

Elevated Allergen-Induced IL-13 Secretion Predicts IgE Elevation in Children Ages 2–5 Years

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It is unclear if early immune responses to allergens, specifically Th1 and Th2 cytokine production, predict later immune responses, including increased IgE levels. In a group of children ($n = 151$) with a parental history of allergy or asthma followed from ages 2 through 5 years, we examined IL-13, IL-4, and IFN- γ secretion by peripheral blood mononuclear cells in response to phytohemagglutinin (PHA), and to dust mite (Der f 1), cockroach (Bla g 2), and cat (Fel d 1) allergens in relation to elevated IgE. Elevated IgE was defined either as a positive IgE-specific response to at least one allergen (dust mite, cockroach, cat, and ovalbumin) or as an elevated total IgE level above a specified cut-off value. In multivariate logistic regression models including 181 observations made between the age of 2 through 5 years and accounting for repeated measures, we found an association between increased IL-13 secretion in response to Der f 1 and elevated IgE (odds ratio [OR] = 1.21, 95% confidence interval [CI] = 1.09–1.34). Age did not modify this relationship. No association was found between allergen-induced IFN- γ secretion and IgE production. Among the group of children with measurements made at age 4–5 ($n = 70$), IL-13 in response to Der f 1 ($p = 0.046$), and IL-4 in response to PHA ($p = 0.04$) were increased among children with elevated IgE. In a smaller subset of children with measurements made at both age 2–3 and age 4–5 ($n = 36$), IL-13 levels at age 2–3 were also significantly increased in response to Der f 1 ($p = 0.01$) and Fel d 1 ($p = 0.002$) among those with elevated IgE at age 4–5. In a group of children ages 2–5 years, there is an association between IL-13 and elevated IgE.

KEY WORDS: Children; IFN- γ ; IL-13; IL-4; IgE; PBMCs.

INTRODUCTION

Increased serum levels of total and allergen-specific IgE are common characteristics of allergic diseases (1, 2). Since the delineation of type 1 (Th1) and type 2 (Th2) CD4⁺ T cells in mice (1989) (3) and in humans (1994) (4), evidence has largely shown that activated Th2 lymphocytes and the elaboration of certain cytokines such as interleukin (IL)-4, IL-13, and IL-5 are responsible for the cascade of eosinophil activation and IgE production necessary for allergic inflammation (5–7). Conversely, Th1 cytokines such as interferon- γ (IFN- γ) decrease IL-4- and IL-13-dependent IgE production (8), and some evidence suggests that IFN- γ is reduced in atopic as compared with normal individuals (9, 10). Conceptually, Th1 IFN- γ is known to counterregulate Th2 immune response and Th2 cytokines (IL-4, IL-13, IL-5) are known to activate a cascade of events that are necessary for inflammation; however, the relationship of IL-13 and IFN- γ to IgE production in children is less well understood than that in adults or in animal models.

We previously showed a cross-sectional association of increased allergen-induced IL-13 secretion with elevation of IgE as early as 2–3 years of age in children at risk for asthma or allergy (11). In that study, we also found a weaker, less consistent association between reduced IFN- γ and elevated IgE. Taking advantage of having repeated measures of cytokines and IgE levels, in this follow-up study, we hope to further delineate the relationship of allergen-induced secretion of IL-13 and IFN- γ to IgE production over time. We hypothesized that increased allergen-induced IL-13 secretion would predict elevated IgE at both the early and later stages of life. First, we examined the relationship between allergen-induced cytokines

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and elevated IgE using all observations obtained between ages 2 and 5 years. Next, in a cross-sectional analysis at age 4–5, we determined the difference in allergen-induced cytokine levels between children with and without elevated IgE. Finally, in a smaller subset of children with observations at both age 2–3 and age 4–5, we performed a prospective analysis comparing differences in allergen-induced cytokine levels at age 2–3 among children with and without elevated IgE at age 4–5.

METHODS

Study Cohort

The 505 term infants with a parental history of asthma or allergy were enrolled between September 1994 and August 1996 in the Epidemiology of Home Allergens and Asthma Study, an ongoing longitudinal birth and family cohort. Detailed inclusion criteria have been described previously (12). In brief, families were not approached if the index child had a major congenital anomaly, or was in the neonatal intensive care unit, or if the mother was younger than 18 years old or could not speak English or Spanish. Informed consent was obtained from the parents for blood collection and longitudinal follow-up. Home allergen levels were measured at a home visit conducted when the infants were 2–3 months old. We administered telephone questionnaires to the child's caretaker every 2 months during the first 2 years of life, and every 6 months thereafter. Of the 505 children, 7 were excluded because they were followed for less than 4 months during their first year of life. This study was approved by the Brigham and Women's Hospital Human Research Committee.

