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T-helper type 2 polarization among asthmatics during and following pregnancy

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Summary

Background—Asthma is the most common medical condition during pregnancy. While increased production of T helper cytokines has been reported to occur in both asthma and pregnancy, the effect of T-helper type 2 (Th2) polarization on asthma symptoms during pregnancy has not been well-characterized.

Objective—We hypothesized that systemic Th2 cytokine and chemokine polarization occurs among asthmatics to a greater extent during their pregnancy, and is associated with more severe asthma and increased Th2 polarization in the newborn.

Methods—Fifty-six pregnant asthmatics were recruited from prenatal clinics affiliated with New York Presbyterian Hospital. Systemic production of interleukin-4, interferon-γ, eotaxin and IP10 were measured by intracytoplasmic staining or ELISA at recruitment, peripartum and post-partum, and in the cord blood. The frequency of asthma symptoms was measured by questionnaires and compared with Th biomarkers.

Results—The chemokine ratio (IP10/eotaxin) declined over the course of pregnancy (from 3.3 ± 1.3 to 1.4 ± 0.2, $P = 0.016$), but IP10 and eotaxin increased post-partum. The decrease in the chemokine ratio was associated with more frequent asthma symptoms. A non-significant trend towards decreased interferon-γ and increased interleukin-4 production was detected. Cord blood eotaxin levels correlated with maternal levels ($r = 0.35$, $P = 0.03$). Other peripartum biomarkers were not associated with Th2 polarization nor with subsequent respiratory symptoms in the newborn.

Conclusions—IP10/eotaxin declined over the course of pregnancy and was associated with worse asthma symptoms. Alterations of Th1/Th2 chemokine balance during pregnancy may identify women prone to more severe asthma during pregnancy.

Keywords

asthma; asthma symptoms; pregnancy; T helper chemokines; T helper cytokines

Introduction

Asthma is the most common medical condition occurring during pregnancy, and its variable pattern during this period has been well described [1,2]. Asthma severity during pregnancy has been known to worsen, remain the same, or improve, in an equal proportion of women [3]. Importantly, increased asthma severity during pregnancy is associated with greater morbidity

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in the mother and newborn [4]. Nonetheless, how pregnancy modulates asthma still needs to be elucidated.

In general, atopic asthma is characterized by the increased production of T-helper type 2 (Th2) cytokines [5–8]. Up-regulated systemic Th2 cytokine production in association with increased symptoms has been documented in some [6], but not all [9] studies. More recently, systemic Th2 chemokine upregulation has been reported, and similarly associated with the diagnosis of asthma and allergic rhinitis (AR), as well as worse disease severity in atopic dermatitis [10, 11]. Plasma concentration of the Th2 chemokine thymus and activation-regulated chemokine (TARC) also has been reported to be elevated in childhood asthma [12].

Pregnancy is another condition that has been characterized by Th2 polarization [13,14]. It has been postulated that skewing away from the production of Th1 cytokines and towards the production of Th2 cytokines may help the survival of the foetus [15] and reduce the risk for preeclampsia [14]. Interestingly, despite this description, previous studies only have compared systemic Th2 cytokine production cross-sectionally across groups of pregnant in comparison with non-pregnant women [13,14]. Most recently, Tamasi and colleagues reported greater numbers of IFN-γ and IL-4 cytokine producing T cells among pregnant asthmatic women when compared with pregnant non-asthmatic, and to asthmatic non-pregnant women along with a negative effect of maternal asthma on the birth weight of newborns [16]. However, longitudinal evaluation of Th2 polarization during and following pregnancy with repeated measures has not been reported. Furthermore, the effect of Th2 polarization on asthma symptoms during pregnancy, and on Th2 polarization in the newborn, has not been well-characterized. Systemic chemokine regulation during pregnancy, particularly among pregnant asthmatics, also has not been studied.

