

Allergen-induced cytokine production, atopic disease, IgE, and wheeze in children

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Background: The early childhood allergen-induced immune responses associated with atopic disease and IgE production in early life are not well understood.

Objective: We assessed the relationship of allergen-induced cytokine production by PBMCs to both atopic disease and to IgE increase in a cohort of children with a parental history of allergy or asthma (n = 112) at a median of 2 years of age. We examined cockroach (Bla g 1)-induced, house dust mite (Der f 1)-induced, and cat (Fel d 1)-induced cytokine secretion, including secretion of IFN- γ , IL-13, IL-10, and TNF- α . We investigated whether distinct cytokine patterns associated with atopic disease can be detected in immune responses of children.

Methods: PBMCs were isolated, and allergen-induced cytokine secretion was analyzed by means of ELISA. Atopic disease was defined as physician- or nurse-diagnosed eczema or hay fever. Increased IgE was defined as an IgE level of greater than 35 U/mL to dust mite, cockroach, cat, and egg white or a total IgE level of 60 U/mL or greater.

Results: Compared with children without atopic disease, children with atopic disease had lower Der f 1 ($P = .005$) and Bla g 2 ($P = .03$) allergen-induced IFN- γ levels. Compared with children without increased IgE (n = 95), those with increased IgE (n = 16) had higher Der f 1-induced ($P = .006$) and Fel d 1-induced ($P = .005$) IL-13 levels and lower Bla g 2-induced ($P = .03$) IFN- γ levels. Compared with children with neither atopic disease nor repeated wheeze, children with both atopic disease and repeated wheeze had lower levels of allergen-induced IFN- γ ($P = .01$ for Der f 1 and $P = .02$ for Bla g 2) cytokine secretion.

Conclusion: In young children at risk for asthma or allergy, decreased allergen-induced IFN- γ secretion is associated with

atopic disease and, in some cases, with increased IgE levels. Increased allergen-induced IL-13 secretion is most strongly associated with early life increase of IgE. (*J Allergy Clin Immunol* 2003;112:1072-7.)

Key words: Children, cytokines, IFN- γ , IL-13, allergy, atopy, cockroach, dust mite

The prevalence of asthma is increasing.¹ The beginnings of asthma and allergy are usually manifested by age 3 years.^{2,3} Allergic disease is associated with childhood asthma, but the onset of eczema, hay fever, asthma, and their associated immune responses in children remains incompletely defined. Analysis of early life immune responses to common allergens is warranted to devise prevention or therapeutic strategies for children. T_H1 (eg, IFN- γ and TNF- α) and T_H2 (eg, IL-4, IL-5, IL-10, and IL-13) cytokines produced in response to environmental allergens are presumed to play a significant role in immune modulation. In children, prior studies suggest that decreased IFN- γ levels are associated with atopic disease.⁴⁻⁸ The relationship of other allergen-stimulated production of T_H1 or T_H2 cytokines with atopic immune response in children appears less clear and might be influenced by environmental factors, including the age of the child, the stimuli used, and the allergens encountered in early life. Previously, in a birth cohort at risk for asthma or allergy because of parental history, we examined the relationship of allergen-induced PBMC lymphoproliferative response to both home allergen levels and to repeated wheeze symptoms at a median age of 2 years.⁹ In this same cohort we now assess the relationship of allergen-induced PBMC cytokine production to atopic disease, repeated wheeze, and IgE production. We examine cockroach (Bla g 1)-induced, house dust mite (Der f 1)-induced, and cat (Fel d 1)-induced cytokine secretion, including IFN- γ , IL-10, IL-13, and TNF- α . We investigate whether distinct cytokine patterns can be detected in immune responses of children at a median age of 2 years. We postulate that the production of specific cytokines in children might be associated with atopic disease, wheeze, and increased IgE levels.

