Maternal Cytokine Profiles during Pregnancy Predict Asthma in Children of Mothers without Asthma

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Abstract

Little is known about whether maternal immune status during pregnancy influences asthma development in the child. We measured cytokine production in supernatants from mitogen-stimulated peripheral blood immune cells collected during and after pregnancy from the mothers of children enrolled in the Tucson Infant Immune Study, a nonselected birth cohort. Physician-diagnosed active asthma in children through age 9 and a history of asthma in their mothers were assessed through questionnaires. Maternal production of each of the cytokines IL-13, IL-4, IL-5, IFN- γ , IL-10, and IL-17 during pregnancy was unrelated to childhood asthma. However, IFN- γ /IL-13 and IFN- γ /IL-4 ratios during pregnancy were associated with a decreased risk of childhood asthma ($n = 381$; odds ratio [OR], 0.33; 95% confidence interval [CI], 0.17–0.66; $P = 0.002$; and $n = 368$; OR, 0.36; 95% CI, 0.18–0.71;

$P = 0.003$, respectively). The inverse relations of these two ratios with childhood asthma were only evident in mothers without asthma ($n = 309$; OR, 0.18; 95% CI, 0.08–0.42; $P = 0.00007$; and $n = 299$; OR, 0.17; 95% CI, 0.07–0.39; $P = 0.00003$, respectively) and not in mothers with asthma ($n = 72$ and 69 , respectively; P for interaction by maternal asthma = 0.036 and 0.002, respectively). Paternal cytokine ratios were unrelated to childhood asthma. Maternal cytokine ratios in mothers without asthma were unrelated to the children's skin-test reactivity, total IgE, physician-confirmed allergic rhinitis at age 5, or eczema in infancy. To our knowledge, this study provides the first evidence that cytokine profiles in pregnant mothers without asthma relate to the risk for childhood asthma, but not allergy, and suggests a process of asthma development that begins *in utero* and is independent of allergy.

Keywords: asthma; children; cytokines; maternal; pregnancy

The etiology of childhood asthma remains elusive. Although both genetic variants and environmental exposures have been implicated (1), the nature of their etiologic contributions is far from being completely characterized. The role of allergy as a causative factor in asthma is also unclear. Because of the many studies that have shown strong associations of asthma with allergy phenotypes, asthma is widely

considered an allergic disease (2). However, asthma often occurs in individuals who are allergen skin-test negative (3). Furthermore, although the direct association of total serum IgE with asthma prevalence is quite striking (4), cord blood or very early IgE levels are at best only weakly related to the development of asthma (5, 6), suggesting that IgE may not be a primary etiologic driver of disease development.

Because type 2 cytokines are known to influence IgE synthesis, investigators have performed mitogen stimulation of immune blood cells to investigate whether type 2 cytokine bias occurs in subjects with asthma. Here, studies have also differed, with some suggesting that type 2 cytokines are related to skin-test reactivity rather than asthma *per se* (7, 8). In our own previous study, we assessed early-life

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Clinical Relevance

Our study identifies a risk factor for asthma that is specific to children of mothers without asthma. Cytokines produced by mitogen-stimulated immune cells from pregnant women were strongly associated with asthma in their children, a relation that was evident for children of mothers without asthma but not for those of mothers with asthma. More specifically, among children born to mothers without asthma, maternal production of IFN- γ relative to IL-13 and to IL-4 during pregnancy was inversely related to asthma in the child. This relationship was independent of childhood allergy phenotypes, suggesting a process of asthma development not shared by allergy. To our knowledge, this is the first report of a maternal risk factor for asthma that is specific to children of mothers without asthma.

cytokine production from peripheral blood immune cells and examined the relation to subsequent diagnosis of asthma in the Tucson Infant Immune Study, a longitudinal birth cohort that was enrolled nonselectively with regard to family history of asthma or allergy (9). We found that mitogen-induced production of IL-13 (but not IL-4, IL-5, or IFN- γ) from immune cells very early in life was associated with subsequent asthma, and that the IL-13 relation was strengthened by adjusting for IFN- γ production. Also, after adjustment for IL-13, IFN- γ was found to be inversely related to asthma. These cytokine relations to asthma at ages 2–9 years were observed in immune cell samples obtained from the children at age 3 months, but not in later samples. Although such results might suggest a type 2 allergy association with asthma, IL-13 relative to IFN- γ at 3 months did not show a relation to subsequent IgE levels. Thus, this early-life cytokine production related to asthma development independent of IgE suggests that asthma processes may already be underway by 3 months of age, and that the prevailing conceptualization of type 2 cytokines as representing only allergy may be too narrow.