We hypothesized that systemic Th2 cytokine and chemokine polarization does occur among asthmatics to a greater extent during their pregnancy, and is associated with more severe asthma. In addition, we hypothesized that increased Th2 polarization during pregnancy may be associated with increased Th2 polarization in the newborn. To address our hypotheses, we tracked the association between asthma symptoms with systemic chemokine levels, and intracytoplasmic IL-4 and IFN-γ measurements, during and following pregnancy. We also investigated the associations between maternal and cord blood cytokine and chemokine production and subsequent respiratory symptoms in the newborn.

Methods

Subjects

The cohort consisted of 56 pregnant African American and Hispanic women with a reported history of physician-diagnosed asthma and currently receiving medical therapy for asthma (e.g. bronchodilators, inhaled corticosteroids). They were recruited from prenatal clinics affiliated with Columbia University (New York Presbyterian Hospital and Allen Pavilion) during their first or second trimester. Exclusion criteria included age less than 18 years and use of parenteral or oral corticosteroids, cigarette smoking, immunotherapy, or other immunosuppressive medications taken within 4 weeks of entry into the study. The study was approved by the Institutional Review Board, and written informed consent was obtained from all participants. Investigators played no role in the usual obstetrical care provided to the participants, with the exception of offering the influenza vaccine as recommended by the Center for Disease Control to participants pregnant during the influenza season [17]. The subjects underwent phlebotomy at the time of recruitment (‘pregnancy’) (late second trimester or early third trimester; mean 26.7 week gestation +0.7 standard error [SE]), ‘peripartum’ (within 1 day post-partum), and in a subset (n = 20), ‘post-partum’ (minimum 3 months post-partum, mean 4.9 ± 0.6 months post-partum). Cord blood was collected at delivery, as described previously [18].

Asthma severity and infant respiratory questionnaire

Asthma severity was evaluated by a questionnaire that addressed clinical parameters that included the nature and frequency of day-time and night-time symptoms (cough, wheezing, shortness of breath) in the preceding 4 weeks, emergency room and urgent care visits for asthma exacerbations, use of systemic steroids, and need for increase in rescue doses of inhaled β-adrenergic agonists. All medication use also was recorded. The questionnaire was administered during pregnancy, peripartum, and post-partum. At recruitment the frequency of these symptoms 4 weeks before pregnancy also was queried.

A detailed 23 point questionnaire was administered to the infant's primary caretaker over the telephone at 3, 6, 12 and 18 months after birth. Details regarding the onset of eczema and other atopic diseases, upper and lower respiratory tract symptoms, environmental exposures to allergens and secondary cigarette smoke, and dietary practices were queried.

Intracellular cytokine staining

Maternal peripheral blood mononuclear cells (PBMCs) and cord blood mononuclear cells (CBMCs) were separated by density centrifugation from whole blood as described [17]. For intracytoplasmic staining of IL-4, PBMCs and CBMCs were stimulated with IL-2 (10 ng/mL), IL-4 (2.5 μg/mL), soluble anti-CD3 (10 ng/mL) and soluble anti-CD28 (2 ug/mL) for the first 2 days followed by IL-2 and IL-4 for the next 3 days. Subsequently cells were stimulated with phorbol 12-myristate 13-acetate (PMA) 50 ng/mL, and ionomycin (1.25 μg/mL) for 4 h and stained immediately or frozen at −80 °C. For IFN-γ, isolated PBMCs and CBMCs were stimulated immediately with PMA and ionomycin at the aforementioned doses for 5 h and stained immediately or frozen at − 80°. In both cases, brefeldin A (10 μg/mL) was added for the last two hours, as described [19,20]. Freshly stimulated or thawed cells were subsequently washed with staining buffer (Dulbecco's PBS without Ca or Mg (DPBS) (Fisher Scientific, Morris Plains, NJ, USA) with 1% fetal calf serum (FCS) (Atlanta Biologicals Corp, Lawrenceville, GA, USA), 0.1% Sodium Azide), and incubated with 10% human sera (Sigma Aldrich, St Louis, MO USA) for 30 min at 4 °C to block non-specific Fc binding. Cells then were stained with the cell surface marker CD4-Biotin (Caltag Laboratories, Burlingame, CA, USA) at 1 : 100 dilution followed by strepavidin-cychrome (BD Pharmingen, San Jose, CA, USA) at 1 : 200 dilution, and CD3-FITC (Caltag Laboratories) at 1 : 33 dilution, for 30 min in dark at 4 °C. Cells then were washed with staining buffer, permeabilized (1% FCS and 1% Saponin in DPBS) for 20 min at 4 °C, rewashed, and stained with anti-IFN-γ-PE or IL-4-PE (BD Biosciences San Jose, CA, USA) at 1 : 100 dilution. Isotype staining (mouse IgG_1) was used as a negative staining control. Hick 1 cells and Hick 2 cells (BD Biosciences) were used as a positive staining control for IFN-γ and IL-4 respectively. Using CellQuest software (Becton Dickinson, Franklin Lakes, NJ, USA), lymphocytes were gated according to forward and side scatter properties, and $CD3^+CD4^+$ staining cells were selected for cytokine measurements. Quantification of cytokine staining was expressed as percentages of $CD3^+CD4^+$ T cells staining with their respective cytokine antibody, after subtracting any background staining detected by the isotype control.

