

Original article

Association of IL-5 cytokine production and *in vivo* IgE levels in infants and parents

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Background: Total IgE in human subjects tracks strongly from birth onward through unknown mechanisms. Regulation of IgE might occur in relation to adaptive immune cytokine production. *In vitro* studies have assessed the role of individual cytokines in regulating IgE production in human subjects.

Objective: We sought to investigate the association between IgE levels *in vivo* and the capacity of the individuals to produce adaptive immune cytokines.

Methods: Blood samples from participants in the Tucson Infant Immune Study (children at birth and at 3 and 12 months of age, fathers, and mothers before and after delivery) were assessed for percentage of eosinophils and plasma total IgE levels.

IFN- γ , IL-4, IL-5, IL-13, and IL-10 levels were measured in supernatants of mitogen-stimulated PBMCs and examined cross-sectionally for relation to cytokine production by using simple regression, multiple regression with cytokines only and with other known predictors of IgE levels, and longitudinally by means of random effects modeling.

Results: After adjusting for eosinophils and other predictors, IL-5 production (but not that of other cytokines) was associated directly with total IgE levels in children at 3 months ($P = .009$) and 12 months ($P = .011$) of age but not at birth. The IL-5/IgE association was present also in fathers ($P = .040$) and in mothers, both during pregnancy ($P < .001$) and after delivery ($P = .030$).

Conclusions: This study indicates that mitogen-stimulated IL-5 production is associated with *in vivo* total IgE levels, independent of the production of other cytokines and circulating eosinophils.

Clinical implications: Understanding the regulation of IgE *in vivo* might help elucidate the development of allergic responses in individuals. (J Allergy Clin Immunol 2007;119:1016-1023.)

Key words: IgE, cytokine, eosinophils, allergic diseases

Abbreviation used

IIS: Infant Immune Study

IgE antibody levels in serum or plasma are detectable in nearly all human subjects but are strongly increased in relation to a variety of diseases, including allergic diseases, such as asthma¹ and atopic dermatitis,² and many parasitic diseases.³ Much effort has been put into unraveling the factors involved in regulating IgE antibody levels. There is clear evidence that total IgE is under a strong genetic influence,^{4,5} and epidemiologic studies have demonstrated that IgE levels in human subjects show a strong tracking tendency, even from birth,⁶⁻⁸ although birth levels are not strongly predictive of subsequent allergic disease.⁹ It therefore seems that maintaining *in vivo* homeostasis of IgE levels represents a highly regulated process, the mechanisms of which still remain to be fully elucidated. Many different factors, both genetic and environmental, are likely to be involved in a complex network of interactions.

In vitro studies have focused on the role of cytokines in the molecular events that lead to IgE synthesis. These studies have highlighted the roles of T_H2 cytokines, such as IL-4, IL-13, and IL-5, in inducing B-cell isotype switching to IgE (reviewed by Gould et al¹⁰). Few studies, however, have investigated the association between IgE levels *in vivo* and cytokine production in the population at large, most having focused on allergic subjects, on cytokine responses to specific allergens, or both.

The aim of our study was to evaluate the *in vivo* association between mitogen-induced cytokine production from blood mononuclear cells and total serum IgE levels in infants at different ages and in their parents in a birth cohort study in Tucson, Arizona.

METHODS

Study population

The subjects included in this study are members of families participating in the Infant Immune Study (IIS). Begun in 1997, the IIS is an ongoing birth cohort study aimed at investigating immune system maturation in relation to the development of allergic disease in childhood. Pregnant women who planned to seek health care from collaborating physicians were enrolled at 32 to 35 weeks of gestation.

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The study was approved by the University of Arizona Institutional Review Board. Informed consent was obtained from the mothers at enrollment.

A total of 94.4% (458/485) of the children enrolled in the IIS study had IgE data available at least at 1 point in time (birth and 3 and 12 months) and were therefore included in our analyses. Data available in the IIS dataset were analyzed retrospectively for this study. No differences in sex, ethnicity, parental asthma or allergic rhinitis, or parental education were observed between children included in this study and those for whom no IgE data were available.

