Sex-related differences in immune development and the expression of atopy in early childhood

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Background: Sex and age are known to influence the clinical expression of asthma and allergic diseases.
Objective: We sought to evaluate whether immune response profiles also vary by sex and age.
Methods: We performed a prospective birth cohort study (Childhood Origins of Asthma) designed to evaluate interactions among age, sex, immune responses, and virus infections on the development of asthma and allergic diseases. Two hundred eighty-nine subjects were enrolled at birth, and 275 maintained prospective follow-up for 3 years. Cytokine response profiles at birth, 1, and 3 years of age; rates of wheezing, atopic dermatitis, and viral illnesses; and biomarkers of atopy, including total and specific IgE levels and peripheral eosinophil counts, were evaluated.
Results: PHA-induced IFN-γ responses were higher in boys at 1 year of age (median, 35 vs 19 pg/mL; $P < .001$) and at 3 years of age (median, 282 vs 181 pg/mL; $P = .07$). Among children who wheezed during the third year of life, boys had increased IFN-γ, IL-5, and IL-13 responses at age 3 years ($P < .001$, $P = .008$, and $P = .01$, respectively). Boys also demonstrated increased rates of sensitization ($P = .05$ at year 1), total IgE levels ($P = .03$ at year 1 and $P = .006$ at year 3), and peripheral eosinophil counts (2.62 vs 1.85; $P = .05$ at year 3).
Conclusion: Sex-specific differences in immune responses develop during early childhood; some of these differences developmentally proceed, whereas others occur in parallel to the clinical expression of various atopic phenotypes.
Clinical implications: The differential expression of atopic diseases between boys and girls in early childhood is accompanied by sex-specific differences in immune response profiles. (J Allergy Clin Immunol 2006;118:1375-81.)

Key words: *Cytokine, IFN-γ, IL-5, IL-13, IL-10, sex, wheezing, atopy, allergic sensitization, birth cohort*

Abbreviations used
COAST: Childhood Origins of Asthma
RSV: Respiratory syncytial virus

Boys have a higher prevalence of wheezing in early childhood caused, in part, by differences in airway physiology. At as early as 16 weeks' gestation, female fetuses demonstrate earlier lung maturation, with advanced mouth movements and lung phospholipid profiles with respect to male fetuses.[1,2] After birth, female lungs have a lower specific airway resistance and a higher specific airway conductance, despite the fact that male lungs are on average slightly larger.[3-6] Clinically, girls demonstrate throughout childhood a consistently higher threshold response to methacholine and a decreased prevalence of airway hyperresponsiveness,[7,8] although these relationships reverse after adolescence.[9-12] Prior attempts to explain these physiologic differences have been speculative and have focused primarily on the anatomic changes occurring during puberty.[13] Recently, sex hormones have also been implicated in the increased airway hyperresponsiveness noted in adolescent girls.[13] Despite these observations, the effect of hormonal influences on immune development and respiratory health in young children remains poorly understood. As such, this led us to hypothesize that sex-specific differences in immune development also contribute to different rates of wheezing in preschool boys and girls.

Using a birth cohort at high risk of allergic diseases, asthma, or both, we have previously reported on differences in immune response profiles both in relationship to markers of atopy[14] and to the type and severity of viral infections during infancy.[15] To address the current hypothesis, we have now expanded our analyses of these preschool children to determine whether there are sex-specific patterns of cytokine responses that are associated with the clinical expression of atopy, wheezing, or both.