METHODS

Subject recruitment and study protocol

We have previously described the detailed protocol for cohort recruitment.¹⁰ In brief, 505 children with a parental history of asthma or allergy were enrolled in a birth cohort study designed to

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examine the effects of allergen exposure in early life on the development of asthma. The Brigham and Women's Hospital Human Research Committee approved the study. Informed consent was obtained from the parents for blood collection and longitudinal follow-up. Mothers in the greater Boston metropolitan area delivered at a large Boston hospital were screened with the following questions: (1) Have you ever had asthma, hay fever, or allergies? (2) Has the biologic father of your child ever had asthma, hay fever, or allergies? Mothers responding yes to either question were asked to complete a screening questionnaire. One month after birth, the mothers were contacted by telephone and asked to respond to a second questionnaire. Families were not approached if the index child was premature, had a major congenital anomaly, or was in the neonatal intensive care unit or if the mother was less than 18 years old or could not speak English or Spanish. Every 2 months until 2 years of age, an additional telephone questionnaire was administered to the primary care provider. Information was obtained regarding respiratory and allergic symptoms and diagnoses in the child and various home characteristics. After age 2 years, a health questionnaire was administered every 6 months.

Allergen-induced cytokine measurements

From the 505 children, 112 were selected for isolation of PBMCs and measurement of cytokine responses to stimulation with allergens and the mitogen PHA. As described previously,⁹ selection of these 112 patients was based on the home allergen levels measured during the age 2- to 3-month home visit. The goal was for the subset of children to have had a wide range of early infant exposure to the allergens with which their mononuclear cells were stimulated at 2 to 3 years of age. At approximately 2 years of age (range, 18-32 months), blood was drawn for this study.

By means of Ficoll-Hypaque centrifugation, PBMCs were isolated as previously described.¹⁰ PBMCs were immediately processed and not cryopreserved. 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: Der f 1 (dust mite, 30 μ g/mL), Bla g 2 (cockroach, 30 μ g/mL; Martin Chapman, Charlottesville, Va), and Fel d 1 (cat, 1000 U/mL). Optimal concentrations of the allergens were determined in a dose-response analysis (data not shown). Supernatants were harvested at 24 and 60 hours after allergen stimulation. On the basis of the optimization of the detection of cytokine levels, IL-10 and TNF- α secretion was measured in the 24-hour sample, and IFN- γ and IL-13 secretion was measured in the 60-hour sample. The lower limits of detection for each of the measured cytokines were as follows: IL-10, less than 3 pg/mL; TNF- α , less than 5 pg/mL; IFN- γ , less than 2 pg/mL; and IL-13, less than 7 pg/mL. The ELISA was performed according to the manufacturer's instructions (Endogen). Supernatant quantities were limited, and IFN- γ levels were measured as the first priority. Consequently, fewer children had measurements for IL-10, IL-13, and TNF- α . The cytokine values presented represent net release in that we did not subtract media levels. Complete blood cell counts and eosinophil counts were obtained by the Clinical Laboratories, Brigham and Women's Hospital.

IgE analysis

Total and allergen-specific IgE measurements were obtained from serum samples by using the UNICAP System (Pharmacia). Specific IgE antibodies were assayed for mite, cat, cockroach, and egg white, with each allergen attached to a cellulose sponge (ImmunoCAP). Under the conditions of allergen access, the addition of 0.05 mL of the subject serum allows specific IgE antibodies in the serum to bind to the allergen. After washing, the bound IgE is detected with purified antibody to IgE conjugated to a fluorescent

TABLE I. Population characteristics

Characteristic	N (%)
Sex	
Male	68 (60.7)
Female	44 (39.3)
Atopic disease	
No	58 (51.8)
Yes (total)	54 (48.2)
Eczema*	48
Itchy rash $\geq$ 6 mo†	10
Pollen allergy*	1
Hay fever–allergic rhinitis*	6
Atopic disease, no; repeated wheeze, no	41 (36.6)
Atopic disease, no; repeated wheeze, yes	17 (15.2)
Atopic disease, yes; repeated wheeze, no	39 (34.8)
Atopic disease, yes; repeated wheeze, yes	15 (13.4)

*Physician- or nurse-diagnosed disease: of the 7 children with physician-diagnosed hay fever or pollen allergy, 4 did not have eczema or itchy rash for 6 months or greater.

†Report of an itchy rash that had come and gone but did not completely resolve for at least 6 months and that occurred in the past 12 months.

enzyme label. This allows the specific IgE to be quantified. Total IgE measurement involves the same procedure using a sponge coated with antibody to IgE. The results are given as international units of IgE. An individual was considered to have increased IgE if he or she had one or more specific allergen sensitizations (defined as a level >0.35 IU/mL) or had a total IgE level of 60 IU/mL or greater. Total IgE levels of 60 IU/mL or greater represent levels greater than the 90th percentile of all measurements.

