phytohemagglutinin SI dichotomized at 2 or at the median for phytohemagglutinin.

Results—Maternal smoking during pregnancy, inadequate or excessive maternal weight gain during pregnancy, neonate black race/ethnicity (compared with white), and Apgar score less than 8 were each independently associated with increased cord blood mononuclear cell proliferative responses to stimulation with Bla g 2 and/or Der f 1. Maternal history of asthma was associated only with increased lymphoproliferative response to ovalbumin stimulation.

Conclusions—Distinct fetal and perinatal exposures and black race/ethnicity may be associated with increased cord blood lymphoproliferative responses. The implications of these findings for future development of allergy or asthma are, as yet, unknown.

INTRODUCTION

Asthma is the most prevalent chronic disease of childhood in industrialized countries.[1,2] Genetic, developmental, immunologic, and environmental factors all appear to play roles. A

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maternal history of asthma is likely linked to all of these factors and is a predictor of childhood asthma.[3]

The fetal period may be a critical time for influence of a child's immune system. One factor that likely affects the fetal immune system is maternal and fetal antigen exposure. Recognition and response of neonatal T lymphocytes to antigens have been previously demonstrated.[4–6] Fetal antigen exposure may be affected by maternal allergy status[7] and maternal exposure to high concentrations of allergen. Mothers with high cat allergen exposure also have high levels of IgG to cat and pass this specific antibody to the fetus. The implications of the in utero transfer of IgG to inhalant allergens for asthma development is a subject of active investigation.[8]

Also, additional factors such as exposure to cigarette smoke, maternal weight gain during pregnancy, and race/ethnicity may be influential in immune development.[9–14] Improved understanding of cord blood immune responses and an association with maternal in utero influences, such as maternal history of asthma or smoking, may help elucidate the understanding of immune development. This information may help direct future prevention strategies for subsequent development of childhood allergy and asthma.

Previous literature regarding the influence of maternal asthma and allergy on cord blood mononuclear cell (CBMC) proliferative response to antigens and mitogens is heterogeneous.[15,16] While previous investigations have determined that antigen-specific T-cell priming can occur, the influence of maternal asthma and allergy on the presence and degree of CBMC proliferative response to antigens, including house dust mite, cockroach (Bla g 2), birch pollen, grass, β-lactoglobulin, and ovalbumin, has varied.[4–6,17–19] In a study of 223 neonates from Scotland, the magnitude of CBMC proliferative responses to timothy grass pollen and/or house dust mite increased in association with family history of atopic disease and/or maternal smoking.[20]

We investigated the association of maternal and perinatal exposures such as maternal smoking, maternal history of asthma, eczema, hay fever, maternal weight gain during pregnancy, neonatal race/ethnicity, and Apgar scores with CBMC proliferative responses to the antigens Bla g 2, Der f 1, ovalbumin, and mitogen phytohemagglutinin.

METHODS

Study Participants

Study participants were a subgroup of participants from Project Viva, a pregnancy/birth cohort study under way in the Boston, MA, metropolitan area.[21] Enrollment for this study occurred from April 1999 through July 2002. Expectant mothers were enrolled at their initial clinical prenatal visit. Participants were interviewed and completed questionnaires in the first and second trimesters of pregnancy, as well as at the time of delivery. Venous umbilical cord blood was obtained at the time of nonemergent deliveries. This study was approved by the Institutional Review Boards of Brigham and Women's Hospital and Harvard Pilgrim Health Care. Informed consent was obtained from mothers, including cord blood collection and longitudinal follow-up of their offspring.

Cord Blood Samples, Cell Preparation, and Lymphocyte Proliferation

Cord blood samples were collected from the umbilical vein at delivery (n = 427). Samples were placed in heparinized tubes and processed within 24 hours. The CBMCs were isolated by density-gradient centrifugation with Ficoll-Hypaque Plus (Pharmacia, Uppsala, Sweden) after dilution in phosphate-buffered saline. Cells were washed in RPMI 1640 and diluted in 10% human serum (Biowhittaker, Walkersville, MD) to a concentration of 5×10^6 cells/mL. For the lymphocyte proliferation assay, 0.5×10^6 cells per well were cultured in quadruplicates in