The possibility that asthma might have its origins in infancy or even *in utero* is consistent with findings that maternal asthma is a stronger risk factor than paternal asthma for asthma in the child (10). Further support for the *in utero* origins of asthma comes from reports of associations between childhood asthma and other maternal pregnancy conditions and exposures, including maternal age, smoking, infectious illness, preeclampsia, stress, weight gain, and exposure to farm animals (11–24). Here, we asked whether mitogen-stimulated cytokine production from circulating immune cells from pregnant mothers would show a relation to the development of asthma in their children, and if so, whether this relation would differ by maternal asthma status. Finding such an immune function link would not in itself implicate the maternal cytokines as directly affecting the fetus, but it would, nonetheless, address the possibility that a trajectory to childhood asthma may begin *in utero*, especially if the relation were limited to pregnancy.

Methods

Study Population

The Tucson Infant Immune Study is a prospective population-based birth cohort study of immune system maturation and its relation to the development of asthma and allergy. Participating mothers and fathers to be, who were not selected for asthma or allergy, agreed to enroll their children in the study during prenatal visits at three sites in Tucson between 1996 and 2004, as previously described (25). This research was approved by the Institutional Review Board of the University of Arizona, and written informed consent/assent was obtained from/for all subjects.

Cytokine Production and Total IgE Measurement

Blood samples were obtained from mothers during the third trimester of pregnancy ($n = 470$, mean weeks gestation: 39.5 [SD = 1.2], mean age 29.4 [SD = 6.1] years). Production of IFN- γ , IL-13, IL-4, IL-5, IL-10, and IL-17 was measured by ELISA from peripheral blood mononuclear cells (PBMCs) stimulated with Con A/PMA (26) (for additional details, *see* the data supplement). Blood samples from infants

and mothers when the infants were 3 months old (2.9 ± 1.2 mo), from fathers at enrollment, and from the mother, father, and child when the child was 5 years of age were processed and assayed for cytokine production as described above. Total IgE was measured in maternal third-trimester sera and age 5 child plasma (method described in the data supplement).

Asthma, Allergen Skin-Test Reactivity, Allergic Rhinitis, and Atopic Dermatitis in the Children

Data regarding asthma diagnosis, symptoms, and medication use were obtained from questionnaires completed when the children were 2, 3, 5, and 9 years old. Childhood asthma was defined as a physician diagnosis with symptoms or medication use in the past year at any of these time points, as previously described (27). A child was considered to have “no asthma” if physician-diagnosed asthma was never reported. Some analyses were repeated with childhood asthma as the outcome but limited to ages 5 and/or 9 years. Assessment of skin-test reactivity is described in the data supplement, as is the ascertainment of physician-diagnosed allergic rhinitis at age 5 and atopic dermatitis at age 1.

Parental Asthma, Skin-Test Reactivity, and Demographics

Physician-diagnosed maternal and paternal asthma was ascertained by questionnaire at enrollment and at the age 5 visit (for additional details, *see* the data supplement). A parent was considered to be skin-test positive if she or he had at least one positive skin-test reaction (as described in the data supplement for children). Questionnaires at enrollment also provided maternal age, ethnicity, education, parity, smoking, and number of children in the household.

Statistical Analysis

Log₁₀ transformation of cytokine ratios provided normal distributions. For some analyses, IFN- γ /IL-13 ratios were divided into quartiles. Chi-square tests, Fisher’s exact tests, and linear regression and logistic regression models were used for analysis. For multiple comparisons, the α level was adjusted by a Bonferroni correction (0.05/number of comparisons). Receiver operating characteristic (ROC)

curve analyses are reported as the area under the curve. Stata 14.0 or SPSS 24 was used for analysis.

Results

Mother-child pairs with complete data regarding maternal cytokine production from third-trimester samples, maternal asthma, and asthma in the child were included in this analysis ($n = 394$). When compared with those without complete data ($n = 88$), the included mother-child pairs were more likely to come from nonsmoking households and the mothers were older, were more likely to be non-Hispanic white, and had lower total IgE levels and more years of education. The included mother-child pairs did not differ from those without complete data with respect to maternal asthma, atopy, sex of the child, parity, or number of children in the household (see Table E1 in the data supplement).

