# Adaptive cytokine production in early life differentially predicts total IgE levels and asthma through age 5 years

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**Background: Although it has been postulated that allergic disease is associated with a predominance of $T_H2$ cells, whether IgE levels and asthma might differ in their relation to early-life cytokine production is not known.**

**Objective: We sought to assess the relationship between first-year adaptive immune cytokine production with asthma and total IgE levels through age 5 years in a nonselected birth cohort.**

**Methods: Mitogen (concanavalin A/phorbol 12-myristate 13-acetate)–stimulated IL-4, IL-5, IL-13, and IFN-γ levels were measured in supernatants from cord blood mononuclear cells and PBMCs at birth, 3 months, and 12 months. Total serum IgE levels and physician-diagnosed active asthma were assessed at 1, 2, 3, and 5 years. Longitudinal models that adjust for both $T_H1$ and $T_H2$ cytokine production were used to determine relations of outcomes.**

**Results: Relations of cytokines to total IgE levels and asthma were strikingly different. Total IgE levels through age 5 years were positively associated with 12-month IL-4 ($P < .001$), IL-5 ($P < .001$), and IL-13 ($P = .02$) levels when adjusted for IFN-γ levels and inversely associated with 12-month IFN-γ levels after IL-4 adjustment ($P = .01$). Active asthma through age 5 years was positively associated with 3-month IL-13 levels adjusted for IFN-γ (odds ratio, 2.6; $P < .001$) and inversely associated with 3-month IFN-γ levels adjusted for IL-13 (odds ratio, 0.5; $P = .001$). These relations were strongest for nonatopic asthma.**

**Conclusion: Total IgE levels and active asthma through age 5 years are associated with adaptive cytokine production in early life, although relations vary temporally and with regard to the relative importance of individual cytokines. (J Allergy Clin Immunol 2011;128:397-402.)**

***Key words:*** *Asthma, IgE, cytokines, IL-4, IL-5, IL-13, IFN-γ, children*

According to the classical $T_H1/T_H2$ paradigm, a healthy immune system derives in part from a balance between cell production of $T_H1$- and $T_H2$-type cytokines, whereas manifestations of allergy, including those that accompany asthma, are associated with a predominance of $T_H2$-type cytokines. Although several more recently described cell types and regulatory mechanisms indicate that additional factors might contribute to maintaining the balance, the paradigm of a $T_H2$ shift *per se* remains a hallmark of allergic disease, as reviewed by Jutel and Adkis.[1] This concept developed after the demonstration of differential cytokine production among murine-derived T-cell clones[2] and subsequent studies with human cells and animal models demonstrating separate roles for the cytokines produced by these cell types, as reviewed by Street and Mosmann.[3] Further murine and human *in vitro* studies of the $T_H$ cell paradigm have demonstrated that the $T_H1$ cytokine IFN-γ and the $T_H2$ cytokine IL-4 act to downregulate one another, as reviewed by Paludan,[4] and thus an inverse association between $T_H2$ and $T_H1$ cytokine production might be expected.

However, we and others have recently shown that mitogen-stimulated production of $T_H1$ and $T_H2$ cytokines is strongly *positively* correlated for subjects in a general population sample.[5,6] This strong correlation suggests that any skewing among allergic subjects might be quite subtle. Furthermore, associations of $T_H2$ cytokines with allergic outcomes might either be due to high absolute amounts of a particular cytokine or to high production of $T_H2$ cytokines relative to production of $T_H1$ cytokines. Thus from a methodological standpoint, assessment of the effects of $T_H1$ and $T_H2$ cytokines through models adjusted for one another might provide more information than considering a single cytokine in isolation.

