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ORIGINAL ARTICLE

Maternal Cytokine Production During Pregnancy and the Development of Childhood Wheezing and Allergic Disease in Offspring Three Years of Age

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Allergic diseases are multifactorial; they develop from complex interactions between genes and the environment. The immunological bias toward atopy and asthma might be established during *in utero* development of the fetal immune system. We prospectively investigated the association between maternal cytokine changes during pregnancy and the development of childhood wheezing and atopy at three years of age. Blood samples from 90 pregnant women were assayed for TNF- α , TGF- β , IFN- γ , IL-4, IL-6, and IL-2 at 18 weeks of gestation and at 6 weeks after delivery. Telephone interviews were performed and a questionnaire administered to assess wheezing and allergic disease in the children. The serum total IgE and specific IgE to eggs, milk and dust mites were measured. Maternal IFN- γ , TNF- α and TGF- β levels significantly decreased during pregnancy compared to the levels after delivery. However, the IL-4 levels did not change. Maternal TNF- α and IFN- γ levels were decreased both before and after delivery in children with reported wheezing. Individual maternal IL-4 levels, before delivery, were higher than after delivery in the children that developed wheezing. There were no significant differences in maternal cytokine levels between children with and without asthma. In children with atopy, the maternal IFN- γ /IL-4 ratio, during the first trimester, had a tendency to decrease compared to the children without atopy, whereas the maternal IL-2 levels at 6 weeks after delivery were increased. A first pregnancy showed higher concentrations of IL-4 before and after delivery than did women with multiple pregnancies. Maternal cytokine levels begin to change toward a Th2 immunity starting in the first trimester. A stronger Th2 immune response during the first trimester of pregnancy is associated with childhood wheezing and atopy at three years of age, and a first pregnancy.

Keywords wheezing, atopy, allergic disease, cytokine, risk factors

INTRODUCTION

Over the last decade, the prevalence of allergic disease has rapidly increased worldwide. These increases have been particularly marked in children and in urban areas. The rapidity of the increase has suggested the influence of changes in the environment and/or lifestyle rather than genetic predisposition. Many studies have tried to identify the risk factors associated with the development of allergic disease (1–4). Recent research has started to focus on the possible influence of the antenatal environment as an important factor in the development of atopy and asthma (5). However, the relative influence of antenatal environmental factors remains unknown.

Atopic diseases are characterized by the skewing of the T helper (Th)1/Th2 balance away from Th1 toward the allergy-promoting Th2 cells. Cytokine dysregulation and Th1/Th2 imbalance might be established during the *in utero* development of the fetal immune system in genetically susceptible individuals; it would be present at birth and influence the immunological responses to various environmental stimuli after birth. Although the factors that determine the degree of immune competence at birth and during early infancy are poorly understood, prenatal cytokines are thought to play an important role in controlling the functional maturation of the developing fetal immune system (6).

It has been postulated that maternal immunity polarizes toward Th2-dominance during pregnancy. Maternal serum interferon (IFN)- γ levels decrease as the gestational age advances (7). When the maternal immune response has a Th1-predominance, IFN- γ or tumor necrosis factor (TNF- α), this leads to a spontaneous abortion or a preterm delivery (8, 9). In pregnant asthmatics, IFN- γ and IL-4-producing T cell subsets are increased compared to non-pregnant asthmatics (10).

To date, a number of studies have investigated the predictive or risk factors for allergic disease. Although several studies on cord blood IgE levels and PHA-stimulated cytokine-response profiles have been performed to determine early predictive markers for allergic disease (11), there have been no consistent results. Currently, the best predictor available for allergic disease is the family history.

No prior report has attempted to determine the association between the maternal circulating cytokine profiles during pregnancy and after birth with the development of allergic disease in offspring. We hypothesized that pregnancy-induced immune alterations may affect the development of allergic disease in offspring, and investigated the maternal cytokine changes during pregnancy, in association with the development of wheezing and allergic disease during early childhood.

METHODS

Experimental Design

Ninety pregnant women were randomly recruited during the first trimester of pregnancy (at 16 weeks of gestation) at their first antenatal clinic visit at the Kangbuk Samsung Hospital between 2002 and 2003. After obtaining written

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informed consent, blood samples were collected at 16 weeks of gestation and 6 weeks after delivery. Serum samples were stored at -80°C until the cytokine assays were performed. After delivery, the offspring were regularly followed in the pediatric outpatient clinic at scheduled visits for vaccination and physical examinations.