METHODS

Study subjects

After obtaining written informed consent, 289 subjects were enrolled at birth in the Childhood Origins of Asthma (COAST) study at a single research site beginning in November 1998 and ending in May 2000[16]; 285 of these were followed prospectively for at least

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TABLE I. Prevalence of risk factors by sex

	Boys (n =155)	Girls (n =120)	*P* value
Older siblings (%)	54	58	.67
Mom allergy (%)	85	80	.43
Mom asthma (%)	43	42	.92
Dad allergy (%)	76	85	.08
Dad asthma (%)	29	31	.77
Dog in home at birth (%)	35	35	.97
Cat in home at birth (%)	28	32	.57
Exclusively breast-fed first 6 mo (%)	35	29	.33
Passive smoke (%)	21	29	.17
Birth weight (kg), median (25th, 75th)	3.6 (3.2, 3.9)	3.5 (3.1, 3.7)	.02
Day care (%)	45	49	.59

1 year, and 275 had completed the evaluation at year 3. At least 1 parent was required to have demonstrable aeroallergen sensitization (defined as one or more positive skin test responses), a history of physician-diagnosed asthma, or both to qualify for participation. Details of the study design have been described previously.[14,16,17] This study was approved by the University of Wisconsin Human Subjects Committee.

Clinical definitions

Atopic dermatitis[14] was defined as ''physician diagnosed'' either on the basis of documentation by a health care provider on the medical record or parental report of physician-diagnosed atopic dermatitis on the historical questionnaires. A wheezing respiratory illness during infancy was defined as meeting 1 or more of the following criteria: (1) physician-diagnosed wheezing at an office visit; (2) an illness for which the child was prescribed short- or long-acting β-agonists, long-term controller medications, or both; or (3) an illness given the following specific diagnoses: bronchiolitis, wheezing illness, reactive airway disease, asthma, or asthma exacerbation. Wheezing history in the third year of life was documented by questionnaires at the third-year protocol-scheduled visit that asked the parent whether his or her child had ever wheezed during the past year. The nuances in using wheezing histories reported by both parents and health care providers in COAST and other studies as surrogates for clinician-diagnosed asthma have been discussed previously.[18]

Nasal lavage samples

Nasopharyngeal mucus samples were collected during scheduled clinic visits (at 2, 4, 6, 9, 12, 18, and 24 months of age) and during times of acute respiratory illnesses. Parents notified a study coordinator when their child had a respiratory tract infection, and a respiratory symptom scorecard (maximum score, 31) was completed.[19] Symptoms were scored based on the following scoring system: fever (ie, temperature ≥100° F; 1 point); cough (mild = 1 point, moderate = 2 points, severe = 3 points); rhinorrhea (mild [suction 0-4 times per day or wipe every 2 hours or less] = 1 point, moderate-to-severe [suction ≥5 times per day or wipe ≥1 time per hour] = 2 points; hoarseness (1 point); duration of illness greater than 4 days (1 point); apnea (3 points); wheezing (5 points); retractions (5 points); tachypnea (5 points); and cyanosis (5 points). If the symptom score was 5 or greater, signifying a moderate-to-severe upper respiratory tract infection, lower respiratory tract infection, or both, a ''sick visit'' was scheduled, and nasal lavage was performed and processed as previously described.[19] The collection and handling methods of these samples have been described previously.[19] Episodes of viral recovery during scheduled protocol visits were defined as ''infections,'' whereas those obtained with associated symptom scores of 5 or greater were defined as ''illnesses.'' Children attended an average of 5.83 of the 7 scheduled clinic visits in the first 2 years of life.

Viral diagnostics

Nasal specimens were analyzed for respiratory viruses, including respiratory syncytial virus (RSV), rhinovirus, influenza types A and B, parainfluenza virus types 1 through 4, adenovirus, and nonrhinovirus picornaviruses, by using the standard technique.[19] Samples were also evaluated for rhinovirus RNA by means of semi-nested RT-PCR.[19,20] In addition, RSV serology was performed on plasma samples obtained at age 1 year.[15]

Collection of blood samples

Cord blood and 1-year and 3-year peripheral blood samples were collected as described previously.[14]

Mononuclear cell stimulation

Mononuclear cells were stimulated with PHA (5 μg/mL) or incubated in medium alone, as described previously.[14]

Cytokine ELISA

Levels of IFN-γ, IL-5, IL-10, and IL-13 in supernatants were evaluated by using an ELISA (PharMingen, San Diego, Calif). The manufacturer's protocol was followed, except for reduction of the sample volume to 50 μL. The sensitivities are as follows: IFN-γ, 4.6 pg/mL; IL-5, 1.8 pg/mL; IL-10, 7.7 pg/mL; and IL-13, 1.5 pg/mL. The samples were analyzed in duplicate, and median values are reported.