Questionnaires

Information regarding parental demographic characteristics, parental history of respiratory diseases, and parental smoking was collected from questionnaires completed by parents at enrollment, before delivery, and when the child was 3 months old, when the breast-feeding status of the child was also assessed. Parents were considered to have asthma, allergic rhinitis, or both if at the time of enrollment they reported ever being given a physician's diagnosis of asthma, allergic rhinitis, or both. Because almost all children (92%) in the cohort were breast-fed at least for some time, children were dichotomized based on whether they were breast-fed for more or less than 3 months. Parents were asked whether their child had a physician diagnosis of eczema by questionnaire when the child was 12 months old.

IgE, eosinophils, and cytokines

In children, blood was collected at birth (from the umbilical cord), at 3 months of age (2.8 ± 1.2 months [mean $\pm$ SD]), and at 12 months of age (12.9 ± 1.6 months). Blood specimens were drawn from the mothers during pregnancy (approximately 2 weeks before delivery) and again when the child was 3 months old, at which time blood samples were also obtained from fathers.

Total serum IgE levels were assayed fluorometrically with the Pharmacia AutoCAP System (Pharmacia Diagnostics, Kalamazoo, Mich). The percentage of eosinophils was obtained from a differential count of white blood cells.

Cord blood mononuclear cells or PBMCs were separated by means of standard density gradient centrifugation and then stimulated with 10 μ g/mL concanavalin A and 10 ng/mL phorbol myristate acetate (Sigma Chemical Co, St Louis, Mo) for 18 to 24 hours. Levels of IFN- γ , IL-4, IL-5, IL-13, and IL-10 were assayed in the concanavalin A/phorbol myristate acetate-stimulated cell-culture supernatants with quantitative colorimetric sandwich ELISA kits (R&D Systems, Inc, Minneapolis, Minn, for IFN- γ , IL-4, IL-5, and IL-10; Diaclone Research, Cell Sciences, Inc, Norwood, Mass, for IL-13). The thresholds of detection for the assays were as follows: IFN- γ , 15.6 pg/mL; IL-4, 0.25 pg/mL; IL-5, 7.8 pg/mL; IL-13, 3.12 pg/mL; and IL-10, 7.8 pg/mL.

Statistical methods

A step approach was applied to each population group (ie, children, mothers, and fathers) to study the independent association between cytokine production by PBMCs and total serum IgE levels *in vivo*. In each step all subjects who had the required data were included in the analysis. First, a marginal analysis was performed, in which each cytokine was tested individually against the corresponding IgE levels. Those cytokines that showed an association with total IgE (at the level of $P < .10$) in marginal analysis were then included in a stepwise multifactorial model to test the independent effect of each cytokine (cytokine-only analysis).

As an additional step, those cytokines that were still significant in the cytokine-only multifactorial analysis ($P < .10$) were included in a final stepwise model with other possible predictors of IgE levels to adjust for their potential confounding effect.

Random effects models were used to develop longitudinal multivariate models with total IgE as the dependent variable to adjust for the serial correlation within subjects with multiple observations (ie, mothers and infants).

The following predictors of IgE levels were tested in the population groups. For the child at all 3 ages (birth and 3 and 12 months), predictors included maternal and paternal IgE, parental history of asthma, parental history of allergic rhinitis, sex, and ethnicity. For cord blood, additional predictors included gestational age and maternal smoking during pregnancy. For 3 and 12 months, additional predictors included age, breast-feeding status, and parental current smoking status. For the mothers, predictors included age, ethnicity, smoking (ever), eosinophils, asthma, allergic rhinitis, and breast-feeding status. For the fathers, predictors included age, ethnicity, smoking (ever), eosinophils, asthma, and allergic rhinitis.

In children at 3 and 12 months of age, in mothers, and in fathers, simple and multiple linear regression was used to assess the association between individual predictors (cytokines and covariates) and total serum IgE levels. Because of the high proportion of undetectable IgE values in cord blood, censored (Tobit) regression was used in the analysis in children at birth. Tobit regression is an efficient method for estimating the relationship between an explanatory variable and a truncated or censored continuous dependent variable because it estimates the probability of a dependent variable being at or less than a limit.¹¹

Log-transformed total IgE values showed the best approximation to normal distribution and were therefore used in the analyses. Cytokine levels were categorized into tertiles and used as ordinal variables in the analyses in mothers (prenatal and postpartum), in fathers, and in children at 12 months of age. In children at birth and at 3 months of age, however, the high proportion of undetectable values (>50%) for some of the cytokines made the comparison of the upper quartile versus the lower 3 quartiles combined the only consistent categorization possible for all cytokines tested. For consistency purposes, the latter categorization was also applied to all cytokines included in the random effects model in children.