Definitions

Atopic disease was defined as a positive questionnaire response to any of the following: (1) a report of a physician or nurse's diagnosis of eczema, hay fever, or allergic rhinitis by 2 years of age; (2) a report of a physician or nurse's diagnosis of an inhalant allergy (dust mite, cockroach, pollen, animals, or mold); or (3) a report of an itchy rash that had come and gone but did not completely resolve for at least 6 months and that occurred in the past 12 months (validated question for eczema used in the International Study for Asthma and Allergy in Childhood).¹¹ Children were divided into 2 groups (ie, those with [$n = 54$] and without [$n = 58$] atopic disease) to assess whether cytokine production differed by atopic disease history (Table I). Repeated wheeze was defined as 2 or more reports of wheeze in the first 2 years of life. In the Tucson birth cohort study the combination of atopic disease (eczema or allergic rhinitis) and repeated wheeze is a stronger predictor of asthma than atopic disease or wheeze alone.¹² Children were divided into 4 groups to assess whether cytokine production differed on the basis of both atopic disease and repeated wheeze history: (1) no atopic disease, no repeated wheeze ($n = 41$); (2) no atopic disease, repeated wheeze ($n = 17$); (3) atopic disease, no repeated wheeze ($n = 39$); and (4) atopic disease, repeated wheeze ($n = 15$). Eosinophil count was dichotomized into 80th percentile or greater versus less than the 80th percentile. *Increased IgE* was defined as having specific IgE antibody levels of 0.35 U/mL or greater to dust mite, cockroach, cat, or ovalbumin or having a total IgE level of 60 U/mL or greater.¹³

Statistical analysis

The distribution of cytokine levels was statistically skewed, with a significant number of nondetectable values. Therefore the median, rather than the mean, cytokine levels were compared for different groups of children classified on the basis of atopic disease, repeat-

TABLE II. Cytokine levels in response to PBMC stimulation by allergens and mitogen

Cytokine stimulant*	Median cytokine level (pg/mL)			Median cytokine level (pg/mL)		
	No atopic disease (n = 58)	Atopic disease† (n = 54)	Wilcoxon‡ P value	No IgE increase (n = 95)	IgE increase† (n = 16)	Wilcoxon P value
IFN- γ						
Der f 1	194.6 (n = 54)	28.6 (n = 50)	.005	86.2 (n = 89)	35.6 (n = 14)	.57
Bla g 2	94.0 (n = 49)	44.6 (n = 49)	.03	83.3 (n = 85)	16.5 (n = 12)	.03
Fel d 1	53.5 (n = 49)	53.0 (n = 49)	.67	55.8 (n = 85)	32.1 (n = 12)	.34
PHA	537.2 (n = 54)	480.2 (n = 50)	.80	531.9 (n = 89)	320.4 (n = 14)	.22
IL-13						
Der f 1	19.7 (n = 43)	11.0 (n = 35)	.49	12.6 (n = 67)	46.6 (n = 10)	.006
Bla g 2	2.5 (n = 43)	5.1 (n = 35)	.49	3.4 (n = 67)	5.1 (n = 10)	.43
Fel d 1	0.2 (n = 43)	6.2 (n = 35)	.09	0.24 (n = 67)	34.9 (n = 10)	.005
PHA	1710.0 (n = 43)	1509.0 (n = 35)	.74	1601.0 (n = 67)	1751.0 (n = 10)	.93
IL-10						
Der f 1	76.6 (n = 45)	62.2 (n = 39)	.26	68.1 (n = 73)	91.1 (n = 10)	.32
Bla g 2	315.4 (n = 40)	187.3 (n = 38)	.04	237.8 (n = 69)	412.9 (n = 8)	.35
Fel d 1	231.3 (n = 40)	134.2 (n = 38)	.35	153.5 (n = 69)	256.4 (n = 8)	.21
PHA	171.8 (n = 45)	130.0 (n = 39)	.42	143.0 (n = 73)	186.2 (n = 10)	.35
TNF- α						
Der f 1	664.5 (n = 29)	875.4 (n = 27)	.21	701.3 (n = 50)	1057.0 (n = 6)	.22
Bla g 2	830.1 (n = 29)	899.4 (n = 27)	.42	826.4 (n = 50)	1162.0 (n = 6)	.16
Fel d 1	596.0 (n = 29)	519.0 (n = 27)	.58	523.1 (n = 50)	780.0 (n = 6)	.22
PHA	851.3 (n = 29)	859.5 (n = 27)	.94	851.1 (n = 50)	957.1 (n = 6)	.54

*Allergens used to stimulate PBMCs included Der f 1 (dust mite), Bla g 2, (cockroach), and Fel d 1 (cat). The nonspecific mitogen used was PHA.