Allergen-specific IgE

Total and allergen-specific IgE levels were determined as previously described.[14] Allergen-specific IgE values of 0.35 KU/L (class I) or greater were considered positive, and the sensitivity for detection of total IgE was 2 KU/L.

Statistical analysis

Comparisons by sex of birth weight, moderate-to-severe illnesses, and the number of scheduled-visit viral recoveries were conducted by using the Wilcoxon rank sum test. Dichotomous outcomes, including demographic risk factors, were compared by sex by using the χ^2 test for association. Comparisons of continuous outcomes (cytokine production: cord blood, year 1, and year 3; total IgE levels and eosinophil counts: year 1 and year 3) were carried out by using linear models of rank-transformed responses. Comparisons of dichotomous outcomes (RAST results, atopic dermatitis, and wheeze: year 1 and year 3) were conducted by using logistic regression models. Comparisons of cytokine production by sex were also analyzed in the presence of other factors, including parental asthma, parental allergy, breast-feeding, presence of older siblings, passive smoke exposure, presence of cat or dog in home, day-care attendance, allergen sensitivity, birth weight, and number of viral illnesses, by using linear models. A 2-sided *P* value of .05 or less was regarded as statistically significant.

RESULTS

Patient population

Two hundred eighty-nine children were enrolled at birth, with 275 having complete data at year 3. The prevalence of risk factors by sex is presented in Table I.

TABLE II. Cytokine responses by sex* (boys, n = 155; girls, n = 120)

	Cord blood				Year 1				Year 3			
			P value†				*P* value				*P* value	
	Boys	Girls	Univariate	Multivariate	Boys	Girls	Univariate	Multivariate	Boys	Girls	Univariate	Multivariate
IFN-γ	66 (31, 152)	53 (31, 105)	.10	.27	35 (19, 56)	19 (11, 38)	<.001	.004	282 (140, 645)	181 (117, 626)	.07	.28
IL-5	58% detectable	50% detectable	.30	.78	152 (75, 254)	136 (67, 240)	.28	.82	263 (164, 383)	170 (104, 303)	.002	.005
IL-10	103 (59, 165)	96 (57, 169)	.86	.64	91 (60, 162)	90 (49, 158)	.29	.74	449 (329, 831)	428 (270, 909)	.63	.57
IL-13	270 (154, 452)	301 (159, 492)	.35	.20	242 (123, 378)	235 (120, 410)	.96	.50	330 (185, 532)	241 (156, 437)	.05	.02

*Median values are reported with quartiles (25th percentile, 75th percentile).
†*P* values refer to comparisons of responses between boys and girls for each time point evaluated; univariate analysis and multivariate analysis include risk factors.

TABLE III. Allergic sensitization, total IgE levels, peripheral eosinophil counts, and wheezing by sex

	Year 1			Year 3		
	Boys*	Girls*	*P* value†	Boys	Girls	*P* value
Positive RAST result (%)						
Any allergen	33	22	.05	46	36	.12
Any aeroallergen	16	8	.06	32	21	.06
Any food allergen	27	20	.19	35	28	.26
Milk	11	12	.78	25	23	.64
Egg	20	13	.14	20	13	.13
Peanut	14	5	.02	8	6	.59
Dermatophagoides pteronyssinus	3	2	.43	11	8	.49
Dermatophagoides farinae	2	3	.74	9	4	.13
Alternaria species	4	0	.08	17	6	.01
Dog	5	6	.76	13	10	.51
Cat	8	3	.07	18	8	.03
Total IgE (KU/L), median (25th, 75th)	16 (6.8, 36)	12 (5.1, 22)	.03	38 (14, 95)	22 (8.9, 45)	.006
Eosinophils, mean (SD)	2.79 (1.95)	2.35 (1.74)	.10	2.62 (2.56)	1.85 (1.77)	.05
Atopic dermatitis (%)	47	38	.15	46	36	.10
Wheeze (%)	33	30	.61	34	22	.02

*Boys, n = 155; girls, n = 120.
†*P* values refer to comparisons of responses between boys and girls.