The close association of asthma with circulating IgE levels in human subjects[7] together with all of the animal research implicating IgE, $T_H2$ mechanisms, or both as driving allergic inflammation in airways has produced the implicit assumption that asthma is driven, at least in part, by IgE and that IgE is driven by $T_H2$ cytokines. Many of the studies that are presumed to support these relations have examined relations with allergy outcomes occurring concurrently, but such studies do not provide information on temporal relations and might not capture fluctuations in symptoms or age-related effects. It is clear that $T_H2$ cytokines are involved in immunoglobulin class-switching, but in relation to asthma, they might also have direct effects on the airways. Thus cytokine production in early life might be influential, either by initiating these processes or by marking subjects in whom these processes have already been initiated, thereby having different relations with IgE levels and asthma. Unfortunately, information about relations between early cytokine production during or before development of disease in unselected populations is limited.

This study assessed relations between first-year cytokine production and both development of asthma and total IgE levels in childhood, comparing results from single-cytokine analyses with those from models that adjust for both $T_H1$ and $T_H2$ cytokines. We

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hypothesized that if increased levels of IgE develop separately from rather than drive asthma, as has been suggested,[8,9] early cytokine production might be differentially related to these outcomes, either temporally or in the relative importance of individual cytokines. In consideration of previous reports of strong positive correlation, these relations might be most evident in models that adjust for both $T_H1$ and $T_H2$ cytokines.

## METHODS

### Study design and population

The Infant Immune Study is a prospective birth cohort study of immune system maturation and its relation to the development of asthma and allergic disease in childhood. Participants were healthy children born to women who planned to obtain care for their newborns from collaborating pediatricians, as previously described.[10]

### Cytokine measurements

Blood specimens were obtained at birth (from the umbilical cord, n = 397) and by means of venipuncture at 3 months (2.9 ± 1.2 months, n = 363) and 1 year (1.1 ± 0.1 years, n = 364). Lymphocyte stimulations were performed on PBMCs and cord blood mononuclear cells separated by means of standard density gradient centrifugation techniques, as previously described.[6] Cells were stimulated with 10 μg/mL concanavalin A (Pharmacia, Piscataway, NJ) and 10 ng/mL phorbol 12-myristate 13-acetate (Sigma Chemical Co, St Louis, Mo). Supernatant fluids from these cultures and unstimulated controls were harvested after 18 to 24 hours and stored at −70°C for later cytokine testing. The supernatants were assayed for IL-4, IL-5, IL-13, and IFN-γ production by using commercially available kits (Genzyme, Minneapolis, Minn). Cytokines with more than 20% undetectable values at a given age were treated as dichotomous variables (detectable vs undetectable). All other cytokines were treated as continuous variables, with undetectable values being assigned the value at the limit of detection (0.25 pg/mL for IL-4, 7.8 pg/mL for IL-5, 3.13 pg/mL for IL-13, and 15.6 pg/mL for IFN-γ).

### Outcome measurements

Total and allergen-specific IgE levels were measured from blood specimens obtained at 1 year (1.1 ± 0.1 years, n = 364), 2 years (2.1 ± 0.2 years, n = 310), 3 years (3.2 ± 0.3 years, n = 295), and 5 years (5.1 ± 0.4 years, n = 278) by using the Pharmacia AutoCAP assay (Pharmacia/Upjohn, Kalamazoo, Mich) until its discontinuation in 2006 and subsequently by using Immulite 2000 (Siemens Medical Solutions, Los Angeles, Calif). Samples analyzed on both instruments (n = 25) yielded a correlation coefficient of 0.995. Specific IgE levels were measured for 6 inhalant allergens (*Alternaria* species, *Dermatophagoides farinae*, Bermuda grass, careless weed, and olive and mulberry tree) and 2 foods (ovalbumin and β-lactoglobulin). Each allergen-specific IgE was categorized as detectable or undetectable at each age based on a cutoff of 0.25 IU/mL, with atopy defined as detectable specific IgE for 1 or more allergens at any age. Children were defined as nonatopic if they had at least 1 negative result at 1 or more ages and never had positive results.

Asthma was assessed based on parent-completed questionnaires at ages 1 year (1.1 ± 0.1 years, n = 423), 2 years (2.1 ± 0.2 years, n = 362), 3 years (3.2 ± 0.3 years, n = 371), and 5 years (5.1 ± 0.4 years, n = 373). Specifically, parents were asked if their child ever had asthma, at what age their symptoms last occurred, and at what age they were first told by a physician that the child had asthma. For this analysis, asthma was defined at each age as physician-diagnosed asthma with reported symptoms in the last year.