†Atopic disease was defined as a positive questionnaire response to any of the following: (1) a report of a physician or nurse's diagnosis of eczema, hay fever, or allergic rhinitis by 2 years of age; (2) a report of a physician or nurse's diagnosis of an inhalant allergy (dust mite, cockroach, pollen, animals, or mold); or (3) report of an itchy rash that had come and gone but did not completely resolve for at least 6 months and that occurred in the past 12 months. IgE increase is defined as having specific IgE antibody levels of greater than 35 U/mL to dust mite, cockroach, cat, or ovalbumin or having a total IgE level of 60 U/mL or greater.

‡The Hodges-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 2 groups.

ed wheeze experience, or both. The statistical significance of comparisons of differences in cytokine levels between groups was determined by using 2-sample Wilcoxon tests and by estimating CIs.¹⁴ These methods are based on the ranks of the pairwise differences between the 2 samples and do not require distributional assumptions for their validity. The *P* values and interval limits were computed exactly by using the package exactRankTests in the R language (The Comprehensive R Archive Network, 2001). By using this package to estimate the median difference in cytokine levels between groups, the Hodges-Lehmann method was used to estimate the median cytokine level for each group of children and to estimate the median (95% CI) 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 2 different groups (eg, those with vs without allergic disease) calculated as the median of the pairwise differences between the values in each group. On the basis of literature suggesting that allergic disease or asthma might be predicted by IFN- γ production that was decreased relative to the production of pro-inflammatory T_H2 cytokines, the ratio of IL-10, IL-13, and TNF- α secretion relative to IFN- γ production was also calculated.¹⁵

RESULTS

Population characteristics

Population characteristics have been described previously.⁹ The children in our cohort with cytokine measurements did not differ significantly from the children without cytokine measurements (results not shown). Of the

112 children with cytokine measurements, 54 had atopic disease, and 58 did not (Table I). Of those with atopic disease, 48 of 54 had physician- or nurse-diagnosed eczema, 3 had physician- or nurse-diagnosed hay fever without eczema, 1 had physician- or nurse-diagnosed pollen allergy without hay fever, and 2 had an itchy rash that did not completely resolve over at least a 6-month period without a physician or nurse's diagnosis of eczema. Compared with the children without atopic disease, the proportion of children with atopic disease who had increased IgE was higher but not significantly so (odds ratio, 1.93; 95% CI, 0.65-5.74). Of the 16 children with increased IgE, 10 had atopic disease (eczema or hay fever). Absolute eosinophil counts tended to be higher in those with atopic disease. Of those with atopic disease, 33% had an eosinophil count in the 80th percentile or greater compared with 13% in the group without atopic disease (*P* = .03).

Atopic disease, repeated wheeze, and cytokine levels

Displayed in Fig 1 are the distributions of cytokine measurements obtained after mitogen and allergen-specific stimulation in PBMCs for all subjects. The distributions are mostly single tailed. We did not find that sex, number of siblings, use of daycare, or family history were confounders of the relationship of cytokines to atopic disease, wheeze, or both in this subset of children.