Allergen-Induced Cytokine Measurements

Of the 498 children with follow-up information, 151 had cytokines measured either at age 2–3 or age 4–5. The goal in choosing these subjects was to maximize variability in early life exposure to the allergens with which their cells were to be stimulated.

As previously described, peripheral blood mononuclear cells (PBMCs) were isolated by means of Ficoll–Hypaque centrifugation (11–13). PBMCs were immediately processed and not cryopreserved. Aliquots of 5×10^5 cells were incubated in quadruplicate in a 96-well microtiter plate with 100 μ l of media alone, media with PHA (10 μ g/ml), or media with the following antigens: dust mite (*Der f 1*; 30 μ g/ml), cockroach (*Bla g 2*; 30 μ g/ml) (Martin Chapman, Charlottesville, VA) and cat (*Fel d 1*; 1000 U/ml). Optimal concentrations of the allergens

were determined in a dose-response analysis (data not shown). Supernatants were harvested at 24 h and 60 h after allergen-stimulation. Based on the optimization of the detection of cytokine levels, IFN- γ , IL-4, and IL-13 secretions were measured in supernatants harvested 60 h after allergen-stimulation. Cytokine analyses were performed with commercially available ELISA kits according to the manufacturer's instructions (Endogen). The lower limit of detection for IFN- γ is <2 pg/ml, for IL-4 is < 2 pg/ml, and for IL-13 is <7 pg/ml.

IgE Measurements

Serum samples were assayed for total IgE and specific IgE antibodies to dust mite, cat, cockroach, and ovalbumin using the UNICAP System (Pharmacia, Uppsala, Sweden). Elevated IgE was defined as an IgE-specific response (≥ 0.35 IU/ml) to at least one allergen or a total IgE level above a specified cut-off value. A total IgE cut-off value ≥ 60 IU/ml was used for age 2–3 and ≥ 100 IU/ml was used for age 4–5. Since total IgE levels increased with age, a higher cut-off value for total IgE was used at age 4–5 based on previous reports showing that total IgE levels >100 IU/ml were associated with allergic sensitization, and risk of asthma (14, 15).

Analyses

In the longitudinal analyses using all observations available from ages 2 through 5 years, we fitted logistic regression models using generalized estimation equations (GEE) developed by Zeger and Liang (16). The GEE methods accounted for within-subject correlation among repeated measures of IgE and cytokines. We also adjusted for potential confounding variables and determined if age modifies the relation between cytokines and IgE by creating interaction terms between age (in months) and cytokine levels. The distribution of cytokine levels was statistically skewed, with a significant number of nondetectable values; therefore, cytokine values were logarithmically transformed in logistic regression analyses.

In cross-sectional analyses, median cytokine levels measured at either age 2–3 or age 4–5 were compared for different groups of children classified by elevated IgE (yes/no) at age 4–5. As in our previous analysis (11), the Hodges–Lehmann method was used to estimate the median cytokine level for each group of children and to estimate the median 95% confidence interval of the differences in the cytokine levels in the children from the different groups. The Hodges–Lehmann median is a measure of the central location of the differences between values (in this case, cytokine levels) from two different

groups (e.g., those with elevated IgE vs. those without), calculated as the median of the pair-wise differences between the values in each group. The statistical significance of comparisons of differences in cytokine levels between groups was determined with two-sample Wilcoxon tests. Computation of the *p* values and interval limits were done using the exact RankTests package in the R language (The Comprehensive R Archive Network, 2001). Logistic regression analyses were performed using SAS, version 8.