96-well round-bottom tissue-culture plates (Corning, New York, NY) for 3 days at 37°C in a 5% carbon dioxide incubation chamber. At the start of the culture, cells were stimulated with 30 μg/mL of Bla g 2, 30 μg/mL of Der f 1 (Indoor Biotechnologies, Charlottesville, VA), 100 μg/mL of ovalbumin, or 5 μg/mL of phytohemagglutinin (Sigma Aldrich, St Louis, MO). The indicated doses and time points were chosen after optimization experiments with dose-and time-response curves. We tested all reagents for endotoxin contamination by limulus assay. Endotoxin content was low (<0.01 EU/mL), and functional ability to stimulate CBMCs was not relevant at these concentrations. Cells were pulsed with 1-μCi [^{3}H]-thymidine for an additional 8 hours. Cells were harvested with a Tomcat Mach II harvester (Wallac, Turku, Finland) onto filter plates, which were read in a β-counter. Samples were run in quadruplicate, and mean counts per minute of stimulated and unstimulated samples were calculated.

Definition of Predictor Variables

We determined maternal and paternal history of asthma, eczema, and hay fever during the first trimester interview. We asked expectant mothers and fathers, "Has a health professional such as a doctor, physician assistant, or nurse practitioner ever told you that you had asthma, eczema, or hay fever?" Additional potential covariates, including maternal body mass index (BMI), smoking history, neonate sex, and race/ethnicity, were determined through written questionnaires, interviews, and review of medical records. Maternal prepregnancy weight and height were obtained at the time of enrollment, and we calculated BMI as the ratio of weight in kilograms divided by the square of height in meters. Maternal smoking status was determined during the first and second trimester of pregnancy and at delivery, when the mother was asked if she had ever smoked, in the last 3 months and currently. Maternal weight gain during pregnancy was calculated subtracting the prepregnancy weight from the weight before delivery. Adequate, inadequate, and excessive weight gain were defined using the 1990 Institute of Medicine criteria guidelines,[22] which are based on prepregnancy BMI and weight gain: for a prepregnancy BMI of less than 19.8, recommended weight gain is 12.5 to 18 kg; prepregnancy BMI of 19.8 to 26.0, recommended weight gain is 11.5 to 16 kg; prepregnancy BMI of more than 26.0, recommended weight gain is 7 to 11.5 kg; and prepregnancy BMI of more than 29.0, recommended weight gain is 6 to 11.5 kg.

Neonate birth weight was recorded at the time of delivery. The 5-minute Apgar score (assigning a score of 0 to 2 to heart rate, respiratory effort, muscle tone, reflex irritability, and color) was abstracted from birth record and dichotomized between a total score of 8 or higher vs less than 8, since a score less than 8 is considered abnormal and reason for resuscitation. Neonate race was determined based on parental report of race, which was collected at the first trimester interview. If both parents were white, the child was classified as being white. If either parent was black, the child was classified as being black. If no parent was black but at least one parent was Hispanic, the child was classified as being Hispanic. If no parent was black or Hispanic but at least one parent was Asian, the child was classified as being Asian.

Definition of Outcome Variables and Data Analysis

The SI was calculated as the ratio of mean counts per minute of stimulated lymphocytes divided by mean counts per minute of unstimulated lymphocytes. The data from each sample were reviewed, and if significant differences from usual laboratory conditions were found or if phytohemagglutinin SI was more than 3 SDs below the geometric mean (phytohemagglutinin SI <5) (n = 8), which could represent an absence of phytohemagglutinin stimulation or an occult immunologic dysfunction, data were excluded from analyses. Proliferative responses to Bla g 2, Der f 1, and phytohemagglutinin were natural log transformed to normalize distribution of the data. Proliferative response to ovalbumin did not normalize with natural log transformation; we used the Wilcoxon rank sum test and χ^2 test to evaluate predictors of ovalbumin SI as a continuous ranked or a dichotomous outcome. We used linear and logistic

multivariate regression to evaluate predictors of Bla g 2 SI, Der f 1 SI, and phytohemagglutinin SI, evaluating them also as continuous or dichotomous outcomes. All multivariate models included maternal history of asthma, as well as maternal smoking, race/ethnicity, and BMI (covariates with a possible association [$P \leq .20$] in the multivariate analysis with antigen-specific SI). In dichotomous analyses, data were divided at an SI of 2 or greater vs less than 2, taking into account the range and median value for the outcomes and previous literature suggesting an SI greater than 2 or 3 as a positive response.[5,17,19,23–25] SAS statistical software, version 8.2 (SAS Institute Inc, Cary, NC) was used for all analyses.