The 2-sided α level for statistical significance was set at .10 for marginal univariate and multifactorial analyses and at .05 for the final adjusted stepwise regression analyses and the final random effects model.

RESULTS

Total IgE levels increased dramatically during the first year of life from birth (median, 0.10 IU/mL; interquartile range, 0.10-0.35; $n = 380$) to a several-fold increase by 3 months of age (geometric mean, 1.3 IU/mL; 95% CI, 1.1-1.5; $n = 324$) to an almost 7-fold increase by 12 months of age (geometric mean, 9.3 IU/mL; 95% CI, 7.9-10.9; $n = 264$). Children with IgE data at birth only did not differ from those with IgE data at 12 months of age in the prevalence of eczema at 12 months (data not shown). The proportion of undetectable (<0.24 IU/mL) IgE values was 58.2% (221/380) in cord blood and 2.2% (7/324) at 3 months, whereas all IgE values were detectable above the minimum threshold level by the age of 12 months.

Total IgE levels were measured in 434 mothers during pregnancy (geometric mean, 26.1 IU/mL; 95% CI, 22.4-30.2) and in 348 fathers (geometric mean, 50.1 IU/mL; 95% CI, 43.0-58.4). Here also, participants did not differ in prevalence of allergic disease based on availability of cytokine and IgE data. IgE levels in parents did not differ

TABLE I. Association between cytokine production and total serum IgE levels in the child at birth and at 3 and 12 months (regression analysis)

	Univariate analysis		Multifactorial analysis*	
	Regression coefficient (95% CI)	P value	Regression coefficient (95% CI)	P value
Cord blood cytokines†				
IFN- γ	−0.066 (−0.119 to 0.021)	.085	†	†
IL-4	−0.068 (−0.234 to 0.099)	.4	—	—
IL-5	−0.249 (−0.426 to −0.072)	.006	−0.252 (−0.434 to −0.069)	.007
IL-13	−0.120 (−0.316 to 0.075)	.3	—	—
IL-10	0.109 (−0.054 to 0.273)	.2	—	—
3-mo cytokines‡				
IFN- γ	−0.008 (−0.148 to 0.131)	.9	—	—
IL-4	0.157 (0.021 to 0.292)	.023	0.115 (−0.036 to 0.193)	.060
IL-5	0.172 (0.134 to 0.410)	<.001	0.145 (0.085 to 0.306)	.003
IL-13	0.117 (−0.026 to 0.261)	.2	—	—
IL-10	0.116 (−0.021 to 0.252)	.2	—	—
12-mo cytokines§				
IFN- γ	−0.033 (−0.124 to 0.056)	.5	—	—
IL-4	0.118 (0.030 to 0.207)	.009	†	†
IL-5	0.130 (0.043 to 0.216)	.003	0.253 (0.109 to 0.396)	.060
IL-13	0.034 (−0.056 to 0.123)	.5	—	—
IL-10	−0.030 (−0.118 to 0.057)	.5	—	—

*N = 271 for children at birth, n = 283 for children at 3 months, and n = 215 for children at 12 months.

†Variable was excluded from the model ($P > .1$).

‡For these variables, 2 groups (upper quartile vs lower 3 quartiles combined) were compared.

§For these variables, tertiles were used as ordinal variables.

by the time of year at which blood was drawn (data not shown).