RESULTS

Population Characteristics

Among the 498 subjects with follow-up information in this cohort, 151 had cytokines measured either at age 2–3 or age 4–5. Among these 151 subjects, 31 (21%) had a doctor's diagnosis of asthma by age 5. Some of the baseline characteristics of these children are depicted in Table I. There were no statistical differences in family history of allergy or asthma, household income, or race between these 151 subjects as compared to the 347 subjects who did not have cytokine measurements. However, there were more boys than girls who had cytokines measured than not. Of the 111 children with cytokines measured at age 2–3, 14.4% (*n* = 16) had elevated IgE. Among the 16 subjects with elevated IgE at age 2–3, 7 met the criteria by just having elevated total IgE $\geq$ 60 IU/ml, 4 had an IgE response to dust mite, 4 had an IgE response to ovalbumin, and 1 had IgE response to both cat and ovalbumin. Of the 70 children with cytokine measured at age 4–5, 21.4% (*n* = 15) had elevated IgE. Among the 15 subjects with elevated IgE at age 4–5, only 1 met the criteria based on total IgE $\geq$ 100 IU/ml alone, 6 had an IgE response to dust mite, 3 had an IgE response to cat, and 5 had IgE responses to multiple allergens with dust mite response in all 5 children and cockroach response only in 1 child. In a subset of children who had repeated measures of IgE (*n* = 36), four more children developed elevated IgE and none had lost their specific IgE between the first and second IgE analysis.

Cytokine Levels and IgE

We had previously shown the distributions of cytokine values for children at age 2–3 (11). Here we showed the distributions of IL-13, IL-4, and IFN- γ values obtained after allergens and mitogen stimulation of PBMCs for all subjects at age 4–5 (Fig. 1). The distributions of cytokine values at age 4–5 were mostly sin-

Table I. Characteristics of the 151 Subjects with Cytokine Measurements at Either Age 2–3 or Age 4–5

	<i>N</i>	%
Gender		
Male	96	63.6
Female	55	36.4
Income		
High ($\geq$ \$ 50K)	110	72.8
Medium (\$ 30–\$ 49K)	26	17.2
Low ($\leq$ \$ 29K)	12	8.0
Unknown ^a	3	2.0
Race		
White	115	76.2
Black	14	9.3
Hispanic	10	6.6
Others	12	7.9

^aUnknown indicates a “don't know” response to the question.

gle tailed similar to the distributions observed at age 2–3. For IL-4, more than 60% of the values were below the lower limit of detection for each of allergens (*Fel d 1*, *Der f 1*, *Bla g 2*) with the exception of PHA where there were no values below the lower limit of detection.

In logistic regression models analyzing 181 observations obtained between ages 2 and 5 years from 151 subjects and accounting for repeated measures, we found a significant association between IL-13 and IgE production (Table II). In unadjusted logistic regression models, increased IL-13 secretion in response to *Der f 1* was associated with elevated IgE (OR, 1.20; 95% CI, 1.06–1.36). This association was unchanged after adjusting for age, gender, race, and levels of exposure to home allergens at 2–3 months of age (OR, 1.21; 95%CI, 1.09–1.34). Age (in months) did not modify the association between IL-13 and IgE (data not shown). Allergen-stimulated IFN- γ secretion was not associated with IgE production in logistic regression models.

In cross-sectional analyses comparing cytokine levels between different groups of children, we found that at age 4–5, IL-13 level in response to *Der f 1* (*p* = 0.046) was increased among children with elevated IgE (*n* = 15) as compared with those without elevated IgE (*n* = 55) (Table III). Interestingly, in a subset of children with measurements made at both age 2–3 and age 4–5 (*n* = 36), IL-13 levels at age 2–3 were also increased in response to *Der f 1* (*p* = 0.01) and *Fel d 1* (*p* = 0.002) among children with elevated IgE (*n* = 9) but not among children without elevated IgE (*n* = 27) at age 4–5 (Table IV). Levels of allergen-stimulated IFN- γ measured at both age 2–3 and age 4–5 were not significantly different in children with and without elevated IgE at age 4–5. We