RESULTS

Table 1 demonstrates the baseline characteristics for the 427 children who had measurements for ovalbumin- and phytohemagglutinin-induced lymphocyte proliferation. Of these 427 children, 375 also had measurements for Bla g 2– and Der f 1–induced lymphocyte proliferation. Proliferative responses ranged from 498 (fifth percentile) to 9,671 cpm (95th percentile) (median, 2,725 cpm) for unstimulated samples, from 963 to 17,839 cpm (median, 3,759 cpm) for Bla g 2 stimulation, from 1,261 to 23,562 cpm (median, 4,682 cpm) for Der f 1 stimulation, from 821 to 11,854 cpm (median, 3,218 cpm) for ovalbumin stimulation, and from 14,185 to 258,173 cpm (median, 114,916 cpm) for phytohemagglutinin stimulation. A positive proliferative response was defined as an SI of 2 or greater. The distribution of SI is reported in Table 2.

Maternal history of asthma was associated with increased cord blood lymphocyte proliferation to ovalbumin (median SI, 1.21 vs 1.08; $P = .007$; Table 3). In analyses expressing ovalbumin SI as dichotomy, offspring of asthmatic mothers were more likely to have an ovalbumin SI of more than 2 than offspring of nonasthmatic mothers (21% vs 11%; $P = .02$; data not shown). No additional measured factors were found to be confounders of the association of maternal asthma with response to ovalbumin. Although weak univariate associations were found for maternal asthma and proliferative response to other allergens (Table 3), these associations did not hold after adjustment for other cofactors (Table 4 and Table 5).

Univariate analysis suggested that Bla g 2– and Der f 1–stimulated lymphoproliferative responses were increased in association with maternal smoking during pregnancy (Table 3). Inadequate or excessive maternal weight gain also appeared weakly associated with increased allergen (Bla g 2, ovalbumin) or phytohemagglutinin responses. In addition, black (compared with white) race/ethnicity and 5-minute Apgar score of less than 8 (suggesting fetal stress) predicted increased allergen lymphoproliferative responses. Increased responses to phytohemagglutinin stimulation were also associated with black race/ethnicity and with inadequate maternal weight gain during pregnancy. Neither maternal nor paternal history of eczema, hay fever, and asthma were associated with Bla g 2, Der f 1, or phytohemagglutinin-stimulated lymphocyte proliferation (data not shown). In addition, we found no associations of maternal age, maternal prepregnancy BMI, neonate birthweight, gestational age, or neonatal intensive care unit stay with either antigen or phytohemagglutinin-induced responses (Table 3 and data not shown).

We also found that maternal smoking during pregnancy, neonate black race, and decreased 5-minute Apgar score below 8 were associated with increased Bla g 2 and Der f 1 SI in linear multivariate regression models that controlled for maternal asthma, maternal BMI, and maternal weight gain during pregnancy (Table 4). In addition to having higher allergen-stimulated responses, compared with children with white race/ethnicity, those with black race/ethnicity had significantly higher phytohemagglutinin-stimulated proliferative responses. In these multivariate regression analyses, antigen-and phytohemagglutinin-stimulated lymphoproliferative responses also tended to be higher for those with either inadequate or

excessive weight gain, although the significance of the associations varied. Results were similar in logistic regression analyses, with Bla g 2 and Der f 1 dichotomized at 2 and phytohemagglutinin dichotomized at the median (40), but they also suggested a small but significant increase in the odds of a phytohemagglutinin SI of more than 40 with increasing maternal BMI (Table 5).

DISCUSSION

In this study, we examined the impact of maternal influences on neonatal immune responses to antigens and the mitogen phytohemagglutinin. Maternal smoking during pregnancy, inadequate or excessive maternal weight gain during pregnancy, neonate black race/ethnicity (compared with white race/ethnicity), and Apgar score less than 8 were each associated with increased CBMC proliferative responses to stimulation with Bla g 2 and/or Der f 1. Maternal history of asthma was associated with increased lymphoproliferative response to ovalbumin stimulation.