Immunoglobulin E and chemokine assays

Sera were isolated from clotted blood, aliquoted, and frozen for future use. Total IgE was measured by immunoradiometric assay (Total IgE IRMA; Diagnostics Products Corp. Los Angeles, CA, USA). Sera IP10 and eotaxin levels were measured using Human Eotaxin and Human IP10 systems (Immunoassays QuantikineKits R & D Systems, Minneapolis, MN, USA) systems according to the manufacturer's instructions and as described. The level of detection (LOD) for IP10 was 1.67 pg/mL and for eotaxin was 5 pg/mL.

Statistical analysis

Nonparametric statistical analyses of the chemokine and cytokine measurements were performed using Mann–Whitney *U* across groups (e.g. maternal vs. cord blood biomarker levels), Wilcoxon tests for paired data across time-points (pregnancy vs. post-partum), and spearman ρ for correlations (e.g. cumulative symptom scores with biomarker levels). Cytokine and chemokine values measured below the LOD were replaced with values halfway between 0 and the LOD of the assay. An ascending score of 1 (no symptoms)–5 (daily symptoms) was assigned to each symptom of cough, wheeze, and shortness of breath based on its weekly frequency. A similar score of 0 (no use) and 1 (used) was ascribed to use of systemic steroids, rescue medication and urgent visits for asthma breakthrough symptoms in between the time-points when the questionnaires were administered. To compare asthma severity between two time-points (e.g. pregnancy vs. peripartum, peripartum vs. post-partum), the scores for the three symptoms were summed at one time-point (i.e. 'cumulative symptom score') and analyzed across time-points using repeated measures testing. Based on the cumulative symptom score, the subjects were categorized into those who improved (defined as decrease in cumulative symptom score between the two time-points), remained the same (no change in cumulative symptom score), or worsened (increase in cumulative symptom score between the two time-points). Additional analyses then were performed by grouping participants according to those that improved, remained the same, or worsened and comparing with biomarker levels (individual cytokine, chemokine measures and their ratios). Two participants who received systemic steroids for asthma exacerbation at the time of delivery were excluded from further analysis. Unless otherwise specified, means and their standard errors are summarized. A two-tailed *P*-value less than 0.05 was considered statistically significant.

Results

Participants

Characteristics of the study population are shown in Table 1. The cohort consisted predominantly of Hispanic women with age range 20−43 (26.7+0.63) years. Total IgE levels ranged between 6.5−759 (mean 280.7 ± 32.57) IU/mL. Based upon the cumulative symptom scores for whom sufficient data were available ($n = 52$), an equal percent (approximately 33%) of women improved, worsened and remained the same throughout the course of pregnancy. With a potential maximum of 15, the mean cumulative symptom score before pregnancy was 7.95 ± 0.42, during pregnancy was 8.87 ± 0.53, at peripartum was 7.35 ± 0.5 greater than 3 months post-partum was 10 ± 3.3.