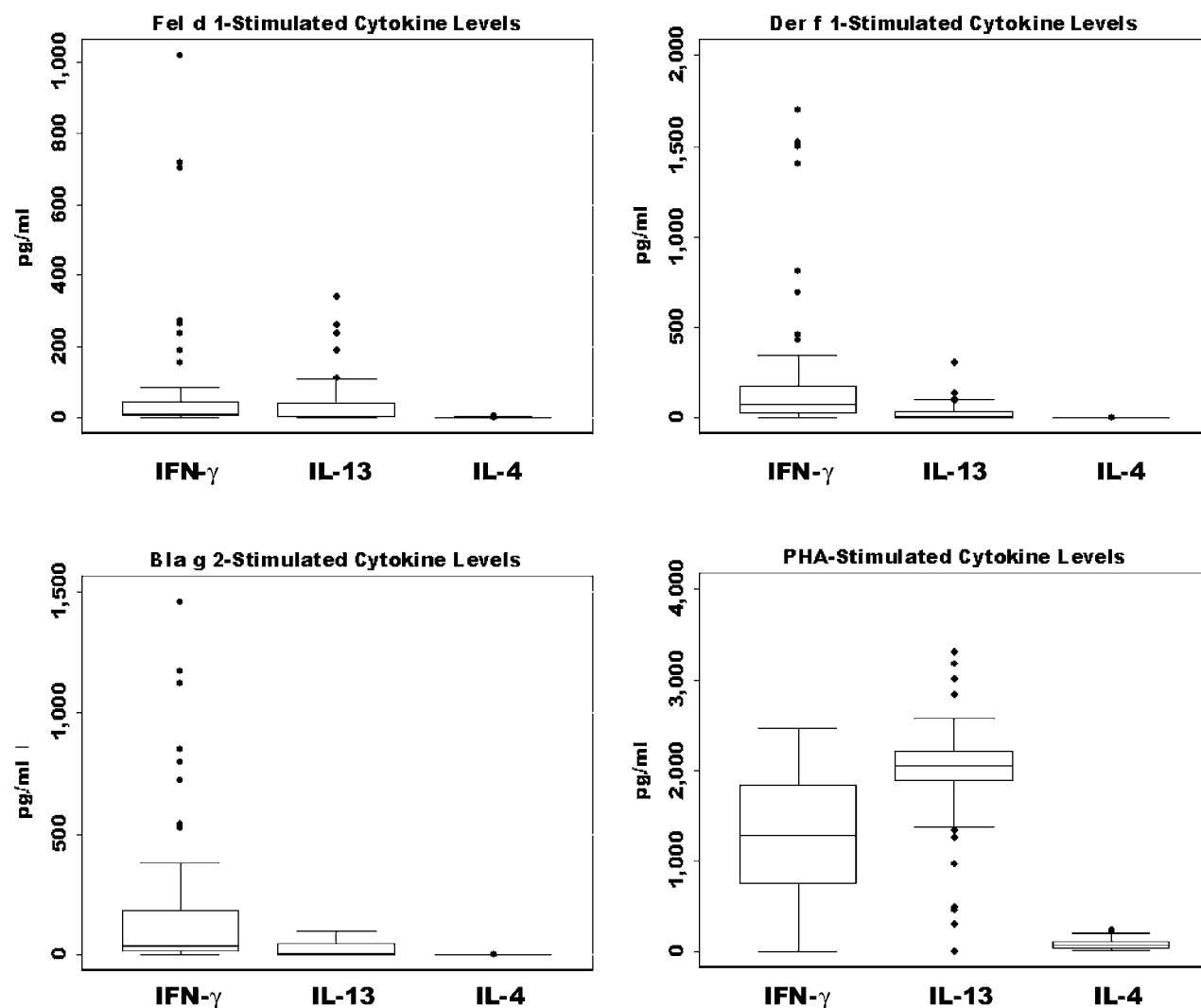


Fig. 1. Production of IFN- γ , IL-13, and IL-4 cytokines in PBMCs among 70 subjects after stimulation with allergens (Der f 1, Bla g 2, and Fel d 1) and a mitogen, phytohemagglutinin (PHA). PBMCs were obtained from blood samples collected between ages 4 and 5 years. IFN- γ , IL-13, and IL-4 secretions were measured in supernatants 60 h after allergen-stimulation. The median is represented by the black bar. The upper and lower boundaries of the box represent the 25th to 75th percentiles of the data, respectively. Observations <1.5 times the height of the box beyond either quartile are displayed within the whiskers. (•) represents outliers.

share the experience of other investigators in finding that in the 2–5 age range, IL-4 is produced in such small amounts that they were usually below detectable limits of secretion. However, we were able to measure IL-4 levels among the 70 subjects at age 4–5 and as noted in Fig. 1, most allergen-stimulated IL-4 values were below the lower limit of detection except for PHA-stimulated PBMCs. Among the subjects with IL-4 measured at age 4–5, we found that the median IL-4 level in response to PHA was 103.5 pg/ml among children with elevated IgE ($n = 12$) as compared with 62.5 pg/ml among those without elevated IgE ($n = 49$) with a significant p value of 0.04.