For children of black ethnicity and (more weakly) for inadequate weight gain during pregnancy, we found a more generalized increased T-cell responsiveness that included responsiveness to the mitogen phytohemagglutinin and to the allergens. Our data raise the possibility (whether genetic or environmental in etiology) that differential neonatal immune responses may underlie, in part, the higher incidence of asthma in black Americans.[11,12] Although lymphocyte proliferation in a healthy cohort of newborns of various racial groups has not been extensively examined, one study detected increased expression of the costimulatory molecules CD80/86 in African American adults following mitogen and antigen stimulation.[26] CD80/86 are crucial molecules in T-cell activation and whether differences in costimulatory molecules occur in different race/ethnicity groups is under investigation.

Although there is extensive literature on the effects of cigarette smoking on neonatal lung function and development,[27,28] previous studies evaluating the effects of maternal cigarette smoking on cord blood immune responses are limited. A prior study suggested that a trend for increased cord blood proliferative responses to concanavalin A, purified protein derivative, timothy grass, and house dust mite extract has been found in mothers who were smoking during pregnancy.[20] Maternal smoking can harm the human placental development by changing the balance between cytotrophoblast proliferation and differentiation,[29] and several studies report that cigarette smoke extracts in vitro can stimulate cell proliferation[30] not only of lymphocytes[31,32] but also of lung epithelial cells.[33] The cigarette component tobacco-related glycoprotein has been previously shown to induce peripheral T-cell proliferation.[31,34] Whether preferential placental transfer of tobacco-related glycoprotein over the immunosuppressants nicotine and polycyclic aromatic hydrocarbons could explain increased lymphoproliferation in CBMCs from smoking mothers remains to be determined.[35,36] Importantly, neonates of maternal smokers have higher expression of the T_H2 cytokine interleukin 13 to both house dust mite and ovalbumin stimulation[14] and a higher risk of developing asthma or atopy in childhood.[37] Follow-up investigations in this cohort will offer the possibility to determine whether heightened lymphoproliferative responses in neonates are also associated with increased incidence of atopic diseases.

Inadequate or excessive maternal weight gain during pregnancy and an Apgar score of less than 8 likely represent a heterogeneous group of exposures that may represent sources of stress or proinflammatory conditions that may also heighten lymphoproliferative responses.[10] For example, weight development in pregnancy may be associated in part with leptin-dependent mechanisms, which have been suggested to be acute stress–related hormones.[38] Also, leptin is known to activate different signaling pathway and can induce cell proliferation.[39] Maternal weight gain may also represent differences in diet that influence the lymphoproliferative

responses.[20,40] Decreased Apgar scores may represent a marker for induction of proinflammatory cascades, eg, triggered by activation of NF-κB, which is responsible for the induction of several inflammatory genes and shown to be differentially regulated in cord blood.[41,42] However, the exact mechanism by which weight gain in pregnancy or other perinatal factors such as Apgar score can influence fetal immune responses have to be further examined.

Prior studies investigating antigen-specific lymphocyte proliferation of neonates at high vs low risk of atopy have been diverse in their findings. Increased proliferative responses to house dust mite Der p, Der extract,[15,17,20] timothy grass, purified protein derivative, β-lactoglobulin,[15,16] and ovalbumin[16] are in accordance with our findings. Heightened proliferative responses to the mitogen concanavalin A[20] have been reported in neonates at higher risk of atopy, but lower proliferative responses have also been shown.[23] Also, Miller et al[19] found no association between maternal history of atopy and antigen-induced proliferation in CBMCs. Those heterogenous findings may be influenced by different exposure to antigens,[20,24] race/ethnicity, or potential other covariates such as cigarette smoking as reported in our study.

This study has the following limitations. The generalizability of the results on maternal asthma and cord blood lymphoproliferative response to ovalbumin may be limited, in part because of relatively small numbers of mothers with asthma and an ovalbumin SI of more than 2. The lack of consistency in the data (in that maternal responsiveness did not predict significantly heightened responsiveness to other stimulants) also tempers our interpretation of the association of maternal asthma with ovalbumin response. The lack of records on maternal use of steroids may have limited our ability to investigate associations of maternal asthma with lymphoproliferative responses, taking into account the use of anti-inflammatory medications. This may have biased results to the null. We did not have documentation of administration of steroids to the fetus, but our sample was biased toward healthy normal deliveries in that we generally could not get cord blood during emergent, difficult, or premature deliveries. Two children in our cohort were sick after delivery—one was treated for presumed sepsis and necrotizing enterocolitis and one for hyaline membrane disease. Elimination of these children from analyses (sensitivity analyses) showed no decrease in the magnitude of the effects of the predictors of interest but showed a slight decrease in the precision of the estimate for an Apgar score of less then 8. We do not know the extent to which the heightened responsiveness of children of black race/ethnicity to allergen and mitogen stimulation represents an inherited characteristic vs responses to unmeasured exposures (perhaps related to poverty or discrimination) encountered more commonly by blacks compared with whites in our cohort.