The prevalence of active physician-diagnosed asthma among children increased from ages 2 to 9 years: 5.3% at age 2 (18/341), 8.7% at age 3 (30/344), 13.1% at age 5 (46/350), and 15.2% at age 9 (49/323). The cumulative prevalence of active physician-diagnosed asthma in children from ages 2 to 9 years (hereafter referred to as childhood asthma) was 17.8% (70/394). Of the 18 children who received an asthma diagnosis at age 2, 16 had active symptoms at a subsequent age. The prevalence of asthma was higher among children of mothers with asthma than among those of mothers without asthma (29.3% [22/75] vs. 15.1% [48/319], respectively; $P = 0.007$). The prevalence of asthma was similar among children of fathers with and without asthma (20.6% [13/63] vs. 16.4% [50/305], respectively; $P = 0.462$).

Maternal Cytokine Production during Pregnancy in Relation to Subsequent Childhood Asthma

We asked whether production of the cytokines IL-13, IL-4, IL-5, and IFN- γ from PBMCs differed between pregnant mothers whose children subsequently developed asthma and mothers whose children did not, and we found no difference (Table 1, upper section). Because individual cytokine levels produced by mitogen-stimulated PBMCs (both type 1 and type 2 cytokines) are known to show a strong within-subject

Table 1. Relation of Maternal Cytokine Production during Pregnancy to Childhood Asthma

Maternal Cytokines	No Childhood Asthma			Yes Childhood Asthma			P Value*
	n	GM	95% CI	n	GM	95% CI	
IFN- γ	323	4,813	4,144–5,591	70	4,388	3,328–5,784	0.60
IL-13	323	222	198–250	70	300	237–378	0.030
IL-4	313	9.6	8.6–10.8	67	12.5	9.7–16.0	0.057
IL-5	289	81.6	72.1–92.4	63	109	85.0–140	0.049
IFN- γ /IL-13	322	21.5	19.5–23.7	70	14.6	12.0–17.8	0.0009
IFN- γ /IL-4	312	530	481–583	67	354	281–447	0.0008
IFN- γ /IL-5	289	63.1	54.4–73.2	63	43.9	32.4–59.3	0.040
IL-10	318	103	88.5–120	67	142	106–191	0.080
IL-17 [†]	324	200	31.3–452	70	173	54.2–453	0.521
IL-10/IL-17	318	0.64	0.56–0.73	67	0.79	0.60–1.04	0.184

Definition of abbreviations: CI = confidence interval; GM = geometric mean.

* $P < 0.005$ considered significant after Bonferroni correction for multiple comparisons (0.05/10) for differences in maternal cytokine log means by childhood asthma.

[†]Because the IL-17 values included 27.9% undetectable values, these data are presented as the median and interquartile range; groups were compared with the Wilcoxon test.

correlation (26), and yet type 1 and type 2 cytokines are also known to be capable of regulating each other (28), we compared ratios of the type 1 cytokine IFN- γ and

the type 2 cytokines IL-13, IL-4, and IL-5. We found that the maternal cytokine ratios for IFN- γ /IL-13 and IFN- γ /IL-4 were markedly decreased in mothers whose

Table 2. Maternal IFN- γ /IL-13 and IFN- γ /IL-4 Ratios during Pregnancy in Relation to Potential Confounders

	Maternal Cytokine Ratios during Pregnancy							
	IFN- γ /IL-13				IFN- γ /IL-4			
	n	GM	95% CI	P Value*	n	GM	95% CI	P Value*
Maternal asthma								
No	317	21.0	19.1–23.2	0.032	307	516	467–571	0.039
Yes	75	16.5	13.6–20.0		72	406	331–498	
Maternal skin-test positivity								
No	126	21.3	18.5–24.5	0.354	121	567	489–657	0.041
Yes	256	19.5	17.4–21.8		248	463	413–519	
Maternal ethnicity								
Non-Hispanic white	288	20.0	18.1–22.1	0.026	280	505	456–560	0.177
Hispanic	71	24.1	20.1–28.9		66	516	429–621	
Other	15	12.6	6.8–23.3		15	330	160–683	
Previous births								
0	216	18.6	16.5–20.9	0.126	210	466	411–528	0.356
1	114	22.8	19.7–26.4		109	539	463–628	
≥ 2	62	20.7	16.1–26.7		60	512	402–652	
Number of children in household								
0	191	18.5	16.3–21.0	0.298	184	468	410–535	0.465
1	110	21.7	18.6–25.2		107	509	438–591	
≥ 2	70	20.4	16.1–25.7		67	543	425–694	
Household smoking								
No	312	19.6	17.8–21.7	0.358	302	481	435–531	0.278
Yes	75	21.8	17.7–26.9		72	546	440–677	
Maternal education								
≤ 12 yr	69	23.2	18.8–28.8	0.129	66	547	437–684	0.301
> 12 yr	323	19.4	17.7–21.4		313	483	437–533	
Maternal age [†]	392	$\rho = -0.04$		0.477	379	$\rho = -0.05$		0.343
Log maternal serum IgE	391	$r = -0.17$		0.0008	378	$r = -0.16$		0.002