DISCUSSION

Results from cross-sectional and longitudinal studies have consistently demonstrated a strong association between an upregulated Th2 immune response and allergic diseases. However, most longitudinal studies have focused on allergen-stimulated cord blood cytokine responses or peripheral blood cytokine responses before age 2 years in relation to allergies and allergic diseases that manifest at a later age. In this study, we demonstrated a longitudinal and cross-sectional association between increased (Th2) IL-13 secretion and elevated IgE in children ages 2–5 years. More importantly, the association between IL-13 and IgE

Table II. Association of Allergen- and Mitogen-Induced Cytokine Secretion to Elevated IgE^a in 151 Children Ages 2 Through 5 Years

Cytokines	Allergens	OR (95% CI)	
		Unadjusted	Adjusted ^b
IL-13	<i>Der f 1</i>	1.20 (1.06, 1.36)	1.21 (1.09, 1.34)
	<i>Bla g 2</i>	1.07 (0.98, 1.17)	1.06 (0.97, 1.16)
	<i>Fel d 1</i>	1.08 (0.98, 1.19)	1.08 (0.97, 1.20)
	PHA	1.45 (0.91, 2.33)	1.37 (0.95, 1.96) ^c
IFN- γ	<i>Der f 1</i>	1.01 (0.92, 1.11)	0.99 (0.90, 1.10)
	<i>Bla g 2</i>	0.94 (0.86, 1.03)	0.93 (0.85, 1.02)
	<i>Fel d 1</i>	0.98 (0.89, 1.07)	0.99 (0.90, 1.07)
	PHA	1.14 (0.94, 1.38)	1.13 (0.93, 1.36)

^aElevated IgE was defined either as IgE-specific response to at least one allergen (dust mite, cockroach, cat, and ovalbumin) or total IgE ≥ 60 IU/ml at age 2–3 and ≥ 100 IU/ml at age 4–5.

^bModels adjusting for gender, race, age in months, and specific home allergen levels.

^cFor PHA-induced cytokine secretion, multivariate models adjusted for gender, race, and age in months but not home allergen levels.

did not change over time. Our findings reconfirmed two important concepts. First, it supports the concept that activated Th2 lymphocytes and the elaboration of certain cytokines such as interleukin (IL)-4, IL-13, and IL-5 are responsible for the cascade of eosinophil activation and IgE production necessary for allergic inflammation (5–7). Second, it supports the general belief that T-cell priming to aeroallergens occurs at a young age and the patterns recognized in adults are established before the age of 5 years (17, 18). Interestingly we found that among children with elevated IgE, Th2 (IL-13) immune response to house dust mite (*Der f 1*) allergen was present at both age 2–3 and age 4–5 but Th2 response to cat (*Fel d 1*) allergen was present only at age 2–3. A possible reason for this

observed difference in immune response may be related to differential allergen exposure. In this cohort, we had considerably more dust mite than cat allergen and allergy. While we did not find a significant Th2 immune response to cockroach (*Bla g 2*) allergen, there was a suggesting of a weak association between cockroach-stimulated IL-13 and a small elevation in IL-13. The relative weakness of the association likely relates to the lower level of exposure to cockroach; there was also a lower level of cockroach-specific allergy amongst the children in this relatively affluent cohort.

While we found a strong association between elevated IL-13 levels and IgE production, we did not find an association between reduced IFN- γ levels and IgE

Table III. Cross-Sectional Relationship Between Allergen- and Mitogen-Induced Cytokine Secretion and IgE Production in Children at Age 4–5

		Median cytokine level (pg/ml) ^a				
Cytokines	Allergens ^c	No IgE elevation (<i>n</i> = 55)		IgE elevation (<i>n</i> = 15) ^b		Wilcoxon <i>p</i> value
		Median	<i>n</i>	Median	<i>n</i>	
IL-13	<i>Der f 1</i>	14.1	53	33.4	11	0.046
	<i>Bla g 2</i>	19.9	53	15.7	11	0.42
	<i>Fel d 1</i>	22.2	53	11.9	11	0.76
	PHA	1994.0	53	2052.0	11	0.58
IFN- γ	<i>Der f 1</i>	93.2	46	409.2	10	0.28
	<i>Bla g 2</i>	66.5	46	82.7	10	0.80
	<i>Fel d 1</i>	17.8	46	23.5	10	0.44
	PHA	1175.0	46	1472.0	10	0.16

^aHodges–Lehmann method was used to estimate the median cytokine level for each group of children and the median of the differences in the cytokine levels in the children from the two groups.

^bIgE elevation at ages 4–5, was defined as having specific IgE antibodies ≥ 0.35 IU/ml to dust mite, cockroach, cat, or ovalbumin or having a total IgE ≥ 100 IU/ml.