Pregnancies with a preterm delivery were excluded from this study. The respiratory symptoms and allergic disease symptoms were evaluated to document the development of wheezing and allergic disease. Additional studies performed at three years of age (between July and August 2007) included a telephone interview or questionnaire, CAP testing for four common allergens (*D. farinae*, *D. pteronyssinus*, egg white, and milk), total IgE levels, and peripheral blood eosinophil counts. The key questions regarding the prevalence of symptoms of allergic diseases were based on the Korean version of ISAAC questionnaires (12). The study design was approved by the ethics committee of the hospital and an informed consent was obtained from all parents.

Cytokine Assay

Cytokine concentrations of IL-2, IL-4, IL-6, IFN- γ , and TNF- α , tumor growth factor (TGF)- β were evaluated by ELISA (R&D Systems, Minneapolis, MN, USA). The assay was performed according to the manufacture's recommendations. The sensitivities of the ELISAs were as follows: IL-2, 7 pg/mL; IL-4, 10 pg/mL; IL-6, 0.7 pg/mL; TNF- α , 1.6 pg/mL; IFN- γ , 8.0 pg/mL; TGF- β , 4.61 pg/mL. Duplicate wells were run for each sample and the mean values were reported.

Total and Allergen-Specific IgE

To determine total and allergen-specific IgE levels, fluorescence enzyme immunoassays (FEIAs) were performed on plasma samples using an automated instrument, UniCAP100TM (Pharmacia and Upjohn Diagnostics, Uppsala, Sweden). Specific IgE levels were determined for dust mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), milk and egg white. The cutoff value for allergen-specific IgE was 0.34 kU/L and values greater than 0.35 kU/L were considered positive. The cutoff value for total IgE was 2 kU/L.

Wheezing, Allergic Disease and Atopy Definitions

Wheezing, asthma, allergic rhinitis and atopic dermatitis were defined by a physician as documented in the medical record or by parental report of physician-diagnosed wheezing and allergic disease on the medical history questionnaires. Atopy was defined by one or more positive specific IgE or total IgE levels greater than 100 kU/L.

Statistics

Data were analyzed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Differences in cytokine profiles based on the presence or absence of wheezing, allergic disease and atopy at 3 years of age were assessed by the Student's *t*-test. Changes in the maternal cytokine profiles before and after delivery were evaluated by the paired Student's *t*-test.

TABLE 1.—Demographic characteristics of subjects.

Mother (N = 90)	
Mean age (years, mean $\pm$ S.D)	32.0 $\pm$ 0.3
Gestational age (weeks, mean $\pm$ S.D)	39.7 $\pm$ 1.2
History of allergic diseases (numbers (%))	20 (22.2)
Mode of delivery (numbers (%))	
Vaginal	65 (72.2)
Cesarean section	25 (27.8)
Parity (numbers (%))	
Primipara	43 (47.8)
Multipara	47 (52.2)
Child (N = 55)	
Mean age (months, mean $\pm$ S.D (range))	32.2 $\pm$ 3.8 (26–42)
Male (numbers (%))	25 (45.4)
History of wheezing (numbers (%))	16 (29.1)
Asthma (numbers (%))	6 (10.9)
Allergic rhinitis (numbers (%))	10 (18.2)
Atopic dermatitis (numbers (%))	18 (32.7)
Atopy (numbers (%))	9 (16.4)
Family history of allergic diseases (numbers (%))	16 (29.1)
Exposure to smoke (numbers (%))	5 (9.1)
Exposure to pet (numbers (%))	2 (3.6)
Total IgE (kU/L, mean $\pm$ S.D)	
Atopy +	191.0 $\pm$ 18.6
Atopy –	34.9 $\pm$ 4.5
Total eosinophil counts (/mm ³ , mean $\pm$ S.D)	
Atopy +	463.3 $\pm$ 48.7
Atopy –	290.0 $\pm$ 30.4

RESULTS

Clinical Characteristics

The mean maternal age was 32 years and the mean gestational age at delivery was 39.7 ± 1.2 weeks (38–42 weeks). Forty-three pregnant women out of 90 were primigravidas and the remaining 47 were multiparous. Twenty mothers (20%) had a history of allergic diseases, and there was no history of maternal smoking during pregnancy. Twenty-nine pregnancies (32.2%) had premature rupture of the membranes (PROM).