Definition of abbreviations: CI = confidence interval; GM = geometric mean.

* P based on one-way ANOVA unless otherwise indicated.

[†]Nonparametric Spearman ρ .

children developed asthma compared with mothers whose children did not develop asthma (Table 1, middle section). No such difference, however, was observed for the IFN- γ /IL-5 ratio (Table 1). The differences in the maternal IFN- γ /IL-13 and IFN- γ /IL-4 ratios in relation to asthma in the child were retained when asthma was limited to 5 and/or 9 years (Table E2).

Because recent studies have suggested that IL-17 expression is increased in severe asthma (29, 30), and that IL-10 may have a protective role in this disease (29, 31, 32), we also assayed production of these cytokines by maternal PBMCs. Individually, the production of these cytokines did not relate to childhood asthma (Table 1, lower section). These two cytokines showed a strong positive correlation ($\rho = 0.58$), and yet cells producing IL-10 have been shown to be capable of negatively regulating IL-17

responses (33, 34). Therefore, we also examined whether the maternal IL-10/IL-17 ratios might differ for mothers whose children did or did not develop asthma; however, no such difference was evident (Table 1).

The Relation of Maternal IFN- γ /IL-13 and IFN- γ /IL-4 Ratios during Pregnancy to Childhood Asthma Differs by Maternal Asthma Status

Factors that might relate to maternal IFN- γ /IL-13 and IFN- γ /IL-4 ratios (Table 2) and/or childhood asthma (Table 3) were examined as possible confounders. Of all the factors examined, only maternal asthma fit the traditional definition of a confounder in that it related to both maternal cytokine ratios and childhood asthma. However, because we had identified maternal asthma *a priori* as a potential effect modifier, we examined

the relation of maternal IFN- γ /IL-13 and IFN- γ /IL-4 ratios to childhood asthma separately for children of mothers with and without asthma. For the adjusted models that follow, we then conservatively included any factor that was related to *either* the maternal cytokine ratios *or* childhood asthma as covariates. When stratified by maternal asthma, the inverse relations of maternal IFN- γ /IL-13 and IFN- γ /IL-4 to childhood asthma were found to be present only for children of mothers without asthma (Table 4). No relations were evident for children of mothers with asthma (Table 4). The interactions of maternal asthma status and maternal ratios in relation to childhood asthma were statistically significant ($P = 0.036$ for IFN- γ /IL-13 and 0.002 for IFN- γ /IL-4). This inverse relation of cytokine ratios for mothers without asthma to childhood asthma prevalence appeared to be roughly linear, as shown in Figure 1, which plots childhood asthma prevalence by quartiles of maternal IFN- γ /IL-13. A similar linearity was observed for quartiles of IFN- γ /IL-4 ratios for mothers without asthma in relation to childhood asthma (data not shown).

The Relation of Maternal Cytokine Ratios to Childhood Asthma Is Independent of Childhood Allergy

In contrast to the relations of maternal IFN- γ /IL-13 and IFN- γ /IL-4 ratios to childhood asthma, these ratios showed no relation to childhood allergic rhinitis, allergen skin-test positivity, or log total IgE at age 5 (Table 5), or to early-life eczema (Table E3). In addition, among the 234 children of mothers without asthma and skin tests at age 5, the relation of maternal ratios to childhood asthma was present in both skin-test-positive and skin-test-negative children, and thus was independent of childhood skin-test reactivity (Table E4).