### Statistical analysis

Total IgE levels and amounts of cytokines produced were log transformed for all analyses. Log-transformed cytokines were converted to *z* scores for comparison of effect sizes across cytokines.

Longitudinal relations between total IgE levels over 1, 2, 3, and 5 years and cytokine production at each of 3 time points (birth, 3 months, and 12 months) were assessed by using mixed models, treating each subject as a random effect to control for within-subject correlation. In each model age at the time of total IgE measurement was included as a categorical variable because log total IgE levels tend to increase with age in childhood, although in a nonlinear fashion. In addition, age-by-cytokine-level interaction terms were included to allow differing effects between cytokine and total IgE levels at each age. Regression coefficients were calculated for the change in total IgE levels per 1 SD in logged units of each cytokine.

Longitudinal relations between cytokine levels and physician-diagnosed active asthma were assessed by using generalized estimating equations to determine the odds of asthma associated with 1 SD change in the production of each cytokine. As with longitudinal models for total IgE levels, generalized estimating equation models for asthma were run both with age-by-cytokine-level interaction terms to calculate the effect of cytokine levels at each age of asthma assessment and without interaction terms to assess an overall effect of cytokine levels on asthma over ages 1 through 5 years. Finally, asthma-by-cytokine analyses were stratified by specific IgE detectability in the first 5 years to assess the possibility of differing relations of early cytokine production to atopic versus nonatopic asthma.

Each of the analyses described above was performed by using 2 approaches designated as unadjusted and adjusted models. For unadjusted models, individual cytokines were entered into each model with no covariates other than age. Adjusted models include IFN-γ and one of 3 $T_H2$ cytokines (IL-4, IL-5, or IL-13) together as covariates.

All analyses were conducted with STATA software, version 10.0 (StataCorp, College Station, Tex). This research was approved by the Institutional Review Board of the University of Arizona, and informed consent was obtained for all subjects.

## RESULTS

Cytokine production from mitogen-stimulated blood mononuclear cells were measured for 450 children at least once in the first year of life (birth, n = 295; 3 months, n = 323; or 1 year, n = 318). Children with cytokine data did not differ significantly from children who lacked cytokine data (n = 32) with respect to sex, ethnicity, household income, day care attendance, parental atopy, or parental asthma status. Of the 450 children with cytokine data, total IgE levels were available at 1, 2, 3, and/or 5 years for 396 (88.0%) children and asthma data at 1, 2, 3, and/or 5 years for 425 (94.4%) children.

### Cytokines

Cytokine production at birth and 3 months in this cohort has been described in detail.[6] Table I provides a summary of descriptive statistics for production of IL-4, IL-5, IL-13, and IFN-γ at birth, 3 months, and 12 months. Of note, production of all cytokines increased significantly with age from 2- to 10-fold by 1 year, with the exception of production of IL-13, which decreased between birth and 3 months ($P < .001$) and then increased between 3 and 12 months ($P < .001$).

### Outcomes

Geometric mean total IgE levels increased with age (1 year, 8.8 IU/mL; 2 years, 14.8 IU/mL; 3 years, 16.8 IU/mL; and 5 years, 22.3 IU/mL). Similarly, the prevalence of active asthma increased with age (1 year, 3.2% [13/407]; 2 years, 5.2% [18/348]; 3 years, 8.1% [29/358]; and 5 years, 12.3% [44/357]).