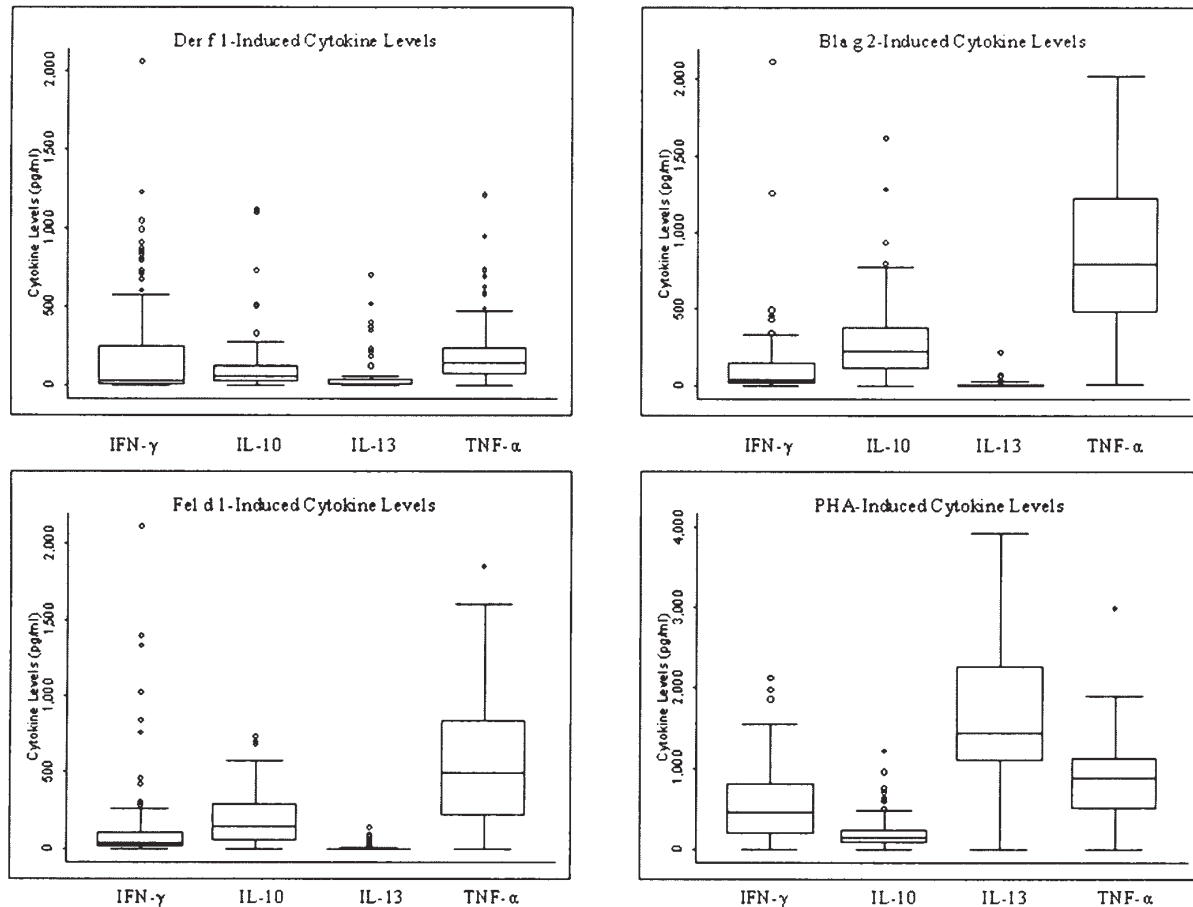


FIG 1. Production of IFN- γ , IL-10, IL-13, and TNF- α cytokines in PBMCs after stimulation with allergen (Der f 1, Bla g 2, and Fel d 1) and mitogen (PHA). PBMCs were obtained from blood samples collected at approximately 2 years of age (range, 18-32 months). IL-10 and TNF- α secretion was measured in the 24-hour sample, and IFN- γ and IL-13 secretion was measured in the 60-hour sample. The median is represented by the black bar. The upper and lower bounds of the box represent the 25th to 75th percentiles of the data, respectively. Observations less than 1.5 times the height of the box beyond either quartile are displayed within whiskers. o, Outliers.

Compared with children without atopic disease (Table II), children with atopic disease had lower Der f 1 ($P = .005$) and Bla g 2 ($P = .03$) allergen-induced IFN- γ levels. Children with atopic disease also had lower Bla g 2-induced IL-10 ($P = .04$) and higher Fel d 1-induced IL-13 ($P = .09$) levels. In the presence of the mitogen PHA or media alone, IFN- γ , IL-10, IL-13, and TNF- α levels were similar between children with and without atopic disease. In general, differences between the median values were larger than the estimated median of the pairwise differences between the observations from the atopic versus the nonatopic groups. For example, as shown in Table II, for Der f 1-induced IFN- γ levels, the difference between 194.6 and 28.6 pg/mL (166 pg/mL) was larger than the estimated median of the pairwise differences (18.7 pg/mL; 95% CI, 2.2-73.0). When the 4 children with only hay fever or pollen allergy and no eczema were excluded from the analyses, the magnitude and the significance of the differences between the atopic and nonatopic groups did not change.