Relation of IFN- γ /IL-13 and IFN- γ /IL-4 Ratios from Maternal Samples Obtained after Delivery and Ratios from Paternal Samples to Childhood Asthma

To determine whether the relations of maternal IFN- γ /IL-13 and IFN- γ /IL-4 ratios to childhood asthma were also evident in maternal samples obtained after delivery, we assessed these ratios in

Table 3. Childhood Asthma in Relation to Potential Confounders

	Childhood Asthma		
	<i>n</i>	%	<i>P</i> Value*
Sex of child			
Male	190	20.0	0.263
Female	204	15.7	
Maternal asthma			
No	319	15.1	0.004
Yes	75	29.3	
Maternal skin-test positivity			
No	126	16.7	0.778
Yes	258	17.8	
Maternal ethnicity			
Non-Hispanic white	290	17.2	0.644
Hispanic	71	18.3	
Other	15	26.7	
Previous births			
0	217	18.4	0.910
1	115	16.5	
≥ 2	62	17.7	
Number of children in household			
0	192	16.7	0.616
1	111	16.2	
≥ 2	70	21.4	
Household smoking			
No	314	15.6	0.084
Yes	75	24.0	
Maternal education			
≤ 12 yr	69	20.3	0.546
> 12 yr	325	17.2	
Maternal age	394	OR, 0.96 (95% CI, 0.92–1.00)	0.055 [†]
Maternal log serum IgE	393	OR, 0.97 (95% CI, 0.67–1.40)	0.855 [†]

Definition of abbreviations: CI = confidence interval; OR = odds ratio.

**P* value based on Pearson chi-square statistic unless otherwise indicated.

[†]*P* value based on logistic regression.

Table 4. Odds of Childhood Asthma Associated with Maternal IFN- γ /IL-13 and IFN- γ /IL-4 during Pregnancy for All Children and Stratified by Maternal Asthma

Maternal Ratios	Childhood Asthma			
	<i>n</i>	OR*	95% CI	<i>P</i> Value†
All mothers‡				
IFN- γ /IL-13	381	0.33	0.17–0.66	0.0017
IFN- γ /IL-4	368	0.36	0.18–0.71	0.0031
Mothers without asthma				
IFN- γ /IL-13	309	0.18	0.08–0.42	0.00007
IFN- γ /IL-4	299	0.17	0.07–0.39	0.00003
Mothers with asthma				
IFN- γ /IL-13	72	2.49	0.54–11.6	0.243
IFN- γ /IL-4	69	4.30	0.86–21.6	0.076

Definition of abbreviations: CI = confidence interval; OR = odds ratio.

*OR estimated for one log unit increase in ratio adjusted for maternal IgE and maternal skin-test positivity.

† $P < 0.0083$ considered significant after Bonferroni correction for multiple comparisons (0.05/6).

‡Adjusting for maternal asthma, maternal IgE and maternal skin-test positivity, the ORs for all mothers' IFN- γ /IL-13 and IFN- γ /IL-4 ratios are 0.35 (95% CI, 0.17–0.70; $P = 0.0029$) and 0.36 (95% CI, 0.18–0.73; $P = 0.0043$), respectively.

samples obtained at 3 months and 5 years after birth of the child. The marked difference in the relations of maternal cytokine ratios to childhood asthma upon stratification by maternal asthma were no longer evident in the maternal samples obtained 3 months postpartum (Table 6). The cytokine ratios for “all mothers” and the subgroups based on maternal asthma tended to show inverse relations to childhood asthma, but significance was reached only for IFN- γ /IL-13 for “all mothers.” No relation of childhood

asthma with maternal cytokine ratios was detected in samples obtained 5 years after the child's birth, and no relation to childhood asthma was evident for paternal cytokine ratios at the time of birth or 5 years later (data not shown).

The Maternal IFN- γ /IL-13 Ratio during Pregnancy and the Child's 3-Month IFN- γ /IL-13 Ratio Are Independently Related to Childhood Asthma

In this same population, we previously observed that the child's 3-month IFN- γ

and IL-13 production levels, when mutually adjusted for each other, were associated with subsequent asthma (reducing and increasing risk, respectively) (9). To assess whether childhood asthma was independently related to the IFN- γ /IL-13 ratio of pregnant mothers without asthma and the child's 3-month ratio, we included both ratios in a single regression model. Both ratios were inversely and independently related to childhood asthma (Table 7).

Employing ROC analysis, we first assessed the capacity of the child's 3-month IFN- γ /IL-13 ratio to predict childhood asthma. When the maternal IFN- γ /IL-13 ratio during pregnancy was added to the analysis, the area under the curve (for childhood asthma prediction) was significantly increased (from 0.67 to 0.77; $P = 0.02$; Figure 2). The relation of the maternal IFN- γ /IL-13 ratio during pregnancy to childhood asthma was not mediated by a path that involved the child's 3-month ratio, as shown by the mediation analysis in Figure E1.