**TABLE I.** Cytokine production at birth, 3 months, and 12 months

| | Birth | | 3 mo | | 12 mo | |
|---|---|---|---|---|---|---|
| | Median (range) | Geometric mean (95% CI) | Median (range) | Geometric mean (95% CI) | Median (range) | Geometric mean (95% CI) |
| IL-4 | 0.52 (0.25-5.07) | * | 1.00 (0.25-19.3) | 1.01 (0.91-1.12) | 2.30 (0.25-30.8) | 2.00 (1.80-2.23) |
| IL-5 | 7.8 (7.8-460) | * | 7.8 (7.8-545) | * | 74.2 (7.8-962) | 68.0 (60.2-78.8) |
| IL-13 | 154 (3.13-1,380) | 138 (123-154) | 123 (3.13-4,210) | 116 (106-126) | 192 (3.13-1,400) | 173 (157-191) |
| IFN-γ | 566 (15.6-15,300) | 409 (349-480) | 774 (15.6-33,500) | 612 (533-703) | 1,470 (15.6-23,000) | 1,080 (915-1,270) |

Values are shown in picograms per milliliter.
*Less than 80% detectability: treated as dichotomous (undetectable/detectable) for all analyses.

## Relations between first-year cytokine levels and outcomes

**Total IgE.** Table II shows regression coefficients for total IgE levels through age 5 years in relation to cytokine production at 3 and 12 months by using longitudinal models. Three-month IL-4 levels significantly predicted subsequent IgE levels in the unadjusted model (1), and this relation was stronger in the IFN-γ–adjusted model (5). Of note, adjustment with IL-4 revealed an inverse relation between 3-month IFN-γ levels and total IgE levels that was not evident before adjustment (5).

Production of $T_H2$ cytokines at 12 months showed even stronger relations to total IgE levels through age 5 years. Total IgE levels through age 5 years were significantly associated with IL-4 and IL-5 in unadjusted models (8 and 9) and with IL-13 in the IFN-γ–adjusted model (14). The inverse relationship between IFN-γ and total IgE levels was not evident in the unadjusted model (11) but again became significant after adjustment with IL-4 (12).

Results from analyses assessing relations of early cytokine production to total IgE levels at each age by including age-by-cytokine interaction terms were not appreciably different from those reported above. However, these analyses do show that the relationships between total IgE levels and early production of all cytokines at 12 months tended to strengthen with age of assessment of total IgE levels (see Table E1 in this article's Online Repository at www.jacionline.org).

There was no association of cytokine production at birth with total IgE levels at 1, 2, 3, or 5 years (data not shown).

**Asthma.** Odds ratios for active asthma through age 5 years associated with first-year cytokine production are shown in Table III. Active asthma was significantly positively associated with 3-month IL-13 levels in the unadjusted model (3), and this relation was considerably stronger in the IFN-γ–adjusted model (7). A nonsignificant inverse relationship between IFN-γ levels and asthma became slightly stronger after adjustment for IL-4 (5) but was markedly affected in the model that adjusted for IL-13 levels (7). Unlike relations with total IgE levels, asthma was unrelated to 12-month cytokine production.

Odds ratios assessed by models, including age-cytokine interactions, were not appreciably different from those described above. However, these results show that relations between 3-month IL-13 levels and active asthma tended to strengthen with age, especially over the first 3 years (see Table E2 in this article's Online Repository at www.jacionline.org).

As was true for IgE levels, there were no consistent relations between cord cytokine levels and asthma (data not shown).

**Stratification by specific IgE detectability.** To assess the possibility of differing relations for atopic and nonatopic asthma with respect to early cytokine production, we stratified asthma-cytokine analyses by atopy in the first 5 years, the prevalence of which was 51% (Table III). The relation of asthma to 3-month IL-13 levels was evident only among nonatopic subjects in the unadjusted model (3). After adjustment, IL-13 and IFN-γ relations remained stronger among nonatopic subjects (7). In contrast, IL-4 levels were significantly positively associated with asthma only among atopic children and only after adjustment for IFN-γ levels (5).