The association between total IgE levels in children and their parents was tested in a simple regression model. Maternal total IgE levels (measured both prepartum and postpartum) were positively associated with children's IgE levels at birth (regression coefficient, 0.158 [$P = .004$] and regression coefficient, 0.225 [$P < .001$], during pregnancy and postpartum, respectively) to children's IgE levels at 3 months (regression coefficient, 0.098 [$P = .032$] and regression coefficient, 0.115 [$P = .019$], respectively) and 12 months (regression coefficient, 0.159 [$P = .003$] and regression coefficient, 0.192 [$P = .001$], respectively). Paternal IgE levels were positively associated with total IgE levels in children at 12 months (regression coefficient, 0.140 [$P = .025$]) and tended to be associated with IgE in cord blood (regression coefficient, 0.131 [$P = .06$]), whereas no association was present between paternal IgE levels and IgE levels measured in children at 3 months.

Cytokine production by mitogen-stimulated cord blood mononuclear cells and PBMCs was assessed to determine whether the capacity to produce cytokines by T_H1 , T_H2 , or both contributed to the regulation of IgE levels (see Table E1 in this article's Online Repository at www.jacionline.org).

Relations of cytokine production to total IgE levels in infants

Table I shows the association between cytokine production and IgE levels for children at 3 ages. At birth, although IFN- γ and IL-5 production showed inverse associations to IgE levels when tested in univariate marginal

analyses, only IL-5 maintained a significant association in a multifactorial model. Even this latter association was no longer evident after adjusting for other potential predictors of IgE levels, leaving parental IgE levels as the only significant predictors of IgE levels in cord blood (Table II).

In children at 3 months of age, IL-4 and IL-5 production were directly associated with IgE levels in univariate analyses, and when they were included in a multifactorial analysis, both associations remained significant (Table I). With further adjusting, IL-5 was the only cytokine to be retained as related to IgE level, together with the child's ethnicity and age, and maternal IgE levels (Table II).

In children at 12 months of age, IL-4 and IL-5 were significantly associated with IgE levels in marginal univariate analyses, whereas only IL-5 remained significant when tested in a multifactorial analysis (Table I). Thus at 12 months also, in the final model IL-5 was the only cytokine that remained associated with the child's IgE level, together with maternal and paternal IgE levels (Table II).

Relation of cytokine production to IgE levels in parents

In mothers during pregnancy and postpartum, IL-4, IL-5, and IL-13 were marginally associated with total serum IgE levels, but only IL-5 maintained significance in the multifactorial model (Table III). After adjustment, IL-5 production remained associated with IgE levels, together with ethnicity and a positive history of asthma and allergic rhinitis during pregnancy and together with eosinophils and a positive history of asthma during the postpartum period (Table IV).

TABLE II. Stepwise multifactorial regression models for predicting children's total serum IgE levels at birth and at 3 and 12 months of age

Variables included	Regression coefficient (95% CI)	P value
Child's IgE in cord blood† (n = 198)		
IL-5	*	*
Maternal IgE	0.201 (0.061-0.341)	.005
Paternal IgE	0.156 (0.010-0.303)	.037
Child's IgE at 3 mo‡ (n = 170)		
IL-5	0.101 (0.025-0.176)	.009
Ethnicity		
Hispanic	0.205 (0.048-0.361)	.010
Other	0.213 (-0.008-0.434)	.068
Maternal IgE	0.159 (0.056-0.263)	.003
Age of the child	1.41 (0.656-2.163)	<.001
Child's IgE at 12 mo§ (n = 135)		
IL-5	0.144 (0.034-0.254)	.011
Maternal IgE	0.193 (0.038-0.347)	.015
Paternal IgE	0.162 (0.027-0.297)	.019

*Variable was excluded from the model ($P > .05$).

†The following variables were tested in the model: IL-5, maternal IgE, paternal IgE, parental history of medically diagnosed asthma, parental history of physician-diagnosed allergic rhinitis, gestational age, sex, ethnicity, and smoking during pregnancy.

‡The following variables were tested in the model: IL-4, IL-5, eosinophils, maternal IgE, paternal IgE, parental history of physician-diagnosed asthma, parental history of physician-diagnosed allergic rhinitis, age, ethnicity, breast-feeding status, and exposure to parental smoking.

§The following variables were tested in the model: IL-5, eosinophils, maternal IgE, paternal IgE, parental history of physician-diagnosed asthma, parental history of physician-diagnosed allergic rhinitis, sex, ethnicity, breast-feeding status, and exposure to parental smoking.

||Non-Hispanic white was used as the reference group.