Other than boys being slightly heavier at birth, there were no differences between boys and girls with respect to parental allergy or asthma, presence of furred pets in the home, passive smoke exposure, day-care attendance, or duration of breast-feeding.

Sex and developmental cytokine response patterns

Median PHA-induced IFN-γ responses were 84% higher in boys when compared with those in girls at year 1 ($P < .001$), with the same trend noted at year 3 (282 vs 181 pg/mL, $P = .07$, Table II). Boys also demonstrated significantly greater PHA-induced IL-5 responses, and the same trend was noted for IL-13 (55% and 37% more, respectively) at year 3. PHA-induced IL-10 responses were similar in boys and girls at all time points.

Sex and atopic outcomes

Boys had increased rates of positive RAST results to any allergen by age 1 year (33% vs 22%, $P = .05$), with specific sensitization to peanut noted at age 1 year (14% vs 5%, $P = .02$) and to *Alternaria* species and cat by age 3 years (17% vs 6% [$P = .01$] and 18% vs 8% [$P = .03$], respectively; Table III). In addition, boys had significantly increased levels of total IgE at age 1 year (median, 16 vs 12 KU/L; $P = .03$) and had increased total IgE levels (median, 38 vs 22 KU/L; $P = .006$) and blood eosinophil counts (2.62 vs 1.85, $P = .05$) at age 3 years. Rates of atopic dermatitis did not differ by sex. Boys were also more likely to wheeze during the third year of life than were girls (34% vs 22%, $P = .02$).

Sex, developmental cytokine response profiles, and atopic outcomes

Cytokine response profiles were analyzed first for the entire cohort overall and second by sex at birth and 1 and 3 years of age to evaluate the relationship between developmental immune response patterns and the evolution of the clinical expression of various atopic phenotypes by three years of age (eg, wheezing, total IgE level,

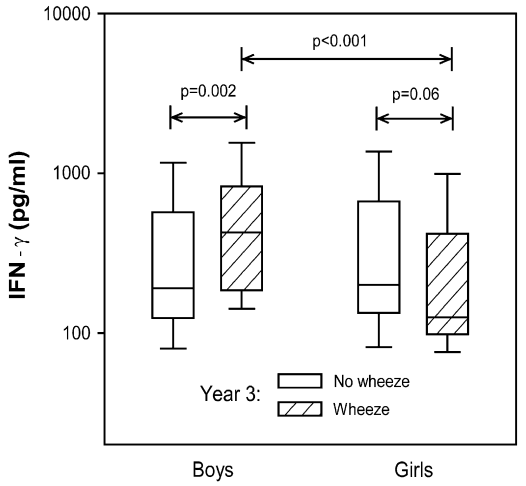

FIG 1. Year 3 median PHA-induced IFN-γ cytokine response by sex and wheezing phenotype. IFN-γ responses are influenced by sex and wheezing status. Boys who wheezed *(hatched bar)* had greater IFN-γ responses than nonwheezing boys *(open bar)* or girls who wheezed *(hatched bar)*. Box plots represent the medians, 25th and 75th percentiles, and 5th and 95th percentiles *(whiskers)*.

allergen-specific IgE level, and atopic dermatitis). There was no relation between PHA-induced IFN-γ, IL-5, IL-10, and IL-13 responses at birth and 1 year of age with wheezing either for the group as a whole or for male or female subjects (data not shown). When these same response profiles were evaluated cross-sectionally at age 3 years in the entire cohort, no differences between wheezing and nonwheezing children were noted. However, when cytokine response profiles were further stratified on the basis of sex, boys who wheezed during this time period were noted to have significantly higher IFN-γ responses compared with girls (median, 426 vs 125 pg/mL; $P < .001$) at 3 years of age. Moreover, the direction of the response was opposite by sex: wheezing boys had significantly higher IFN-γ responses (median, 426 vs 190 pg/mL; $P = .002$), whereas wheezing girls tended to have lower responses (median, 125 vs 200 pg/mL; $P = .06$; wheeze × sex interaction, $P = .0015$; Fig 1). Sex differences in cytokine response profiles in relationship to histories of wheezing during the third year of life were not restricted to IFN-γ: PHA-induced secretion of both IL-5 and IL-13 was also significantly increased in wheezing boys compared with wheezing girls (Table IV).