**TABLE II.** Regression coefficients for log total IgE levels from longitudinal models over 1, 2, 3, and 5 years for individual cytokines (unadjusted) and for $T_H2$ cytokines adjusted for IFN-γ levels (adjusted) at 3 and 12 months*

| | | Total IgE ages 1, 2, 3, and 5 y | |
|---|---|---|---|
| Model | | Coefficient | *P* value |
| | 3 mo Cytokines | | |
| | Unadjusted | | |
| 1 | IL-4 | **0.07** | **.040** |
| 2 | IL-5† | 0.10 | .134 |
| 3 | IL-13 | 0.00 | .968 |
| 4 | IFN-γ | −0.03 | .345 |
| | Adjusted | | |
| 5 | IL-4 | **0.11** | **.005** |
| | IFN-γ | **−0.08** | **.030** |
| 6 | IL-5† | 0.12 | .089 |
| | IFN-γ | −0.04 | .214 |
| 7 | IL-13 | 0.03 | .446 |
| | IFN-γ | −0.05 | .191 |
| | 12 mo Cytokines | | |
| | Unadjusted | | |
| 8 | IL-4 | **0.13** | **<.001** |
| 9 | IL-5 | **0.12** | **<.001** |
| 10 | IL-13 | 0.06 | .086 |
| 11 | IFN-γ | −0.02 | .500 |
| | Adjusted | | |
| 12 | IL-4 | **0.17** | **<.001** |
| | IFN-γ | **−0.09** | **.010** |
| 13 | IL-5 | **0.14** | **<.001** |
| | IFN-γ | −0.06 | .054 |
| 14 | IL-13 | **0.09** | **.016** |
| | IFN-γ | −0.07 | .066 |

Boldfaced values indicate $P < .05$.
*No. at 3 months = 295 to 298; no. at 12 months = 309 to 316.
†Dichotomized: detectable/undetectable.

## Role of adjustment for both $T_H1$ and $T_H2$ cytokines

Fig 1 shows the relation of 3-month IL-13 levels to 3-month IFN-γ levels for subjects with and without asthma. Lines divide the distribution for both IL-13 and IFN-γ levels at the medians to form 4 quadrants. This graph shows the value of considering the effect of IL-13 levels in the context of IFN-γ levels and vice versa by showing (1) the strong correlation between 3-month

**TABLE III.** ORs and P values for active asthma from longitudinal models over 1, 2, 3, and 5 years for individual cytokines (unadjusted) and for $T_H2$ cytokines adjusted for IFN-γ levels (adjusted), at 3 and 12 months for all subjects and stratified by specific IgE detectability through age 5 years*

| | | Active asthma, ages 1, 2, 3, and 5 y | | | | | |
|---|---|---|---|---|---|---|---|
| | | All subjects | | No detectable specific IgE through age 5 y | | Any detectable specific IgE through age 5 y | |
| Model | | OR | *P* value | OR | *P* value | OR | *P* value |
| | 3 mo Cytokines | | | | | | |
| | Unadjusted | | | | | | |
| 1 | IL-4 | 1.3 | .272 | 0.8 | .336 | 1.4 | .206 |
| 2 | IL-5† | 1.1 | .793 | 0.4 | .185 | 1.6 | .299 |
| 3 | IL-13 | **1.9** | **.004** | **2.3** | **.008** | 1.5 | .153 |
| 4 | IFN-γ | 0.8 | .145 | 0.7 | .268 | 0.7 | .259 |
| | Adjusted | | | | | | |
| 5 | IL-4 | 1.6 | .071 | 0.8 | .605 | **1.8** | **.047** |
| | IFN-γ | **0.6** | **.028** | 0.8 | .567 | **0.6** | **.037** |
| 6 | IL-5† | 1.2 | .575 | 0.4 | .241 | 1.7 | .205 |
| | IFN-γ | 0.7 | .127 | 0.8 | .373 | 0.7 | .201 |
| 7 | IL-13 | **2.6** | **<.001** | **3.5** | **<.001** | **1.9** | **.038** |
| | IFN-γ | **0.5** | **.001** | **0.4** | **.009** | **0.5** | **.023** |
| | 12 mo Cytokines | | | | | | |
| | Unadjusted | | | | | | |
| 8 | IL-4 | 1.2 | .302 | 1.1 | .669 | 1.2 | .484 |
| 9 | IL-5 | 1.0 | .844 | 0.9 | .802 | 0.9 | .717 |
| 10 | IL-13 | 1.0 | .884 | 1.0 | .998 | 1.0 | .966 |
| 11 | IFN-γ | 0.9 | .440 | 0.7 | .165 | 1.1 | .814 |
| | Adjusted | | | | | | |
| 12 | IL-4 | 1.3 | .180 | 1.3 | .370 | 1.2 | .490 |
| | IFN-γ | 0.8 | .226 | 0.6 | .099 | 1.0 | .945 |
| 13 | IL-5 | 1.0 | .939 | 1.0 | .866 | 0.9 | .636 |
| | IFN-γ | 0.9 | .445 | 0.7 | .170 | 1.1 | .676 |
| 14 | IL-13 | 1.1 | .620 | 1.3 | .458 | 1.0 | .882 |
| | IFN-γ | 0.8 | .375 | 0.6 | .126 | 1.1 | .745 |