Compared with children with neither atopic disease nor repeated wheeze ($n = 41$), children with both atopic disease and repeated wheeze ($n = 15$) had lower levels of allergen-induced IFN- γ ($P = .01$ for Der f 1 and $P = .02$ for Bla g 2) cytokine levels (Fig 2). The signal for reduced IFN- γ levels in response to Der f 1 stimulation was present but weaker for those with atopic disease and no wheeze compared with that seen in the group with no atopic disease or repeated wheeze.

As well as an absolute reduction in IFN- γ levels, the data also suggested a relative reduction in IFN- γ levels for those with atopic disease compared with levels in those with no atopic disease and in those with atopic disease or repeated wheeze compared with that seen in those with no atopic disease or repeated wheeze. The median IL-13/IFN- γ , IL-10/IFN- γ , and TNF- α /IFN- γ ratios for Der f 1, Bla g 2, and Fel d 1 tended to be higher among those with atopic disease (data not shown). For example, the median Bla g 2-induced TNF- α /IFN- γ ratio was 412.6 for children with atopic disease and 28.4 for chil-

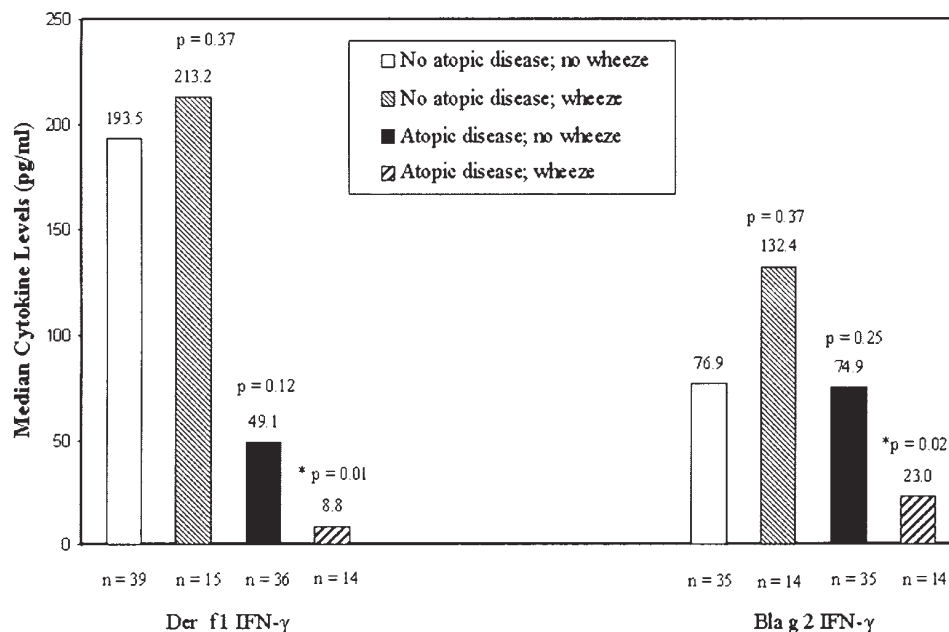


FIG 2. Comparison of dust mite (Der f 1)-induced and cockroach (Bla g 2)-induced IFN- γ levels among 4 different groups of children. The reference group is children with neither atopic disease nor repeated wheeze. Compared with children with neither atopic disease nor repeated wheeze, children with both atopic disease and repeated wheeze had lower median levels of Der f 1-induced IFN- γ ($P = .01$). Children with atopic disease but no repeated wheeze had marginally higher Der f 1-induced IFN- γ production than those with neither atopic disease nor repeated wheeze. Compared with children with neither atopic disease nor repeated wheeze, children with both atopic disease and repeated wheeze had lower median levels of Bla g 2-induced IFN- γ production ($P = .02$).

dren with no atopic disease, suggesting lower IFN- γ production in relation to TNF- α production for the atopic group ($P = .04$, Wilcoxon). In response to Der f 1, median cytokine ratios for the group with atopy, repeated wheeze, or both versus those in the group with no atopy or repeated wheeze were 3022 versus 10 ($P = .07$) for IL-10/IFN- γ , 48 versus 0.8 ($P = .03$) for IL-13/IFN- γ , and 171,800 versus 49.3 ($P = .03$) for TNF- α /IFN- γ .