The relationship of longitudinal alterations in cytokine response profiles and the expression of various atopic markers/phenotypes at age 3 years were also ascertained for total IgE levels, RAST results, and atopic dermatitis. Children in the highest quartile of IL-13 (391.3-1635 units) at 1 year of age had significantly higher IgE antibody levels at age 3 years compared with the children in the lowest quartile (3-121.3 units; median, 290 vs 191 pg/mL; $P = .05$; no differences by sex; see Table E1 in the Online Repository at www.jacionline.org). When evaluated again at age 3 years, significantly increased secretion rates for both IL-5 (median, 285 vs 190 pg/mL; $P = .005$) and IL-13 (median, 355 vs 260 pg/mL; $P = .005$) were observed. When stratified by sex, this association of IgE levels with increased cytokine production was significant only for boys (boys: IL-5, median of 312 vs 216 pg/mL, $P = .002$; IL-13, median of 355 vs 260 pg/mL, $P = .02$; for girls: IL-5, median of 185 vs 169 pg/mL, $P = .54$; IL-13, median of 285 vs 228 pg/mL, $P = .14$). However, when between-sex comparisons were evaluated for these relationships, no significant differences were noted. No significant development effects for either IFN-γ or IL-10 responses on total IgE responses at age 3 years were observed at any of the ages evaluated.

In contrast to total IgE values at age 3 years, no longitudinal or cross-sectional alterations in cytokine response patterns were associated with the development of 1 or more positive RAST results or with atopic dermatitis at this same age (data not shown).

Sex and viral respiratory illnesses and infections

We next compared rates of moderate-to-severe viral respiratory illnesses, with or without wheezing, in boys and girls during the first 3 years of life (Table V). For all viral infections, boys had significantly more illnesses than girls (mean, 6.3 vs 5.0; $P = .004$). This was true for illnesses without (mean, 4.1 vs 3.3; $P = .03$) or with (mean, 2.1 vs 1.7; $P = .04$) the concomitant presence of wheezing. When specific viral causes for these illnesses were evaluated, boys had significantly more illnesses caused by rhinovirus (mean, 2.8 vs 2.0; $P = .02$). Although sex differences in rhinovirus-induced illnesses without wheezing were noted (mean, 2.0 vs 1.3; $P = .005$), the frequency of illnesses that were accompanied by wheezing was similar for boys and girls (mean, 0.7 vs 0.7; $P = .59$). In contrast, there were no differences based on sex related to the frequency or clinical pattern of illness caused by infection with RSV. For illnesses caused by other viruses, boys had significantly more illnesses overall (mean, 2.8 vs 2.2; $P = .004$) and more wheezing illnesses (mean, 1.1 vs 0.7; $P = .008$).

Despite sex-specific differences in illness frequency and severity, the number of infections (based on viral recovery during regularly scheduled visits) observed during the first 2 years of life was not different between boys and girls (mean, 1.1 vs 1.2; $P = .64$).

DISCUSSION

Boys are more likely to have a number of atopic syndromes in early childhood, including atopic dermatitis, allergic sensitization, and wheezing. In addition to confirming these observations in a birth cohort at increased risk for atopic disorders, our results provide novel evidence of distinct patterns of cytokine responses in boys and girls over the first 3 years of life and indicate that the magnitude and direction of these response profiles differ, depending on the outcome measure being prospectively evaluated. At birth, there were indications that boys in our