T-helper type 2 biomarkers during and following pregnancy, and in the newborn

We found a significant decline in the Th1 chemokine ratio (IP10/eotaxin) over the course of pregnancy (i.e. late second trimester/early third trimester until peripartum), in association with a small rise in eotaxin (Table 2). In comparison, we only measured a non-significant trend towards decreased IFN-γ production and increased IL-4 production during the course of the pregnancy. Post-partum, we detected a significant increase in both the Th1 chemokine IP10, and the Th2 chemokine eotaxin. In addition, we also detected a significant correlation in maternal total IgE between the pregnancy and peripartum time-points ($r = 0.892$, $P = < 0.0001$).

While the maternal peripartum and cord blood eotaxin levels correlated significantly ($r = 0.35$, $P = 0.03$), the cord blood eotaxin levels remained significantly greater (Table 2). In contrast, we did not detect any significant correlations between the cord blood and maternal peripartum cytokine measurements.

Asthma severity and T-helper type 2 biomarkers

To determine whether systemic Th2 up-regulation during pregnancy may be associated with worse asthma symptoms, we compared the frequency of reported asthma symptoms with alterations in Th2 biomarkers. We were unable to detect significant associations between individual symptoms (difficulty breathing, wheeze and/or cough) reported during the 4 weeks preceding administration of the questionnaire, and measured cytokines or chemokines during pregnancy, peripartum and/or post-partum. However, when we examined changes in symptomatology (i.e. changes in cumulative symptom score) between different time-points, we found a negative correlation between the change in symptom score and change in chemokine ratio (IP10/eotaxin) over the period between pre-pregnancy and pregnancy assessments (Table 3). These results suggest that worsening of asthma symptoms that occurs during pregnancy, when compared with before pregnancy, is associated with greater Th2 chemokine production. In comparison, we did not find any significant relationship between the cumulative symptom score and changes in biomarker levels between pregnancy and peripartum, or between peripartum and post-partum.

Notably, we were unable to detect significant differences in Th2 biomarker levels when stratifying according to use of oral steroids, use of rescue medications (i.e. bronchodilators), or requirement for urgent care visits during pregnancy.

Effect of influenza vaccination on T-helper type 2 biomarkers and asthma symptoms

Given literature that vaccination may confer a systemic Th1 upregulation [22,23], we asked whether vaccination against influenza alters Th immune pathways during pregnancy and/or improves asthma symptoms. Women were stratified according to vaccination status and cytokine and chemokine levels were measured before and following influenza vaccination, and according to the same time-points studied. No significant differences were detected in the cytokine and chemokine measurements according to vaccination status. However, improved asthma symptoms (defined as decrease in cumulative symptom score) between pregnancy and peripartum time-points were found among 50% of those who received the vaccine in comparison with among 15% of women who were not vaccinated ($P = 0.04$).

T-helper type 2 biomarkers and respiratory outcomes in newborn

Finally, we examined prospectively the relationship between Th2 biomarkers measured among both maternal and cord blood samples at birth with respiratory outcomes in the offspring. Among those offspring for whom follow-up data were available ($n = 38$), we did not find any associations between Th2 biomarkers in the mother during pregnancy, or peripartum, and respiratory symptoms in the child up to age 18 months, nor between Th2 biomarkers in the cord blood and respiratory symptoms in the child.

Discussion

By repeated quantitative analysis of intracellular cytokines and serum chemokine levels, we found evidence of increased Th2 polarization over the course of pregnancy among atopic asthmatics. Greater Th2 polarization during pregnancy appears to be associated with more frequent asthma symptomatology. While the effects of pregnancy on pulmonary function have been well-documented [1,2], our study begins to elucidate the relationships between immunological alterations in Th2 polarization and asthma symptoms during and following pregnancy. Our results confirm and strengthen earlier reports of augmented Th2 polarization among pregnant volunteers when compared with non-pregnant volunteers [13,14,16]. Our data further support that such polarization occurs even among atopic asthmatics. Our study design also has the advantage of reducing intersubject variability by using repeated measures, and

thus supports the conclusion that systemic Th2 polarization tends to occur during pregnancy among atopic asthmatics.