Discussion

This study identified a pattern of cytokine production by immune cells obtained from mothers during pregnancy that predicted a risk for asthma in their children. This pattern was one in which increases in the ratios of IFN- γ /IL-13 and IFN- γ /IL-4 (i.e., more IFN- γ relative to IL-13 or IL-4) were associated with a reduction in the prevalence of childhood asthma. These relations were evident only among children of mothers without asthma, decreased postnatally, did not hold for other cytokines (IL-10 and IL-17), and were not evident for allergy-related outcomes in the children. The finding that the relation of childhood asthma to these cytokine ratios in mothers without asthma diminished after pregnancy suggests that the maternal cytokine ratios may have influenced the fetus, or were associated with other, unknown factors that influenced the fetus in ways that had long-term effects on the child's risk of asthma, but not allergy.

In contrast to mothers without asthma, increasing IFN- γ /IL-13 and IFN- γ /IL-4 ratios in pregnant mothers with asthma did not relate to a reduced risk for childhood asthma. If anything, the data in Table 4

Figure 1. Percent childhood asthma by quartiles of maternal IFN- γ /IL-13 ratios of pregnant mothers without asthma (solid bars) and pregnant mothers with asthma (open bars). Minimum and maximum values for the ratio quartiles: 0.6–11.7, 11.8–20.4, 20.5–38.0, and 38.1–231.

Table 5. Relation of Maternal IFN- γ /IL-13 and IFN- γ /IL-4 Ratios during Pregnancy to Childhood Allergy-Related Traits, for All Children and Stratified by Maternal Asthma

Maternal Ratios	Children at Age 5										
	Allergic Rhinitis				Skin-Test Positive				Total IgE		
	<i>n</i>	OR*	95% CI	<i>P</i> Value [†]	<i>n</i>	OR	95% CI	<i>P</i> Value [†]	<i>n</i>	<i>r</i> [‡]	<i>P</i> Value [†]
All mothers											
IFN- γ /IL-13	336	0.87	0.42–1.84	0.7	288	0.86	0.45–1.64	0.6	241	−0.09	0.14
IFN- γ /IL-4	326	0.56	0.27–1.16	0.12	279	0.84	0.45–1.57	0.6	234	−0.13	0.043
Mothers without asthma											
IFN- γ /IL-13	275	0.65	0.28–1.53	0.3	232	0.83	0.40–1.74	0.6	195	−0.06	0.4
IFN- γ /IL-4	268	0.38	0.16–0.87	0.023	226	0.75	0.37–1.53	0.4	190	−0.13	0.077
Mothers with asthma											
IFN- γ /IL-13	61	3.28	0.59–18.3	0.2	56	1.21	0.30–4.93	0.8	46	−0.13	0.4
IFN- γ /IL-4	58	2.46	0.46–13.2	0.3	53	1.61	0.39–6.67	0.5	44	−0.06	0.7

Definition of abbreviations: CI = confidence interval; OR = odds ratio.

*OR estimated for one log unit increase in maternal cytokine ratio adjusted for maternal total serum IgE and maternal skin-test positivity.

[†]*P* < 0.0028 considered significant after Bonferroni correction for multiple comparisons (0.05/18).

[‡]Pearson correlation coefficient for the relation of maternal cytokine ratio and total serum IgE in children at age 5.

suggest a tendency toward the opposite. These differences in the relation of maternal cytokine ratios to childhood asthma by maternal asthma status were sufficiently marked to generate significant interaction terms when we compared the two maternal asthma groups. We emphasize that this marked difference in the relation of the maternal ratios to childhood asthma was not driven by differences in cytokine ratios between these maternal groups. Although the maternal cytokine ratios were somewhat reduced for mothers with asthma compared with those

without asthma (Table 2), it is important to note that the marked decrease in childhood asthma risk shown in Figure 1 for increasing quartile values of IFN- γ /IL-13 ratios of mothers without asthma was not seen for mothers with asthma, even though the range of ratio values in each quartile was the same for mothers with and without asthma. These results suggest that childhood asthma may develop by different pathways in the children of mothers with asthma and mothers without asthma. It is worth noting that although the childhood asthma risk was lower in

children of mothers without asthma than in those of mothers with asthma, the absolute number of children with asthma in the population was greater among children of mothers without asthma, emphasizing the importance of determining the etiology of childhood asthma in this less well studied group.