TABLE IV. Year 3 cytokine responses by sex and wheezing phenotype

	No wheeze year 3 (boys, n = 102; girls, n = 94)				Wheeze year 3 (boys, n = 53; girls, n = 26)			
			P value†				*P* value	
	Boys	Girls	Univariate	Multivariate	Boys	Girls	Univariate	Multivariate
IFN-γ	190 (125, 541)	200 (137, 655)	.74	.58	426 (188, 800)	125 (99, 312)	<.001	.004
IL-5	256 (154, 361)	171 (110, 337)	.04	.05	269 (175, 412)	158 (98, 269)	.008	.02
IL-10	514 (334, 846)	442 (278, 935)	.91	.80	425 (328, 766)	394 (236, 770)	.29	.33
IL-13	295 (178, 505)	255 (157, 484)	.50	.23	390 (260, 542)	229 (155, 316)	.01	.01

*Median values are reported with quartiles (25th percentile, 75th percentile).
†*P* values refer to comparisons of responses between boys and girls; univariate analysis and multivariate analysis include risk factors.

cohort had enhanced IFN-γ responses (Table II). By age 1 year, these differences were significant, and other disparities emerged, including increased rates in boys of allergic sensitization and increased total IgE levels. By age 3 years, boys were exhibiting enhanced cytokine responses (now both T_H1 and T_H2), increased total IgE levels, and blood eosinophilia. Differences in clinical outcomes were also noted by age 3 years, with boys expressing increased rates of viral illnesses and wheezing. These results corroborate prior findings that boys tend to wheeze more than girls during childhood[21] and that wheezing is more likely to be transient in infant girls.[22] In our cohort the rate of wheezing between years 1 and 3 decreased among the girls, whereas the rate among boys remained stable.

We carefully examined the longitudinal and cross-sectional nature of the relationships among sex, cytokine response profiles, and the development of various atopic and wheezing phenotypic characteristics. When the presence of wheezing during the third year of life was used as the outcome measure, we were unable to discern any relationship between cytokine response patterns at birth or at 1 year of life when the cohort was evaluated as a whole or stratified by sex. In contrast, when these same response profiles were evaluated cross-sectionally at age 3 years, some very interesting patterns emerged. Boys with histories of wheezing had significantly increased PHA-induced IFN-γ, IL-5, and IL-13 responses relative to boys without wheezing. In contrast, girls who wheezed tended to have lower IFN-γ responses compared with girls who did not wheeze (Fig 1).

We next explored whether the sex discrepancy regarding T_H1 (ie, IFN-γ) responses was related to either the frequency or severity of viral respiratory tract infections during the first few years of life. We have previously reported an inverse relationship between PHA-induced IFN-γ responses at the time of birth and the number of viral respiratory illnesses by 1 year of age.[15] In turn, frequent wheezing episodes are associated with enhanced IFN-γ responses at age 1 year, indicating that viral illnesses affect immune development in early life.[23] A novel finding from our continued observations of this cohort through age 3 years is that the numbers of viral illnesses, but not infections, is significantly higher in boys (Table V). Thus although reduced T_H1 responses at birth might predispose all children to an increased risk of viral infections during early life, boys tend to behave differently with regard to both their clinical response to infection (ie, more moderate-to-severe respiratory tract symptoms) and the modulation of their immune response profiles during these exposures (increased T_H1 and T_H2 responses).

TABLE V. Number of early-life moderate-to-severe illnesses and scheduled-visit viral infections by sex*

	Boys	Girls	*P* value
Moderate-to-severe illnesses†			
Any cause			
All	6.3 (4.4)	5.0 (4.4)	.004
Without wheezing	4.1 (3.6)	3.3 (3.2)	.03
With wheezing	2.1 (3.2)	1.7 (3.1)	.04
Rhinovirus			
All	2.8 (2.8)	2.0 (2.4)	.02
Without wheezing	2.0 (2.0)	1.3 (1.4)	.005
With wheezing	0.7 (1.6)	0.7 (1.7)	.59
RSV			
All	0.7 (0.7)	0.8 (0.7)	.74
Without wheezing	0.4 (0.5)	0.5 (0.6)	.31
With wheezing	0.4 (0.6)	0.3 (0.6)	.33
Nonrhinovirus/non-RSV			
All	2.8 (2.1)	2.2 (2.3)	.004
Without wheezing	1.7 (2.0)	1.5 (2.0)	.08
With wheezing	1.1 (1.6)	0.7 (1.6)	.008
Scheduled-visit viral recoveries‡			
Any cause	1.1 (1.1)	1.2 (1.1)	.64
Rhinovirus	0.8 (0.9)	0.9 (0.8)	.75
RSV	0.1 (0.2)	0.1 (0.2)	.48
Nonrhinovirus/non-RSV	0.2 (0.6)	0.3 (0.5)	.80