Fifty-five parents responded to the telephone interview or questionnaire when their children reached 3 years of age. The mean age of the children at the time of blood sampling was 32.2 ± 3.8 months (26–42 months). Twenty-five children were male and 30 were female. Sixteen children out of 55 had a history of wheezing, six children had physician-diagnosed asthma, 10 children had a history of allergic rhinitis, and 18 children had a history of atopic dermatitis. Nine children had a history of atopy. Sixteen children had a family history of allergic diseases and 5 children had been exposed to cigarette smoke after birth. Only 2 children had been exposed to pets in the home. The mean total IgE levels and total eosinophil counts were 191 kU/L and $463.3/\text{mm}^3$ for the children with atopy, whereas they were 34.87 kU/L and $290/\text{mm}^3$ for children without atopy. The normal range of serum IgE level is less than 70 kU/L for 3 years of children and that of total eosinophil count is less than $250/\text{mm}^3$ (Table 1).

Maternal Cytokine Profiles Before and After Delivery

Maternal circulating TNF- α , TGF- β and IFN- γ levels were significantly decreased during the first trimester (81.44 ± 9.533 pg/mL, 1152 ± 55.41 pg/mL, 236.8 ± 17.0 pg/mL, respectively) compared to those analyzed 6 weeks after delivery (106.0 ± 12.62 pg/mL, 1281 ± 55.87 pg/mL, 262.7 ± 20.18 pg/mL, respectively). IL-2, IL-4 and IL-6 levels showed no interval changes before and after delivery. We

TABLE 2.—Comparison of cytokines before and after delivery.

	Before delivery	After delivery	<i>p</i> value
TNF- α (pg/mL)	81.44 $\pm$ 9.53	105.99 $\pm$ 12.62	0.250
TGF- β (pg/mL)	1151.65 $\pm$ 55.41	1281.02 $\pm$ 55.86	0.009
IFN- γ (pg/mL)	236.75 $\pm$ 17.00	262.72 $\pm$ 20.17	0.036
IFN- γ /IL4	1.99 $\pm$ 0.21	2.38 $\pm$ 0.34	0.172
IL2 (pg/mL)	41.09 $\pm$ 5.40	42.69 $\pm$ 5.01	0.548
IL4 (pg/mL)	182.51 $\pm$ 19.07	183.52 $\pm$ 18.17	0.890
IL6 (pg/mL)	40.9 $\pm$ 9.81	61.53 $\pm$ 10.5	0.144

compared the IFN- γ /IL-4 ratio before delivery to the ratio after delivery to evaluate the imbalance between Th1/Th2 immunity. The IFN- γ /IL-4 ratio during the first trimester (1.997 ± 0.213) was much lower than the ratio was after delivery (2.382 ± 0.349), which demonstrated that the Th2-dominant immunity was already established in the first trimester (Table 2).

Maternal Cytokine Profiles in Children with Wheezing

To compare the maternal cytokine changes with the children with and without wheezing, we evaluated the maternal cytokine concentrations before and after delivery in each group. There were associations between the concentrations of maternal TNF- α and IFN- γ , and the development of wheezing in the children at three years of age. In the wheezing group, the maternal TNF- α and IFN- γ concentrations, during pregnancy and after delivery, were significantly lower than those of the children were without wheezing. In addition, when we compared individual IFN- γ /IL-4 ratios before delivery to those after delivery, the IFN- γ /IL-4 ratio before delivery was significantly lower in the wheezing group compared to the group without wheezing. These findings suggest that the Th2-dominant immunity during pregnancy was accentuated in the group with wheezing (Table 3).

TABLE 3.—Maternal cytokine changes before and after delivery in children with and without wheezing.