*OR*, Odds ratio. Boldfaced values indicate $P < .05$.
*All subjects: no. at 3 months = 307 to 310; no. at 12 months = 309 to 316. Subjects with no detectable specific IgE: no. at 3 months = 153 to 155; no. at 12 months = 154 to 159. Subjects with detectable specific IgE: no. at 3 months = 144 to 145; no. at 12 months = 155 to 157.
†Dichotomized: detectable/undetectable.

IFN-γ and 3-month IL-13 levels and (2) that high IFN-γ levels provide protection from asthma primarily among those with low IL-13 levels: only 2.3% (1/44) of children in this group have active asthma at age 5 years compared with 16.2% (35/216) among children from the other 3 quadrants combined ($P = .015$).

## DISCUSSION

This study found that early cytokine production was related to IgE levels and asthma in childhood, with positive associations for $T_H2$ cytokines and inverse associations for IFN-γ levels in a nonselected population followed longitudinally from birth. However, the relations with $T_H1$ and $T_H2$ cytokines differed temporally and by outcome considered. Total IgE levels were most strongly associated with cytokine production at 12 months and showed positive relations with IL-4 and IL-5 levels and an inverse relationship with IFN-γ levels. IL-13 levels related to IgE levels modestly and only after adjustment with IFN-γ levels. In contrast, asthma in unadjusted models was associated only with IL-13 production

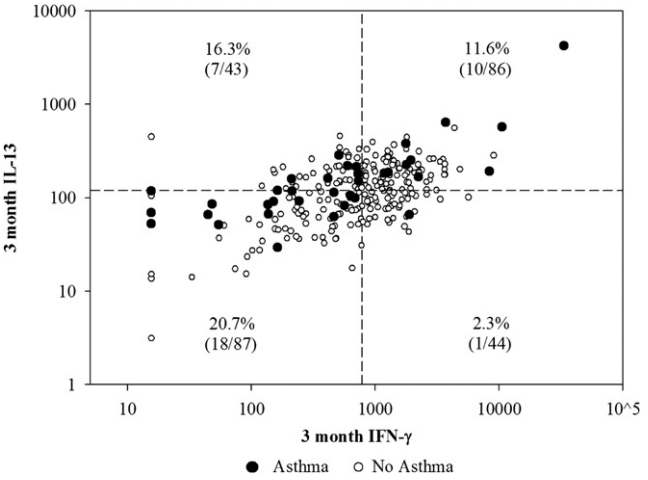


**FIG 1.** Active asthma at age 5 years by 3-month IFN-γ and IL-13 levels, with asthma prevalence shown by quadrants created by dividing IFN-γ and IL-13 values at the median.

and only at 3 months. Adjustment for IFN-γ levels strengthened the relation between asthma and IL-13 levels, and adjustment for IL-13 levels revealed a strong inverse association with IFN-γ levels. Three-month IL-4 levels were also positively associated with asthma, although only for atopic children. Cord cytokine levels showed no consistent association with IgE levels or asthma.