Interestingly, the maternal IFN- γ /IL-13 and IFN- γ /IL-4 ratios during pregnancy were not related to the prevalence of skin-test reactivity, total IgE, or allergic rhinitis in the children. The ratios were also unrelated to atopic dermatitis in infancy (a finding that is consistent with a previous study, albeit with different cell-stimulation conditions [35]), even though this condition is a known risk factor for asthma (36, 37). Furthermore, the relation of the cytokine ratios to asthma in children of mothers without asthma was independent of skin-test reactivity in the children. Thus, we suggest that these maternal ratios may be influencing a developmental pathway in asthma that is separate from allergy. We offer this as an alternative to the prevailing suggestion that allergy *drives* a common asthma endotype. Our speculation that allergy and asthma may develop through separate pathways has been offered in previous reports, including several studies indicating that IgE levels in early life were only weakly related or unrelated to subsequent diagnoses of asthma (3, 5, 6), thus providing no evidence for increased IgE levels preceding the diagnosis. Along

Table 6. Odds of Childhood Asthma Associated with Maternal IFN- γ /IL-13 and IFN- γ /IL-4 Ratios 3 Months after Delivery, for All Children and Stratified by Maternal Asthma

Maternal Ratios 3 Months Postpartum	Childhood Asthma			
	<i>n</i>	OR*	95% CI	<i>P</i> Value [†]
All mothers				
IFN- γ /IL-13	363	0.40	0.21–0.75	0.004
IFN- γ /IL-4	352	0.47	0.25–0.88	0.019
Mothers without asthma				
IFN- γ /IL-13	295	0.39	0.19–0.80	0.011
IFN- γ /IL-4	288	0.44	0.22–0.88	0.021
Mothers with asthma				
IFN- γ /IL-13	68	0.45	0.12–1.66	0.229
IFN- γ /IL-4	64	0.47	0.10–2.15	0.328

Definition of abbreviations: CI = confidence interval; OR = odds ratio.

*OR estimated for one log unit increase in maternal cytokine ratio after adjustment for maternal total serum IgE and maternal skin-test positivity.

[†]*P* < 0.0083 considered significant after Bonferroni correction for multiple comparisons (0.05/6).

Table 7. Risk for Childhood Asthma Associated with Maternal IFN- γ /IL-13 Ratios during Pregnancy and Children's 3-Month IFN- γ /IL-13 Ratio in Single Models, for All Children and Stratified by Maternal Asthma

IFN- γ /IL-13	Childhood Asthma		
	OR*	95% CI	P Value†
All children (n = 270)			
Maternal	0.32	0.14–0.72	0.006
Child 3 mo	0.40	0.20–0.81	0.010
Mothers without asthma (n = 214)			
Maternal	0.12	0.04–0.36	0.0001
Child 3 mo	0.31	0.13–0.73	0.008
Mothers with asthma (n = 56)			
Maternal	4.31	0.68–27.4	0.122
Child 3 mo	0.42	0.09–1.88	0.256

Definition of abbreviations: CI = confidence interval; OR = odds ratio.

*OR estimated for one log unit increase in cytokine ratio after adjustment for maternal total IgE and maternal skin-test positivity.

†P < 0.0167 considered significant after Bonferroni correction for multiple comparisons (0.05/3).

the same lines, Burrows and colleagues showed that although parental asthma was a strong risk factor for childhood asthma, and parental IgE levels showed a strong relation to children's IgE levels in the general population, the parental IgE levels showed at best a limited relation to asthma in the children (38).

The influence on childhood asthma of cytokine production among mothers without asthma was largely limited to pregnancy, and points to the possibility that this period is a critical window. It is possible that IFN- γ /IL-13 and IFN- γ /IL-4 ratios are influenced by maternal environmental exposures during pregnancy, which may

be the true drivers of a reduced or increased risk of childhood asthma. Although we found no evidence that the demographic and environmental exposures measured in our study influenced maternal cytokine production, we obviously did not measure all possible exposures. Other possible endogenous exposures for both the mother and fetus could include hormones of luteal or placental (either maternal or fetal) origin or other pregnancy-related messenger molecules. Several placental hormones have been shown to influence immune cell numbers or function (reviewed in Reference 39), and one of these, relaxin, has been shown to reduce

asthma-like phenotypes when administered to the lungs in animal models (40). Although Xu and colleagues (41) did not find a relation between maternal hormone levels and asthma in the child, their study was limited to samples from early pregnancy and examined only estradiol and progesterone. Although assessment of maternal illnesses (other than asthma), hormonal changes, and other environmental exposures during pregnancy was beyond the scope of our study, our results suggest that this is an important direction for future research.