In summary, maternal smoking during pregnancy, inadequate or excessive maternal weight gain during pregnancy, neonate black race/ethnicity (compared with white race/ethnicity), and an Apgar score less than 8 were associated with enhanced neonatal responses. Continuing efforts to understand the influence of maternal and genetic factors on newborn lymphocyte response to antigens may help elucidate critical pathways leading to atopic disease.

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Table 1

Population Characteristics

Characteristics	No. (%) of population (N = 427)*
Maternal age, y	
Minimum	16
25th percentile	29
50th percentile	32
75th percentile	35
Maximum	44
Maternal prepregnancy BMI	
Minimum	15
25th percentile	21
50th percentile	23
75th percentile	27
Maximum	48
Maternal weight gain during pregnancy†	
Inadequate	70 (17)
Adequate	140 (33)
Excessive	214 (50)
Maternal smoking during pregnancy	
Yes	10 (2)
No (never or former)	365 (85)
Missing	52 (12)
Maternal history of asthma	
Yes	56 (13)
No	371 (86)
Neonate sex	
Female	197 (46)
Male	230 (54)
Gestational age, wk	
Minimum	31
25th percentile	39
50th percentile	40
75th percentile	41
Maximum	43
Neonate birthweight, g	
Minimum	1,786
25th percentile	3,220
50th percentile	3,515
75th percentile	3,855
Maximum	5,329
Neonate race/ethnicity	
White	282 (68)
Black	78 (19)
Hispanic	30 (7)
Asian	27 (6)
5-Minute Apgar score	
≥8	415 (97)
<8	11 (3)

Abbreviation: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters).

*Data are number (percentage) of population unless otherwise indicated. Total sample size may not add up to 427 and total percentage may not add up to 100% because of missing values or rounding.

†Defined according to Institute of Medicine guidelines,[22] using prepregnancy BMI and total weight gain during pregnancy.

Table 2

Antigen and Mitogen-Stimulated Cord Lymphocyte Proliferation Shown as SI

Lymphocyte proliferation response (SI)	No. of participants	Minimum	25th percentile	50th percentile	75th percentile	Maximum
Bla g 2	374	0.34	0.98	1.31	1.95	12.19
SI >2	91					
SI ≤2	283					
Der f 1	374	0.34	1.13	1.69	2.99	19.45
SI >2	146					
SI ≤2	228					
Ovalbumin	427	0.50	0.97	1.09	1.40	12.44
SI >2	52					
SI ≤2	375					
Phytohemagglutinin	427	1.15	20.24	39.42	71.22	458.86
SI >40	213					
SI ≤40	214					

Abbreviation: SI, ratio of mean counts per minute of stimulated lymphocytes divided by mean counts per minute of unstimulated lymphocytes.

Table 3

Univariate Associations of Parental and Child Characteristics With Antigen- and Mitogen-Stimulated Lymphocyte Proliferation as Measured by SI