In fathers IL-5 was the only cytokine to show a significant individual association with total IgE level (Table III), a result that was confirmed after adjusting for other potential determinants of IgE levels. Other significant predictors included in the final model were percentage of eosinophils, paternal allergic rhinitis, and ethnicity (Table IV).

Finally, longitudinal random effects models were created for both children (including data at 3 and 12 months of age) and mothers (including data from prepartum and postpartum) to adjust for possible correlations between repeated measures in both dependent and independent variables. IL-5 production was confirmed to be associated with total IgE levels both in children and mothers (Table V).

DISCUSSION

Data from this nonselected birth cohort showed that in a general population sample, the production of IL-5 by mitogen-stimulated peripheral blood cells is significantly associated with total serum IgE levels both in children and in adults. Our study also confirms previous findings

indicating that maternal IgE levels are associated with IgE levels in cord blood^{9,12-15} but extends this finding to IgE levels at 3 and 12 months. In contrast, paternal IgE levels were significantly associated with IgE levels in the child only at 12 months of age, which suggests that the earlier maternal effect might be at least in part environmental through *in utero* exposures.

IgE levels in human subjects track with an amazingly strong capacity both in adults¹⁶ and in infants right from birth,¹⁷ a phenomenon that likely involves many complex events. Studies in animal models and *in vitro* studies of human cells have identified many regulatory mechanisms of IgE synthesis that might contribute to maintaining IgE levels within the circulation. IL-4 and IL-13 are now regarded as the classical mediators essential to initiating class switch in B cells, and they are produced by the subset of T cells referred to as T_H2 cells. Thus it was rather surprising that we found in a general population sample that total IgE levels are unrelated to production of IL-4 or IL-13 but instead are closely associated with the production of IL-5 both in children and in adults. Moreover, this association, although not evident at birth, seems to develop in the first few months of life and, consequently, appears to involve immune system maturation.

IL-5 is produced by both CD4⁺ and CD8⁺ T cells¹⁸ but is considered a T_H2 cytokine. The broad effects of IL-5 on eosinophils have long been known. IL-5 has been shown to induce eosinophil differentiation,^{19,20} adhesion,²¹ and chemotaxis,²² as well as activation^{23,24} and enhanced survival.²⁵ Although the role of IL-5 in allergic reactions has traditionally been linked to its capacity to induce and maintain eosinophilia, a key pathologic feature of allergic diseases, the concept that IL-5 might also be involved in IgE regulation is not new. In the late 1980s, IL-5 was shown to enhance IL-4-induced IgE production both in murine and in normal human B cells,²⁶ and Purkerson and Isakson²⁷ reported that IL-5 provided a signal required in addition to IL-4 for isotype switching to IgG1 and IgE in mice.

In this regard it is of interest to take some additional observations into account. First, the possibility of a differential regulation of IL-4, IL-13, and IL-5 has been reported. Although these T_H2 cytokines are often regarded as being expressed together, their expression can also be selective, with responses characterized, for example, by IL-4 without IL-5 and IL-5 without IL-4.²⁸ Indeed, recent studies have shown that the transcription factor GATA-3, which is present in T cells, binds to the promoter region of the *IL5* gene in contrast to the *IL4* or *IL13* gene.^{29,30} Moreover, in a recent report by Moss et al,³¹ the authors observed that in Zambian children with measles, IL-5 was regulated differently from IL-4 and IL-13: serum levels were low in affected children compared with those in control children, and total IgE levels were lower in children with measles, despite the high levels of IL-4 and IL-13.

Second, a recent study by Yang et al³² showed that IL-5 cooperates in the lung with eotaxin-2 to regulate IL-13 production. Such observations, together with our results, make it plausible to hypothesize that IL-5, even without having an effect in isolation, might synergize with other

TABLE III. Association between cytokine production and total serum IgE levels in parents (regression analysis)