	Wheezing		<i>p</i> value
	–	+	
TNF- α (pg/mL, wk0*)	102.08 $\pm$ 18.92	46.73 $\pm$ 14.60	0.006
(wk6 [†])	119.17 $\pm$ 17.76	59.39 $\pm$ 14.85	0.035
(wk0/wk6 [‡])	0.95 $\pm$ 0.06	0.78 $\pm$ 0.08	0.120
TGF- β (pg/mL, wk0)	1237.08 $\pm$ 83.67	1145.98 $\pm$ 143.46	0.430
(wk6)	1256.27 $\pm$ 73.79	1454.35 $\pm$ 119.68	0.085
(wk0/wk6)	1.05 $\pm$ 0.08	0.84 $\pm$ 0.10	0.120
IFN- γ (pg/mL, wk0)	260.40 $\pm$ 25.03	172.99 $\pm$ 39.444	0.040
(wk6)	274.53 $\pm$ 30.41	176.42 $\pm$ 45.811	0.030
(wk0/wk6)	1.86 $\pm$ 0.79	1.08 $\pm$ 0.15	0.950
IFN- γ /IL4 (wk0)	2.08 $\pm$ 0.30	1.47 $\pm$ 0.46	0.059
(wk6)	1.92 $\pm$ 0.38	3.54 $\pm$ 1.44	0.380
(wk0/wk6)	2.26 $\pm$ 1.04	0.79 $\pm$ 0.10	0.043
IL2 (pg/mL, wk0)	36.29 $\pm$ 4.01	54.96 $\pm$ 26.83	0.310
(wk6)	42.98 $\pm$ 5.02	55.19 $\pm$ 23.71	0.290
(wk0/wk6)	1.01 $\pm$ 0.09	1.10 $\pm$ 0.15	0.560
IL4 (pg/mL, wk0)	174.45 $\pm$ 15.10	248.44 $\pm$ 90.25	0.840
(wk6)	184.85 $\pm$ 16.02	211.90 $\pm$ 89.44	0.170
(wk0/wk6)	0.97 $\pm$ 0.05	1.91 $\pm$ 0.53	0.013
IL6 (pg/mL, wk0)	29.94 $\pm$ 7.75	25.99 $\pm$ 5.88	0.360
(wk6)	44.57 $\pm$ 8.51	66.16 $\pm$ 29.53	0.510
(wk0/wk6)	2.94 $\pm$ 1.87	0.77 $\pm$ 0.18	0.590

*wk0: 16 weeks of gestation before delivery.

[†]wk6: 6 weeks after delivery.

[‡]wk0/wk6: ratio before delivery to after delivery.

TABLE 4.—Maternal cytokine changes before and after delivery in children with and without allergic rhinitis.

	Allergic Rhinitis		<i>p</i> value
	–	+	
TNF- α (pg/mL, wk0*)	89.26 $\pm$ 17.10	71.23 $\pm$ 19.62	0.870
(wk6 [†])	94.01 $\pm$ 14.49	136.75 $\pm$ 38.05	0.330
(wk0/wk6 [‡])	0.94 $\pm$ 0.06	0.69 $\pm$ 0.10	0.090
TGF- β (pg/mL, wk0)	1196.34 $\pm$ 76.27	1274.65 $\pm$ 206.53	0.610
(wk6)	1338.40 $\pm$ 74.78	1203.60 $\pm$ 90.08	0.180
(wk0/wk6)	0.98 $\pm$ 0.08	1.04 $\pm$ 0.13	0.140
IFN- γ (pg/mL, wk0)	229.47 $\pm$ 23.19	259.71 $\pm$ 59.27	0.820
(wk6)	230.58 $\pm$ 27.07	315.32 $\pm$ 72.54	0.230
(wk0/wk6)	1.78 $\pm$ 0.69	1.00 $\pm$ 0.16	0.590
IFN- γ /IL4 (wk0)	1.61 $\pm$ 0.23	3.21 $\pm$ 0.84	0.040
(wk6)	1.82 $\pm$ 0.36	4.96 $\pm$ 2.12	0.070
(wk0/wk6)	1.97 $\pm$ 0.90	1.20 $\pm$ 0.32	0.630
IL2 (pg/mL, wk0)	44.50 $\pm$ 9.9	29.20 $\pm$ 4.23	0.810
(wk6)	47.77 $\pm$ 9.12	40.96 $\pm$ 9.97	0.890
(wk0/wk6)	1.07 $\pm$ 0.09	0.87 $\pm$ 0.11	0.570
IL4 (pg/mL, wk0)	211.57 $\pm$ 33.52	125.81 $\pm$ 27.84	0.070
(wk6)	206.16 $\pm$ 33.43	132.21 $\pm$ 23.84	0.310
(wk0/wk6)	1.25 $\pm$ 0.19	1.22 $\pm$ 0.30	0.960
IL6 (pg/mL, wk0)	28.93 $\pm$ 6.77	28.16 $\pm$ 8.74	0.710
(wk6)	51.22 $\pm$ 12.40	49.19 $\pm$ 13.91	0.510
(wk0/wk6)	2.64 $\pm$ 1.62	0.82 $\pm$ 0.30	0.600