We recognize that our study has certain limitations. The data obtained from questionnaires are subject to recall bias, and potential confounding effects of season also limit our interpretations. Further, the immunomodulatory effects of stress during parturition potentially can affect the cytokine milieu. The small sample size combined with additional attrition during follow-up limited the certainty with which conclusions can be made.

The effect of asthma on pregnancy has been extensively studied, and poorly controlled asthma has been implicated as a cause of premature births and low birth weight by some [4] but not all investigations [24]. As asthma in young adults often is characterized by increased Th2 polarization, our findings raise the question whether pregnancy-associated Th2 polarization may contribute mechanistically to worse birth outcomes. Our results also introduce the possibility that closer monitoring with early identification and presumably treatment of Th2 polarization may in turn lead to reduced asthma symptomatology and perhaps prevention of asthma-related adverse effects on the fetus. Notably, our data also suggest that immunomodulation of Th2 polarization during pregnancy would require an intervention other than routine vaccination against influenza.

We consistently found increased polarization of Th2 chemokines (as indicated by a decreased IP10/eotaxin ratio) during pregnancy, an aspect not studied thus far. Although it has been clear that the Th2 chemokine eotaxin is elevated in individuals with asthma and eosinophilia, most studies have evaluated its role at local tissues [25]. These include studies of nasal lavage fluid obtained from volunteers with AR that revealed significantly elevated eotaxin levels as compared with controls [26]. Increased eotaxin expression in bronchial mucosal cells 48 h after allergen challenge also has been reported [27]. In contrast, production of the Th1 chemoattractant IP10 has been found to be up-regulated by bronchial mucosal cells following oral corticosteroid use [28]. Systemic eotaxin was up-regulated in atopic lung diseases [29] and IP10 levels up-regulated in non-atopic lung diseases [30]. Only a few researchers have evaluated systemic chemokine levels in association with asthma severity previously [31].

Although the exact mechanisms for Th2 polarization during pregnancy are unclear, one could postulate that elevations in both estrogen and progesterone that develop particularly during the first trimester may contribute. Evidence for this can be found from the multiple studies that have reported associations between higher hormonal levels and asthma symptoms. For example, more frequent asthma symptoms have been reported in association with menstruation [32] and in association with hormone replacement therapy [33,34]. Increased progesterone levels, in both animal [35] and human [33,34] studies, also have been associated with worse asthma symptoms in several, but not all [36] studies. Both hormones have been shown to up-regulate Th2 cytokine production both *in vitro* [37,38] and *in vivo* [39]. As a result, several studies have suggested that pregnancy-associated Th2 polarization may develop because of an advantage conferred on the fetus with respect to avoiding rejection and/or preeclampsia [13, 14,15].

Another goal of this study was to evaluate whether Th2 polarization in the mother affected the child's cytokine or chemokine production at birth, or risk for the development of respiratory symptoms. Maternal atopy has been associated with increased production of IL-5 [40] and decreased IFN-γ production in umbilical cord blood [41,42]. Yet in another study, children born to atopic mothers have attenuated Th1/Th2 cytokine production at birth, but later develop recurrent wheeze and atopic phenotype at 6 years [43]. Our approach was to use intracytoplasmic cytokine staining to examine the cytokine relationships in conjunction with determination of chemokine levels, and prospective follow-up of the cohort using

questionnaires. The differences in our techniques as compared with those of the previous studies may potentially explain the differences in the findings. Our technique for measuring cytokines had the advantage of revealing changes in cytokine patterns at a single cell level, and our strategy of measuring chemokine production offered potentially novel information on additional relevant biomarkers. By doing so, we detected only a significant correlation between maternal post-partum and newborn eotaxin levels, while newborns levels were consistently higher. This latter result contrasts with one earlier study that reported cord blood eotaxin levels to be lower among full-term newborns when compared with their non-asthmatic mothers [44], suggesting that the presence of asthma affects chemokine production in the newborn. Significant relationships between Th2 biomarkers at birth and subsequent respiratory symptoms were not identified here.