IgE and cytokine levels

Compared with children without increased IgE ($n = 95$), those with increased IgE ($n = 16$) had higher Der f 1-induced ($P = .006$) and Fel d 1-induced ($P = .005$) IL-13 levels and lower Bla g 2-induced ($P = .03$) IFN- γ levels.

DISCUSSION

The strongest consistent evidence in infancy for prediction of future atopic disease is not an excess secretion of T_H2 cytokines but a deficiency, specifically in secretion of IFN- γ .⁴⁻⁸ In this study, at a median of 2 years of age, the levels of allergen-induced (Der f 1 and Bla g 2) IFN- γ secretion were significantly lower in children with atopic disease compared with levels in those without atopic disease. The data also suggested a qualitative difference in cytokine patterns between the children with atopic disease and the smaller group of children with increased IgE. Although there was overlap between the 2 groups, significantly decreased IFN-

γ production was more consistently present for the children with atopic disease, whereas significantly increased IL-13 production was more consistently present for the children with increased IgE.

Our data demonstrating an absolute and relative deficiency of allergen-induced IFN- γ in 2- to 3-year-old children with atopic disease with or without repeated wheeze is consistent with those of prior studies.⁴⁻⁸ Children at risk for asthma produced less IFN- γ in response to viral respiratory infections.¹⁶ In children with persistent wheeze, house dust mite-induced IFN- γ secretion in PBMCs was decreased relative to that seen in those in whom atopic disease did not develop.⁵ Our data indicate that both cockroach- and house dust mite-induced IFN- γ secretion is decreased in atopic subjects. In contrast, Fel d 1 stimulation did not result in decreased IFN- γ secretion in atopic subjects. Whether the different cytokine responses elicited by distinct allergens are related to different exposures or immune mechanisms is not well defined. Together, our data indicate that underproduction of IFN- γ secretion to some, but not all, allergens is associated with atopic disease. Interestingly, compared with children with neither atopic disease nor repeated wheeze, levels of allergen-induced IFN- γ were more consistently low for children with both atopic disease and repeated wheeze than for children with only atopic disease and no repeated wheeze. Thus our study supports the notion that decreased allergen-induced IFN- γ secretion is associated not only with atopic disease but perhaps more strongly

with atopic disease in the presence of airways disease. Thus the early life airway and nonairway inflammation associated with these phenotypes might result, in part, through a relative decrease in production of a very specific T_H1 cytokine, IFN- γ .

Expression of IgE in early childhood might result from an increased secretion of IL-13, which can mediate isotype switching to IgE,^{17,18} as well as from relative decreased production of IFN- γ . Prescott et al¹⁹ found that by age 2 years, the subjects who they had defined as atopic had increased allergen-specific IL-13 levels, but their definition of atopic subjects included children with atopic eczema diagnosed by a pediatric allergist or diagnosed on the basis of a positive skin prick test response or a positive RAST test result.

Interpretation of the data presented in this study should include a consideration of the potential limitations. Because we relied on parental report of physician- or nurse-diagnosed disease, there might be diagnostic misclassification of children with eczema, hay fever, or wheeze. The weak relationship between IgE and eczema, hay fever, or both might have resulted, in part, from the absence of IgE data regarding other food and pollen allergy. This is a study of a susceptible population because at least one parent had a history of atopic disease, limiting the generalizability of these results to a general population. However, this specific population is of interest because it is the most vulnerable to development of atopic disease and asthma. As with all analyses of PBMCs, the data presented might not represent findings in the lung. However, we have previously shown that PBMCs can be used to characterize lymphocyte phenotypes associated with asthma exacerbation or after in vivo allergen challenge.²⁰

We present an analysis of the allergen-induced cytokine response in PBMCs from 2-year-old children at risk for asthma or allergy. In this study the onset of atopic disease is associated with decreased dust mite- and cockroach-induced IFN- γ secretion. Increased IgE is associated with increased dust mite- and cat-induced IL-13, as well as with decreased cockroach-induced IFN- γ .

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