	Univariate analysis		Multifactorial analysis*	
	Regression coefficient	P value	Regression coefficient	P value
Maternal cytokines				
during pregnancy‡				
IFN- γ	-0.038 (-0.134-0.058)	.4	—	—
IL-4	0.105 (-0.011-0.200)	.029	†	†
IL-5	0.242 (0.147-0.336)	<.001	0.227 (0.132 to 0.322)	<.001
IL-13	0.130 (0.035-0.226)	.008	†	†
IL-10	0.028 (-0.068-0.124)	.6	—	—
Maternal cytokines				
postpartum‡				
IFN- γ	-0.022 (-0.124-0.080)	.7	—	—
IL-4	0.109 (0.010-0.208)	.032	†	†
IL-5	0.125 (0.022-0.227)	.017	0.101 (-0.014 to 0.217)	.080
IL-13	0.076 (-0.025-0.176)	.14	—	—
IL-10	0.081 (-0.023-0.186)	.13	—	—
Paternal cytokines‡				
IFN- γ	0.032 (-0.077-0.142)	.6	—	—
IL-4	0.078 (-0.033-0.189)	.17	—	—
IL-5	0.157 (0.045-0.269)	.006	0.157 (0.045 to 0.269)	.006
IL-13	0.065 (-0.052-0.182)	.28	—	—
IL-10	0.033 (-0.079-0.145)	.6	—	—

*N = 248 for mothers during pregnancy, n = 204 for mothers 3 months after delivery, and n = 185 for fathers.

†Variable was excluded from the model ($P > .1$).

‡Tertiles were used as ordinal variables for all cytokines.

TABLE IV. Stepwise multivariate regression models for predicting total serum IgE levels in parents

Variables included	Regression coefficient (95% CI)	P value
Maternal IgE during pregnancy* (n = 228)		
IL-5	0.180 (0.081-0.280)	<.001
Ethnicity§		
Hispanic	0.133 (-0.057-0.322)	.169
Other	0.355 (0.069-0.640)	.015
Physician-diagnosed asthma	0.373 (0.165-0.581)	<.001
Physician-diagnosed allergic rhinitis	0.193 (0.015-0.370)	.033
Maternal IgE postpartum† (n = 170)		
IL-5	0.111 (0.009-0.213)	.030
Eosinophils	0.047 (0.005-0.089)	.028
Physician-diagnosed asthma	0.349 (0.142-0.557)	.001
Paternal IgE‡ (n = 166)		
IL-5	0.130 (0.004-0.256)	.040
Eosinophils	0.067 (0.026-0.109)	.002
Physician-diagnosed allergic rhinitis	0.253 (0.030-0.476)	.026
Ethnicity§		
Hispanic	0.200 (-0.040-0.440)	.101
Other	0.403 (0.019-0.787)	.040

*The following variables were tested in the model: IL-5, eosinophils, physician-diagnosed asthma, physician-diagnosed allergic rhinitis, age, ethnicity, and history of smoking.

†The following variables were tested in the model: IL-5, eosinophils, physician-diagnosed asthma, physician-diagnosed allergic rhinitis, age, ethnicity, history of smoking, and breast-feeding status.

‡The following variables were tested in the model: IL-5, eosinophils, physician-diagnosed asthma, physician-diagnosed allergic rhinitis, age, ethnicity, and history of smoking.

§Non-Hispanic white was used as the reference group.

factors *in vivo* to produce either direct effects or to modulate the action of other mediators involved in allergic reactions.

Third, evidence seems to suggest that IL-5 might play an important role in the proliferation and survival of

mature murine B cells,³³ thus acting as a B-cell growth factor,³⁴ and it has been proposed that IL-5 is required for the optimal T cell-dependent activation of specific B-cell subsets in mice.³⁵ It might therefore be possible to hypothesize that IL-5 is more generally involved in

TABLE V. Longitudinal random effects models for predicting total serum IgE levels in children (3 and 12 months) and mothers (prenatal and postpartum)

Variables included	Regression coefficient (95% CI)	P value
Child's IgE*† (n = 222)		
IL-5	0.207 (0.102-0.312)	<.001
Age of the child	0.928 (0.460-1.395)	<.001
Maternal IgE	0.146 (0.055-0.237)	.002
Paternal IgE	0.108 (0.016-0.199)	.021
Maternal IgE‡§ (n = 272)		
IL-5	0.040 (0.012-0.040)	.005
Ethnicity		
Hispanic	0.183 (0.018-0.348)	.029
Other	0.239 (-0.003-0.482)	.053
Physician-diagnosed asthma	0.413 (0.240-0.587)	<.001
Physician-diagnosed allergic rhinitis	0.175 (0.025-0.324)	.022

*For consistency purposes, all cytokines tested in this model were used as binary (upper quartile vs 3 lower tertiles combined; see the Methods section).