The current study shows that relations between maternal immune characteristics during pregnancy and subsequent asthma in the child were evident when we assessed ratios rather than individual cytokines. This characteristic was also a hallmark of our previous work (9), in which an infant's own production of IFN- γ and IL-13 was found to be related to the subsequent development of childhood asthma, *but only when they were assessed relative to each other*. This finding led us to stress that variation in the balance of type 1/type 2 cytokine production appeared to be more relevant to asthma risk than the magnitude of production of individual cytokines.

Despite the similarities between our present findings and those of our previous study (9), we were able to show (Table 7) that maternal cytokine production during pregnancy and the child's 3-month cytokine production were *independently* associated with childhood asthma. The substantial increase in the capacity to predict asthma risk that is contributed by the maternal IFN- γ /IL-13 ratio over that obtained from the child's 3-month ratio was shown by ROC analysis (Figure 2). The independence of these relations to childhood asthma was further shown by a mediation analysis that demonstrated how a direct path for the relation of maternal cytokine ratios to childhood asthma was favored over an indirect path that included the child's 3-month IFN- γ /IL-13 ratio (Figure E1).

Our findings support the possibility that asthma begins *in utero*. We do not have maternal samples from earlier in pregnancy, so we do not know whether the maternal cytokine relation to childhood asthma is limited to late in pregnancy. Only a few studies have examined cytokine production in pregnancy, and these were cross-sectional, limited in group size, and

Figure 2. Receiver operating characteristic (ROC) curves for reduced risk of childhood asthma attributable to the IFN- γ /IL-13 ratios of the children and pregnant mothers without asthma. The naive model includes only the children's 3-month ratio (blue). The full model (red) including the IFN- γ /IL-13 ratios of the pregnant mothers shows a significant increase in the area under the curve (P = 0.02).

inconsistent in their results (42–45). Future studies with a longitudinal design and larger group sizes are needed for clarification.

This study has certain limitations. Our definition of asthma was based on a questionnaire, and although it included a diagnosis made by a physician, it was not contingent upon any direct physiological measurements. The biologic mechanisms underlying the relation of childhood asthma to the capacity of immune cells in pregnant mothers without asthma to produce IFN- γ , IL-13, and IL-4 upon mitogen stimulation are not yet established, and we cannot rule out the possibility of environmental confounders in this relationship. We do not know the cellular source(s) of the cytokines, which may be innate and/or adaptive. We do not assume that the maternal prenatal ratio values reflect fetal exposure to these cytokines *in utero*, as mitogen stimulation reveals a capacity for cytokine production, not *in vivo* levels of cytokines. Whether maternal cytokines cross the placenta has been addressed only in a limited way in

previous studies. In those studies, investigators found that at least some cytokines (although not those studied here) did not cross the placenta, but whether this is the case for all cytokines is not yet clear (46–48). Nonetheless, we previously found no correlation between maternal prenatal cytokine production and cytokine production from cord-blood immune cells (including IFN- γ /IL-13 and IFN- γ /IL-4) (26). Further studies are needed to explore in detail how the capacity of maternal cells to respond to mitogens may reflect or even bring about alterations of the milieu in which the fetus develops—alterations that influence asthma-initiating events without influencing the development of allergy.

In conclusion, a mother's cytokine profile during pregnancy is related to, and thus appears to influence, the health of her child. Specifically, this study showed that higher ratios of IFN- γ /IL-13 and IFN- γ /IL-4 in mitogen-stimulated immune cells from the mother's third trimester of pregnancy were associated with a lower prevalence of childhood asthma. Importantly, these

indicators of a reduction in childhood asthma risk were applicable only to children of mothers without asthma, and were specifically related to asthma risk without an influence on allergy-related phenotypes. The relations of the cytokine ratios of mothers without asthma to childhood asthma diminished after pregnancy. To our knowledge, this is the first report of an immune indicator that is measurable during pregnancy and predicts the risk of subsequent asthma in the child. It is also the first study to describe a risk factor for childhood asthma that is specific to mothers without asthma. ■

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