Many epidemiologic studies have reported associations of allergic outcomes in children with increased production of $T_H2$ cytokines[11-23] and decreased production of $T_H1$ cytokines.[15,19-22,24-27] However, because the majority of these analyses are cross-sectional, considering concurrent relations between cytokine production and allergic outcomes, they cannot identify potential predictive relations of early cytokine production and later allergic outcomes. Furthermore, of the few birth cohort studies, only 1 assessed allergic outcomes after the age of 3 years.[27] Our study extends this body of literature by investigating longitudinally whether $T_H1$ and $T_H2$ cytokine production in early life predicts total IgE levels and asthma through age 5 years in a nonselected population. In addition, cytokine production was measured at 3 time points during the first year of life, which revealed that the predictive relation of cytokine production to allergic outcomes is both age and outcome dependent. Finally, this study expands our previous analysis in this population pertaining to wheeze in the first year of life[24] by considering outcomes through age 5 years and showing that significant cytokine-asthma relations were apparent at 3 but not 12 months.

Our results are consistent with those of other epidemiologic studies that have shown positive relations of total IgE levels with IL-4,[11] IL-5,[11,17,23] and IL-13[23] levels and inverse associations between IFN-γ levels and atopy defined in part by increased total IgE levels.[20,21] These findings are consistent with the mechanistic roles of these $T_H1$ and $T_H2$ cytokines, as IL-4, IL-5, and IL-13 promote, while IFN-γ inhibits IgE synthesis.[28,29] Our finding that relations to total IgE levels were strengthened in adjusted models including both a $T_H2$ and $T_H1$ cytokine can also be interpreted mechanistically. We know that IL-4 both drives IgE synthesis by B cells[28,29] and reduces IFN-γ production by inhibiting the $T_H1$ cell development from which IFN-γ is derived.[4] IL-13 also drives[30] and IL-5 enhances[31] IgE synthesis. In contrast, IFN-γ inhibits both IgE class-switching and $T_H2$

cell growth, as reviewed by Robinson et al,[29] thereby potentially decreasing IL-4, IL-5, and IL-13 levels. Thus any relation between IFN-γ production and total IgE levels becomes more evident if adjusted for $T_H2$ cytokine production and vice versa.

With respect to asthma, this study found that IL-13 levels were positively and IFN-γ levels were inversely associated with both atopic and nonatopic asthma, whereas IL-4 levels were only associated with asthma for atopic children. As suggested by studies relating to IL-13 variants, our finding of a strong relation of asthma to IL-13 levels is not surprising.[32] Moreover, these findings are similar to previous epidemiologic studies that have shown positive relations between IL-13 levels and asthma[13,14] and IL-4 levels and atopic asthma[22] and inverse relations between IFN-γ levels and asthma,[15,26] atopic asthma,[22] and wheeze.[24,27] However, this is the first study in children that examines relations of cytokine levels to asthma stratified by atopic status to our knowledge. Our finding that IL-13 levels were related to both atopic and nonatopic asthma suggests that IL-13 acts independently of mechanisms involved in the allergic response, a speculation supported by studies that find overexpression of IL-13 in airways of both allergic and nonallergic asthmatic subjects.[33,34] These results emphasize the possibility that asthma can develop independently of IgE, as previous epidemiologic findings have suggested.[8,9] Studies in animal models have demonstrated that IL-13 has direct effects on airway tissues, including mucus hypersecretion, airway inflammation, and subepithelial fibrosis.[35] In contrast to IL-13, we found that IL-4 levels were only associated with asthma in the presence of specific IgE sensitization, which corresponds with earlier findings of overexpression of IL-4 among allergic but not among nonallergic asthmatic subjects.[33,34]

We found that adjusted analyses between cytokine production and asthma followed a pattern similar to that for IgE, in that adjustment for IFN-γ levels strengthened the relation with IL-13 and vice versa, again emphasizing that it is the relative rather than absolute production of $T_H1$ and $T_H2$ cytokines that influences risk. The value of adjustment is further demonstrated in Fig 1, which provides a representation of one slice of our asthma data at age 5 years in relation to 3-month IFN-γ and IL-13 production. By showing the effect of IL-13 levels relative to IFN-γ levels and vice versa, we see clearly that only subjects with both low IL-13 and high IFN-γ levels appear to be protected from asthma, a relation that would be missed if we were to examine IL-13 and IFN-γ levels in isolation. Other studies have modeled the effect of $T_H1$ cytokines relative to $T_H2$ cytokines by using ratios as single variables in analyses.[15,22] We opted *a priori* to use $T_H1/T_H2$ cytokine–adjusted models rather than ratios, both because of the questionable validity of results from ratio-based analyses[36] and to better interpret the individual effects of each cytokine, which might be missed if our assessed variables were collapsed to a single ratio.