^cAt age 4–5, allergens used to stimulate peripheral blood mononuclear cells included *Der f 1* (dust mite), *Bla g 2* (cockroach), and *Fel d 1* (cat). The nonspecific mitogen used was PHA (phytohemagglutinin).

Table IV. Association Between Allergen- and Mitogen-Induced Cytokine Secretion at Age 2–3 and IgE Production at Age 4–5 in a Subset of Children with Repeated Measures

Cytokines (age 2–3)	Allergens ^c	Median cytokine level (pg/ml) ^a				Wilcoxon <i>p</i>
		No IgE elevation at age 4–5 (<i>n</i> = 27)		IgE elevation at age 4–5 (<i>n</i> = 9) ^b		
		Median	<i>n</i>	Median	<i>n</i>	
IL-13	<i>Der f 1</i>	8.2	20	63.3	6	0.01
	<i>Bla g 2</i>	5.5	20	5.1	6	0.62
	<i>Fel d 1</i>	1.3	20	36.4	6	0.002
	PHA	1282.0	20	1331.0	6	1.00
IFN- γ	<i>Der f 1</i>	16.5	24	38.8	8	0.33
	<i>Bla g 2</i>	26.9	24	12.7	7	0.54
	<i>Fel d 1</i>	17.8	24	27.2	7	0.43
	PHA	481.4	24	196.4	8	0.08

Note. Analysis was performed only on the children (*n* = 36) with IgE measured at age 4–5 who had cytokine and IgE measured at age 2–3.

^aHodges–Lehmann method was used to estimate the median cytokine level for each group of children and the median of the differences in the cytokine levels in the children from the two groups.

^bIgE elevation at ages 4–5, was defined as having specific IgE antibodies ≥ 0.35 IU/ml to dust mite, cockroach, cat, or ovalbumin, or having a total IgE ≥ 100 IU/ml.

^cAt age 2–3, allergens used to stimulate peripheral blood mononuclear cells included *Der f 1* (dust mite), *Bla g 2* (cockroach), and *Fel d 1* (cat). The nonspecific mitogen used was PHA (phytohemagglutinin).

production in a group of children ages 2–5 years as we did in our previous cross-sectional study of children aged 2–3 (11). In the previous study, we found that attenuated allergen-induced IFN- γ secretion, in some cases, was associated with elevated IgE secretion. In analyses including all children ages 2 through 5, we did not find an association between IFN- γ and IgE production. In a cross-sectional analysis at age 4–5, there was a suggestion that IFN- γ levels were higher among children with elevated IgE as compared to those without, but the results were not statistically significant. In keeping with findings from other groups, the relationship of IFN- γ to allergy or allergic diseases is less clear. The Th1 IFN- γ response may have different roles in the pathogenesis of allergic diseases depending on the age of the individual. For example, prior study results, although not entirely consistent, suggest that attenuated IFN- γ production very early in life is associated with atopy (19), wheeze (20), and atopic dermatitis (21). However, by early childhood, studies showed that it is an elevated rather than attenuated IFN- γ production that is found among atopics as compared to non-atopics (22, 23). Also, in relation to allergic diseases, IFN- γ has been isolated at local sites of inflammation in atopic dermatitis (24) and in airways of animal models of asthma (25). Furthermore, serum IFN- γ was found to be associated with a decline in lung function among adult asthmatics (26). Collectively these data suggest that at birth, IFN- γ may act to antagonize Th2 response, however, by early childhood, this Th1 response may act concurrently with Th2 response in maintaining chronic inflammation character-

istic of allergic diseases (e.g., symptoms of eczema or wheeze).

Our study has some limitations. We can generalize our results only to children with a parental history of allergic diseases or asthma. However, this group of children is most at risk for developing allergy and asthma; therefore, identifying early markers for predicting allergy is particularly important and may help in designing prevention strategies directed specifically to these children. It is possible that not all allergic children will have a specific IgE response to dust mite, cockroach, cat, and ovalbumin; therefore, we included high total IgE values in our definition for elevated IgE to reduce case misclassification. We did perform separate analyses including only children with allergen-specific IgE response and not elevated total IgE and found a similar relationship between allergen-induced IL-13 and elevated IgE (data not shown). Finally, we may not have accounted for other potential unmeasured confounding variables.

Consistent with the hypothesis that from an early age, allergy involves expression of Th2 cytokines, the enhanced allergen-induced secretion of IL-13, particularly in response to *Der f 1*, is a strong predictor of elevated IgE production at ages 2 through 5 years.

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