Characteristics	Bla g 2			Der f 1			Ovalbumin			Phytohemagglutinin		
	R	Geometric mean*	*P*value	*R*	Geometric mean SI*	*P*value	*R*	Median*	*P*value	*R*	Geometric mean SI*	*P*value
Maternal history of asthma												
Yes		1.67	.09		2.07	.20		1.21	.007		41.1	.36
No		1.43			1.81			1.08			36.1	
Paternal history of asthma												
Yes		1.27	.11		1.69	.38		1.15	.19		32.7	.41
No		1.48			1.86			1.08			36.9	
Maternal age	−0.05		.30	−0.02		.74	0.001		.98	−0.06		.24
Maternal prepregnancy BMI	0.05		.32	0.06		.29	−0.02		.69	0.08		.10
Maternal weight gain during pregnancy†												
Inadequate		1.56	.11		1.96	.34		1.12	.28		47.0	.02
Adequate (referent)		1.35			1.77			1.07			33.3	
Excessive		1.50	.12		1.84	.63		1.12	.05		36.2	.43
Maternal smoking during pregnancy												
Yes		2.20	.02		3.39	.004		1.07	.76		50.3	.31
No (referent)		1.44			1.81			1.08			36.4	
Missing		1.45	>.99		1.83	.95	-	1.18	.08	-	43.2	.21
Neonate sex												
Male		1.49	.37		1.89	.41		1.10	.69		37.5	.63
Female		1.41			1.78			1.11			35.8	
Neonate race/ethnicity												
White (referent)		1.38			1.70			1.09			34.4	
Black‡		1.78	.002		2.40	<.001		1.13	.33		57.4	<.001
Hispanic‡		1.52	.37		1.91	.39		1.09	.87		28.8	.33
Asian‡		1.39	.93		1.81	.67		1.06	.48		28.0	.28
5-Minute Apgar score												
≥8 (referent)		1.44			1.81			1.09			36.6	
<8		2.34	.03		2.84	.08		2.05	<.001		40.9	.71

Abbreviation: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); SI, ratio of mean counts per minute of stimulated lymphocytes divided by mean counts per minute of unstimulated lymphocytes.

*For all SI outcomes other than ovalbumin SI, the *P* value is for the *t* test when comparing lnSI for children in 2 discrete categories (eg, maternal asthma yes vs maternal asthma no). For the outcome ovalbumin SI, *P* value is for the Kruskal-Wallis test when comparing SI for children in 2 discrete categories. When evaluating association of lnSI with level of a predictor measured as a continuous variable (eg, gestational age), *P* value is for the Spearman correlation.

†Defined according to Institute of Medicine guidelines,[22] using prepregnancy BMI and total weight gain during pregnancy (see text).

‡Each group compared with whites.

Table 4

Adjusted Geometric Mean SI for Associations of Parental and Child Characteristics With Antigen-Specific Lymphocyte Proliferation*

Variables	Adjusted geometric mean SI (95% CI)	*P*value
Bla g 2 SI (n = 356)†		
Maternal history of asthma		
Yes	2.30 (1.72–2.89)	.76
No	2.23 (1.70–3.09)	
Maternal smoking during pregnancy		
Yes	3.16 (2.06–4.84)	.02
No (referent)	1.80 (1.60–2.58)	
Missing	2.03 (1.35–2.40)	.24
Maternal weight gain during pregnancy‡		
Inadequate	2.41 (1.79–3.24)	.07
Adequate (referent)	2.03 (1.55–2.67)	
Excessive	2.37 (1.81–3.11)	.03
Neonate race/ethnicity		
White (referent)	1.99 (1.53–2.59)	
Black	2.69 (2.02–3.60)	<.001
Hispanic	2.22 (1.59–3.09)	.36
Asian	2.20 (1.57–3.10)	.42
5-Minute Apgar score		
≥8 (referent)	1.72 (1.46–2.03)	
<8	2.98 (1.89–4.68)	.01
Der f 1 SI (n = 356)†		
Maternal history of asthma		
Yes	3.03 (2.16–4.24)	.96
No	3.01 (2.24–4.04)	
Maternal smoking during pregnancy		
Yes	4.94 (3.05–8.00)	.002
No (referent)	2.57 (1.97–3.36)	
Missing	2.17 (1.57–3.00)	.15
Maternal weight gain during pregnancy‡		
Inadequate	3.20 (2.19–4.47)	.22
Adequate (referent)	2.81 (2.07–3.82)	
Excessive	3.06 (2.25–4.16)	.29
Neonate race/ethnicity		
White (referent)	2.57 (1.91–3.46)	
Black	3.80 (2.74–5.28)	<.001
Hispanic	2.91 (2.01–4.24)	.35
Asian	2.92 (1.99–4.29)	.38
5-Minute Apgar score		
≥8 (referent)	2.30 (1.90–2.78)	
<8	3.97 (2.38–6.62)	.03
Phytohemagglutinin SI (n = 407)†		
Maternal history of asthma		
Yes	44.5 (28.6–69.2)	.91
No	43.8 (29.9–64.0)	
Maternal smoking during pregnancy		
Yes	55.7 (27.9–111.3)	.24
No (referent)	38.4 (27.4–53.8)	
Missing	40.2 (26.7–60.6)	.77
Maternal weight gain during pregnancy‡		
Inadequate	52.5 (33.7–81.8)	.05
Adequate (referent)	39.0 (26.0–58.4)	
Excessive	42.0 (28.4–62.2)	.51