*Mean (SD) values are reported.
†During the first 3 years of life.
‡During the first 2 years of life (2 months, 4 months, 6 months, 9 months, 1 year, 18 months, and 2 years).

The relative immaturity of the neonatal immune system has been well characterized, and neonatal IFN-γ responses are significantly attenuated with respect to those seen in adults.[24-26] Recent studies have also shown that newborns at high risk of atopic disease (parental atopy) demonstrate an even greater reduction in polyclonal IFN-γ responses when compared with infants at low risk of allergy.[27-35] The relative T_H1 deficiency among at-risk children might in fact be transient as rebound increases in IFN-γ responses relative to those seen in low-risk children begin

to emerge in the first 2 years of life.[36-38] Our data extend these observations by demonstrating that among children at increased risk for atopic diseases, boys and girls have distinct patterns of immune maturation that can precede (ie, total IgE antibody production) or occur in parallel (ie, wheezing with viral illnesses) with the development of various immunologic and respiratory phenotypic traits.

Although the exact pathophysiologic mechanisms of our observations remain unclear, sex-specific hormonal influences could contribute to these sex ratios during early development. Sex differences in corticosteroid secretion and activity could theoretically explain variations in susceptibility to atopy; however, cortisol levels and patterns of secretion do not appear to differ by sex during infancy.[39] On the other hand, there is conclusive evidence that sex hormones can influence the development of specific lymphocyte populations and cytokine production. For example, lymphocytes are known to express both testosterone and estrogen receptors,[40-43] whereas androgens enhance $CD8^+$ lymphocyte activity[44,45] and are correlated with the activation of IFN-γ–secreting cells in healthy adults.[46] Therefore it is certainly plausible that sex-related differences in sex hormones, in addition to other genetic and environmental influences, modulate the developing immune system and consequently the clinical expression of atopic diseases and wheezing.

The COAST study was designed to evaluate how interactions between immune responses and viral infections in early life affect the development of wheezing illnesses and eventually asthma, and the experimental design has a number of strengths and some limitations in this regard. The strengths of the study include its prospective nature, which enabled the measurement of immune responses before and after viral infections and wheezing episodes; documentation of specific viral pathogens; a comprehensive prospective characterization of atopic phenotypes and biomarkers; and excellent subject retention (98.6% at 1 year). One of the limitations of the study is that the cohort only includes children from families in which at least 1 parent has allergies or asthma. Some previous studies have demonstrated immunologic differences between healthy infants born to atopic versus nonatopic parents.[47,48] Therefore our data must be interpreted keeping in mind that the healthy children in the COAST study, who serve as our comparator group, could be immunologically different than healthy children of nonallergic nonasthmatic parents. Finally, although the overall COAST study is sufficiently powered to evaluate the primary outcome measure, the size of the cohort might have some limitations when evaluating certain secondary outcomes because they are expressed over time in relationship to the observed natural history of allergic respiratory tract disorders (eg, the incidence of wheezing in preschool girls).

In conclusion, previous findings have implicated the importance of dietary, genetic, and environmental factors on the development of allergic sensitization and wheezing. Our data extend these observations by demonstrating that sex-specific atopic characteristics during early childhood are associated with differences in the development of cytokine responses. Continued study of our cohort and others is needed to determine the relative influence of developmentally regulated and sex-specific immune responses on the clinical expression and remission of allergic diseases and asthma throughout childhood.

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