*wk0: 16 weeks of gestation before delivery.

[†]wk6: 6 weeks after delivery.

[‡]wk0/wk6: ratio before delivery to after delivery.

Maternal Cytokine Profiles in Children with Asthma, Allergic Rhinitis and Atopic Dermatitis

There were no significant differences in the maternal cytokine levels between children with and without asthma. The maternal IFN- γ /IL-4 ratios during the first trimester were increased in children with AR compared to those without AR, and the maternal TNF- α levels during the first trimester were decreased in children with AD (Table 4).

Maternal Cytokine Profiles in Children with and without Atopy

The maternal IFN- γ /IL-4 ratios during the first trimester had a tendency to be lower in children with atopy than in those without atopy ($p = 0.055$). This suggests that maternal cytokine dysregulation to a Th2-dominant immunity might be associated with atopy in offspring. The concentrations of IL-2 after delivery were also decreased in the children with atopy (Table 5).

Maternal Cytokine Profiles in Relation to Parity

We evaluated the maternal cytokine levels according to parity. The maternal IL-4 levels, both in the first trimester and after delivery, showed a significant increase in the primigravida compared to the multiparous pregnancies, which indicated enhanced maternal immunity toward the Th2 type in the primigravida (Table 6).

Maternal Cytokine Profiles in Relation to Possible Risk Factors of Allergic Diseases

We investigated the risk factors for allergic diseases such as family history of allergic diseases, history of passive smoking and pet exposure. However, there were no significant risk factors identified. We also searched for relations between possible risk factors of allergic diseases, i.e., a family history of allergic disease, a history of passive smoking, and pet exposure, and the differences in maternal cytokine levels during

TABLE 5.—Maternal cytokine changes before and after delivery in children with and without atopy.

	Atopy –	Atopy +	p value
TNF- α (pg/mL, wk0*)	77.97 $\pm$ 25.03	132.38 $\pm$ 66.29	0.71
(wk6 [†])	95.55 $\pm$ 25.38	145.03 $\pm$ 55.30	0.94
(wk0/wk6 [‡])	0.80 $\pm$ 0.08	1.04 $\pm$ 0.18	0.13
TGF- β (pg/mL, wk0)	968.99 $\pm$ 149.18	1117.35 $\pm$ 148.51	0.55
(wk6)	1308.07 $\pm$ 149.11	1194.46 $\pm$ 130.74	0.37
(wk0/wk6)	0.77 $\pm$ 0.08	0.97 $\pm$ 0.10	0.15
IFN- γ (pg/mL, wk0)	273.90 $\pm$ 36.75	188.97 $\pm$ 30.70	0.20
(wk6)	237.25 $\pm$ 30.76	206.10 $\pm$ 41.33	1.00
(wk0/wk6)	1.32 $\pm$ 0.24	1.06 $\pm$ 0.14	0.71
IFN- γ /IL4 (wk0)	1.85 $\pm$ 0.31	0.99 $\pm$ 0.20	0.05
(wk6)	2.04 $\pm$ 0.68	1.21 $\pm$ 0.277	0.71
(wk0/wk6)	1.27 $\pm$ 0.25	1.01 $\pm$ 0.18	0.50
IL2 (pg/mL, wk0)	77.45 $\pm$ 38.25	42.49 $\pm$ 13.28	1.00
(wk6)	81.20 $\pm$ 31.91	36.98 $\pm$ 15.76	0.02
(wk0/wk6)	0.82 $\pm$ 0.144	1.40 $\pm$ 0.15	0.006
IL4 (pg/mL, wk0)	289.72 $\pm$ 129.44	214.26 $\pm$ 32.80	0.55
(wk6)	281.60 $\pm$ 127.54	198.49 $\pm$ 40.50	0.60
(wk0/wk6)	1.18 $\pm$ 0.18	1.16 $\pm$ 0.14	0.76
IL6 (pg/mL, wk0)	19.95 $\pm$ 5.04	44.19 $\pm$ 19.9	0.41
(wk6)	50.02 $\pm$ 12.10	35.74 $\pm$ 15.10	0.37
(wk0/wk6)	0.45 $\pm$ 0.06	0.95 $\pm$ 0.03	0.08