Variables	Adjusted geometric mean SI (95% CI)	*P*value
Neonate race/ethnicity		
White (referent)	43.1 (29.3–63.3)	
Black	68.8 (45.2–104.6)	<.001
Hispanic	34.4 (20.8–56.8)	.24
Asian	37.3 (22.1–63.0)	.48
5-Minute Apgar score		
≥8 (referent)	42.0 (32.1–55.2)	
<8	46.3 (24.6–87.0)	.75

Abbreviations: CI, confidence interval; SI, ratio of mean counts per minute of stimulated lymphocytes divided by mean counts per minute of unstimulated lymphocytes.

* Models also adjusted for maternal body mass index.

† $\log_e$ transformed.

‡ Defined according to Institute of Medicine guidelines,[22] using prepregnancy body mass index and total weight gain during pregnancy (see text).

Table 5

Odds Ratios for Associations of Parental and Child Characteristics With Antigen-Specific Lymphocyte Proliferation

Variables	OR (95% CI)
Bla g 2 SI (n = 356, SI >2 in 85 participants)*	
Maternal history of asthma	
Yes	1.09 (0.50–2.43)
No (referent)	1.00
Maternal smoking during pregnancy	
Yes	18.26 (3.53–94.42)
No (referent)	1.00
Missing	0.71 (0.30–1.68)
Maternal BMI	1.01 (0.96–1.06)
Maternal weight gain during pregnancy†	
Inadequate	2.27 (1.00–5.15)
Adequate (referent)	1.00
Excessive	2.59 (1.34–5.00)
Neonate race/ethnicity	
White (referent)	1.00
Black	2.97 (1.49–5.92)
Hispanic	1.75 (0.65–4.69)
Asian	2.37 (0.80–7.00)
5-Minute Apgar score	
≥8 (referent)	1.00
<8	6.09 (1.23–30.09)
Der f 1 SI (n = 356, SI >2 in 139 participants)*	
Maternal history of asthma	
Yes	0.86 (0.42–1.73)
No	1.00
Maternal smoking (during pregnancy)	
Yes	6.79 (1.39–33.29)
No (referent)	1.00
Missing	0.52 (0.24–1.12)
Maternal BMI	1.01 (0.97–1.06)
Maternal weight gain during pregnancy†	
Inadequate	1.16 (0.59–2.28)
Adequate (referent)	1.00
Excessive	1.41 (0.84–2.37)
Neonate race/ethnicity	
White (referent)	1.00
Black	2.73 (1.46–5.11)
Hispanic	1.86 (0.80–4.33)
Asian	1.49 (0.60–3.75)
5-Minute Apgar score	
≥8 (referent)	1.00
<8	2.85 (0.61–13.3)
Phytohemagglutinin SI (n = 407, SI >40.1 in 203 participants)‡	
Maternal history of asthma	
Yes	1.04 (0.56–1.92)
No	1.00
Maternal smoking (during pregnancy)	
Yes	1.71 (0.47–6.27)
No (referent)	1.00
Missing	1.26 (0.64–2.50)
Maternal BMI	1.05 (1.01–1.10)
Maternal weight gain during pregnancy†	
Inadequate	1.44 (0.77–2.68)

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Variables	OR (95% CI)
Adequate (referent)	1.00
Excessive	0.94 (0.59–1.51)
Neonate race/ethnicity	
White (referent)	1.00
Black	1.80 (1.01–3.20)
Hispanic	0.61 (0.27–1.36)
Asian	1.47 (0.64–3.36)
5-Minute Apgar score	
≥8 (referent)	1.00
<8	0.76 (0.22–2.62)

Abbreviation: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); CI, confidence interval; OR, odds ratio; SI, ratio of mean counts per minute of stimulated lymphocytes divided by mean counts per minute of unstimulated lymphocytes.

* Dichotomized at 2.

† Defined according to Institute of Medicine guidelines,[22] using prepregnancy BMI and total weight gain during pregnancy (see text).

‡ Dichotomized at median.