Conclusion

In conclusion, increases in biomarkers associated with Th2 polarization, and particularly Th2 chemokine production, can be detected during pregnancy and are associated with increased asthma symptoms. The subset of women previously described as being susceptible to worse asthma during pregnancy [1] may be the same subset that undergoes heightened Th2 polarization during pregnancy. Further testing for Th2 biomarkers, or possibly further examination for genetic polymorphisms in cytokine or hormonal signaling pathways, may be able to identify this subset. In turn this approach may provide an early window when asthma interventions can improve individual and fetal outcomes for pregnant asthmatics.

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Table 1

Characteristics of the study population*

Characteristics	Percentage (n)
Mother's ethnicity	
Hispanic	85.7 (46)
African American	14.3 (8)
Persistent asthma	41.1 (23)
Received influenza vaccine during pregnancy	55.2 (30)
Relatives with history of allergies or asthma	39.3 (22)
IgE above 100 IU/mL	71.2 (40)

* Data received from questionnaires administered to the pregnant mothers at time of recruitment. Frequency of persistent asthma based on National Asthma Education and Prevention Program [21] symptom criteria.

Table 2

Frequency of cytokine producing $CD4^+$ cells (%) and serum chemokine (pg/mL) levels

	Mother			
	Pregnancy	**Peripartum**	**Post-partum**	**Newborn**
IFN-γ	4.03 ± 0.85 (*n* = 36)	2.34 ± 0.43 (*n* = 44)	2.14 ± 0.57 (*n* = 20)	1.98 ± 0.71 (*n* = 28)
IL-4	0.35 ± 0.11 (*n* = 36)	0.84 ± 0.38 (*n* = 48)	0.49 ± 0.19 (*n* = 19)	0.38 ± 0.17 (*n* = 32)
IP-10	90.3 ± 9.1 (*n* = 45)	79.9 ± 7.04 (*n* = 49)	124 ± 17.3[§] (*n* = 15)	274.9 ± 182.5 (*n* = 26)
Eotaxin	58 ± 5.16 (*n* = 47)	66.7 ± 4.8 (*n* = 47)	129 ± 18.1[≠,¶] (*n* = 15)	116 ± 18.7[**] (*n* = 26)
IFN-γ/ IL-4	95.5 ± 31.9	62.3 ± 21.2	29.2 ± 9.3	25.7 ± 8.3
IP10/ eotaxin	3.3 ± 1.3	1.4 ± 0.2[*]	1.1 ± 0.73	2.8 ± 1.5

Data are presented as means + SE and include all samples for which data were available.

Significant *P*-values detected using paired-testing between any two time-points are denoted by the symbol next to the second time-point.

[*] $P = 0.004$: between pregnancy and peri-partum.

[≠] $P = 0.002$: between pregnancy and post-partum.

[§] $P = 0.04$,

[¶] $P = 0.002$: between peripartum and post-partum.

[**] $P = 0.02$: between peripartum and newborn.

Table 3

Correlations between cytokine and chemokine ratios and changes in cumulative symptom scores (a) between before pregnancy and during pregnancy, (b) between pregnancy and peripartum, and (c) between peripartum and post-partum

	Correlation (*r*-value)	*P*-value
(a) Pregnancy		
IFN-γ/ IL-4 ratio*	−0.02	NS
IP10/Eotaxin ratio*	−0.34	0.016
(b) Peripartum		
IFN-γ/ IL-4 ratio†	−0.09	NS
IP10/Eotaxin ratio†	0.05	NS
(c) Postpartum		
IFN-γ/ IL-4 ratio‡	−0.13	NS
IP10/Eotaxin ratio‡	0.1	NS

*Measured during pregnancy.

†Measured peripartum.

‡Measured at least 3 months postpartum.