*wk0: 16 weeks of gestation before delivery.

[†]wk6: 6 weeks after delivery.[‡]wk0/wk6: ratio before delivery to after delivery.

and after pregnancy. However, there were no significant associations. This may have been due to small subject numbers (data not shown).

DISCUSSION

Our findings showed that cytokine profiles during pregnancy were skewed to Th2 immunity and this was established during the first trimester of pregnancy. Changes of maternal immunity are essential to maintain a pregnancy because of the antigenic differences between the mother and the fetus (13). The maternal immunity must promote tolerance to the allogenic fetus, while protecting the host from infection (14). Therefore, the maternal Th2-dominant immunity might be

necessary to maintain tolerance to the fetus (15, 16). In our study, maternal IFN- γ , a Th1 type cytokine, decreased during the first trimester compared to after delivery, whereas IL-4, a Th2 type cytokine, did not change significantly during pregnancy. TNF- α , a proinflammatory cytokine, is also known to be a Th1-polarizing cytokine. Elevated serum or amniotic fluid TNF- α levels are associated with spontaneous abortions and preterm labor, and serum TNF- α is a marker of systemic inflammation in severe asthmatics (17, 18). In the present study, all the subjects had full term pregnancies, and there were no preterm labors or spontaneous abortions. This may have contributed to a decrease in the maternal TNF- α levels during pregnancy in our study.

The maternal serum TGF- β levels were also decreased in the first trimester compared to after delivery. However, this conflicts with another study. TGF- β has been suggested to be an important factor for the maintenance of pregnancy by reducing allogenic rejection (19). TGF- β induces oral tolerance in neonates fed antigen-transferred breast milk (20). However, the roles of TGF- β are diverse and variable under different conditions. With regard to the pathogenesis of asthma and airway remodeling, decreased levels of TGF- β might aid in the reduction of allergic diseases.

We have demonstrated that maternal cytokines were more skewed to the Th2 response in cases where their offspring had wheezing at three years of age. The maternal TNF- α and IFN- γ levels in the wheezing group were lower than in the group without wheezing, both before and after delivery. In the wheezing group, the individual IFN- γ /IL-4 ratios before delivery decreased more compared to after delivery. In addition, the individual IL-4 levels before delivery increased more than they did after delivery. Low IFN- γ response in cord blood cells has been identified to be a risk factor for atopy and allergic diseases (21, 22). Although the timing of allergen sensitization is controversial, it is possible that many environmental factors will also be exerting *in utero* influences (4, 21, 23). We can speculate that a more accentuated Th2-dominant immunity begins during the first trimester of pregnant women with wheezing offspring. This is the first report to demonstrate that the maternal cytokine profiles are associated with the presence of wheezing at three years of age.

However, we could not identify an association between the maternal cytokine levels and asthma in the offspring. In infants with allergic rhinitis, the maternal cytokine profiles were skewed to the Th1 response with a higher IFN- γ /IL-4 ratio in the first trimester. These findings contradict other findings such as wheeze or atopy in the present study. It might be explained by underdiagnosis of asthma or allergic rhinitis at the age of 3, or by the fact that allergic diseases are a syndrome of different clinical manifestation with different target organs.

The maternal IFN- γ /IL-4 ratios during the first trimester tended to decrease in the offspring with atopy compared to those without atopy. The IL-2 levels decreased after delivery compared to the before delivery levels in mothers with atopic offspring. Even though IL-2 is a Th1-response cytokine, similar to IFN- γ , IL-2 is associated with an atopic phenotype. Previous research has found that IL-2 secretion of PHA-stimulated cord blood mononuclear cells is higher in

TABLE 6.—Maternal cytokine changes related to parity.