†The following variables were tested in the model: IL-4, IL-5, cord blood IL-4, cord blood IL-5, eosinophils, maternal IgE, paternal IgE, parental history of physician-diagnosed asthma, parental history of physician-diagnosed allergic rhinitis, age, sex, ethnicity, breast-feeding status, and exposure to parental smoking.

‡The following variables were tested in the model: IL-5, eosinophils, physician-diagnosed asthma, physician-diagnosed allergic rhinitis, age, ethnicity, history of smoking, and breast-feeding status.

§In this model cytokines were used as tertiles (ordinal variables).

||Non-Hispanic white was used as the reference group.

sustaining a response by B cells, the switching of which to IgE might be primarily determined by other factors.

There are some limitations in our study that need to be considered. Cytokine production was measured as the amount of cytokine produced by mitogen-stimulated PBMCs. The relation of this measurement to *in vivo* production is not known. In our study a panel comprising both T_H1 and T_H2 cytokines was tested. Although they represent some of the main mediators known to be involved in allergic reactions, we cannot exclude the possibility that other unmeasured cytokines might be associated with IL-5 production, with IL-9 being one possible candidate,³⁶ and therefore might mediate the significant association that we observe between IL-5 production and total serum IgE levels. We cannot rule out the possibility that the association between IL-5 and IgE is spurious; that is, high levels might be a manifestation of an underlying process in which both increased IL-5 and IgE levels are epiphenomena. Finally, it is possible that increased IgE levels enhance the production of this cytokine through an as-yet-unidentified feedback mechanism.

In conclusion, in this study we observed that IL-5 production is strongly linked to total serum IgE levels in a general population of children and parents. These findings, together with the results of other studies, suggest that the *in vivo* regulation of IgE production might be the result of a complex interaction among many factors and that the role played by IL-5 in allergic diseases might go beyond

its well-known effects on eosinophil maturation and longevity. In addition, the lack of a significant association between cytokines and total IgE levels in cord blood in our population suggests that different factors might contribute to IgE regulation at different stages of immune system maturation, which could help explain the questionable value of cord blood IgE in predicting allergic outcomes later in life.

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TABLE E1. Descriptive statistics for cytokine production in the IIS population

	Child			Mother		Father
	Cord blood	3 mo	12 mo	Pregnancy	Postpartum	
IFN- γ (pg/mL)						
N	271	280	215	278	216	197
Median	545	758	1716	6097	3921	8078
Interquartile range	155–987	281–1380	637–2957	2480–12,921	1617–10,154	3007–13,209
Geometric mean	397	593	1226	*	3098	*
IL-4 (pg/mL)						
N	279	285	217	284	219	199
Median	0.59	0.97	2.32	9.83	10.1	10.7
Interquartile range	0.25–0.93	0.58–1.73	1.38–3.77	5.01–17.9	5.28–22.6	4.14–23.4
Geometric mean	*	*	2.12	8.90	9.38	9.06
IL-5 (pg/mL)						
N	279	284	217	254	208	185
Median	7.8	7.8	77.5	91.39	104.65	43
Interquartile range	7.8–7.8	7.8–33.4	36.4–146	50–170	55.2–175.8	43–92.2
Geometric mean	*	*	71.09	*	88.7	*
IL-13 (pg/mL)						
N	251	273	219	269	219	181
Median	140	118	185	247.62	235	252
Interquartile range	89.2–218	69.3–186	108–284	123–390	120–427	138–425
Geometric mean	*	110	166	204	*	237
IL-10 (pg/mL)						
N	279	285	218	288	206	187
Median	41.2	77.1	60.5	102	57.2	210
Interquartile range	19.2–74.1	38.7–141.2	27.3–138	26.5–244	22.9–199	68.3–407
Geometric mean	*	68.7	*	*	*	143

*Geometric means are provided only for those variables that were normally distributed after log transformation and for which less than 10% of the values were undetectable.