	Parity		p value
	1	≥ 2	
TNF- α (pg/mL, wk0*)	113.80 $\pm$ 25.84	59.16 $\pm$ 11.86	0.080
(wk6 [†])	118.71 $\pm$ 22.40	85.46 $\pm$ 16.00	0.270
(wk0/wk6 [‡])	0.97 $\pm$ 0.08	0.83 $\pm$ 0.06	0.240
TGF- β (pg/mL, wk0)	1131.84 $\pm$ 86.32	1286.50 $\pm$ 114.13	0.270
(wk6)	1371.26 $\pm$ 102.04	1258.57 $\pm$ 76.88	0.240
(wk0/wk6)	0.89 $\pm$ 0.06	1.09 $\pm$ 0.11	0.080
IFN- γ (pg/mL, wk0)	260.83 $\pm$ 34.22	210.04 $\pm$ 26.49	0.320
(wk6)	268.27 $\pm$ 38.68	224.50 $\pm$ 34.64	0.260
(wk0/wk6)	2.20 $\pm$ 1.15	1.10 $\pm$ 0.12	0.640
IFN- γ /IL4 (wk0)	1.82 $\pm$ 0.40	1.98 $\pm$ 0.32	0.410
(wk6)	1.86 $\pm$ 0.51	2.90 $\pm$ 0.84	0.370
(wk0/wk6)	2.62 $\pm$ 1.50	1.07 $\pm$ 0.13	0.420
IL2 (pg/mL, wk0)	53.24 $\pm$ 16.07	30.61 $\pm$ 4.01	0.120
(wk6)	59.84 $\pm$ 14.66	33.70 $\pm$ 4.28	0.170
(wk0/wk6)	1.08 $\pm$ 0.13	0.98 $\pm$ 0.10	0.670
IL4 (pg/mL, wk0)	249.04 $\pm$ 53.08	144.80 $\pm$ 17.33	0.020
(wk6)	249.30 $\pm$ 52.83	138.16 $\pm$ 15.40	0.007
(wk0/wk6)	1.29 $\pm$ 0.31	1.20 $\pm$ 0.13	0.650
IL6 (pg/mL, wk0)	29.08 $\pm$ 7.15	28.52 $\pm$ 9.04	0.190
(wk6)	37.21 $\pm$ 8.42	64.00 $\pm$ 18.63	0.060
(wk0/wk6)	4.06 $\pm$ 2.69	0.63 $\pm$ 0.13	0.008

*wk0: 16 weeks of gestation before delivery.

[†]wk6: 6 weeks after delivery.[‡]wk0/wk6: ratio before delivery to after delivery.

IgE sensitized than in non-sensitized, non-allergic children (24).

Our findings also showed that maternal cytokine profiles changed with parity. The maternal serum of primigravidas had increased IL-4 levels compared to the multiparous pregnancies. This finding suggests an inverse relationship between parity and the degree of Th2 polarization. This may explain, in part, allergic prevalence with different birth order apart from the hygiene hypothesis. The traditional hygiene hypothesis states that the prevalence of allergic disease decreases as the birth order increases due to the different environmental exposures among siblings (25). Our data may add another mechanism to explain the reduced risk of allergic disease with parity; a greater number of pregnancies may be associated with an attenuated maternal allergic state. That is, the mother with more children is less likely to be allergic (26, 27), and the maternal allergic status influences the development of childhood allergic disease (3).

The present study is limited by the relatively low number of subjects enrolled. Nevertheless, our study demonstrates that the patterns of the immunological response start during early pregnancy, and that a pronounced Th2-biased responsiveness predisposed to the development of wheezing and atopy at three years of age among offspring. Unfortunately, we could not identify risk factors that influenced maternal cytokine profiles during pregnancy. Recent studies suggest that maternal smoking can influence fetal immune function and neonatal toll-like receptor-mediated immune responses (28, 29). However, no previous report has shown the associations between changes in maternal cytokine levels, and atopy and allergic diseases in offspring.

In conclusion, our study indicates that maternal cytokine profiles begin to change toward a Th2 immunity during the first trimester, and that a predominant Th2 immune response during the first trimester of pregnancy is associated with primigravidas, childhood wheezing and atopy at 3 years of age.

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