We found no relation of cord blood cytokine production and either asthma or total IgE levels. Although at least 2 studies have found relations between cord blood mononuclear cell IL-13 production and subsequent atopic disease,[18,37] the direction of the relation was inconsistent for these studies. Furthermore, several larger studies corroborate our null results, reporting no associations between allergic outcomes and the cord blood cytokines that we assess in this article.[17,23,38] Our finding that total IgE levels through age 5 years were associated with 12-month and, in the case of IL-4 and IFN-γ, 3-month cytokine production but not with cord blood cytokine production suggests that these mechanisms are not fully determined at birth but rather develop during the first year of life. Similarly, the transient relation we found between 3-month cytokine production and later asthma, which was no longer present at 12 months, suggests there might be an early window of vulnerability that is influential in asthma and that is marked by altered production of IL-13, IFN-γ, and, in the case of atopic asthma, IL-4. The fact that these relations varied depending on the timing of the assessment underscores the value of longitudinal follow-up with repeated measures of both the predictors and the outcomes.

Our study has certain limitations. First, cytokine production from mitogen-stimulated PBMCs is likely to be, at best, a crude reflection of cytokine production within local tissue sites where T-cell stimulation occurs as a result of antigen presentation (or perhaps endogenous mitogen stimulation). Thus the reported associations cannot necessarily be attributed to cause and effect, but might be driven by some common factor that we have not considered. More mechanistic studies are required to determine whether the observed relationships have biologic significance.

Second, our follow-up of outcomes currently ends at age 5 years. Clearly, this age precludes a definitive assessment of asthma.

Third, although we have described the cytokines produced as being $T_H1$ and $T_H2$ cytokines, we acknowledge that there might be additional cell sources other than $T_H$ cells, given that the cell stimulation studies used PBMCs.

Fourth, our study is limited by its focus on $T_H1$ and $T_H2$ cytokines. Although reflecting the state of the science in the 1990s, other more recently discovered cytokines, including regulatory cytokines, might also show differential relations with asthma outcomes.

Finally, we acknowledge that using an 18- to 24-hour window for harvesting supernatant fluids might not optimally capture the capacity to produce particular cytokines. However, we have no reason to suspect that outcome groups would be affected differentially.

In conclusion, our results suggest that total IgE levels and active asthma through age 5 years are associated with adaptive cytokine production in the first year of life but that patterns of relation differ temporally and with regard to the relative importance of individual cytokines. At 3 months, production of IL-13 and IFN-γ was positively and negatively, respectively, associated with asthma, as was production of IL-4 for the subset of atopic children. In contrast, cytokine production at 12 months was associated with total IgE levels, with IL-4 and IL-5 levels showing the strongest relations. These data demonstrate the complex interplay between $T_H1$ and $T_H2$ cytokines in the development of allergic disease.

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**Key messages**

- **Cytokine production in the first year of life shows different relations with total IgE levels and asthma through age 5 years.**
- **Several cytokines at age 1 year associate with IgE levels assessed longitudinally.**
- **Asthma relates directly to IL-13 levels and inversely to IFN-γ levels adjusted for IL-13 levels at 3 months but not to cytokine production later.**

## REFERENCES

1. Jutel M, Akdis CA. T-cell subset regulation in atopy. Curr Allergy Asthma Rep 2011;11:139-45.
2. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 1986;136:2348-57.
3. Street NE, Mosmann TR. Functional diversity of T lymphocytes due to secretion of different cytokine patterns. FASEB J 1991;5:171-7.
4. Paludan SR. Interleukin-4 and interferon-gamma: the quintessence of a mutual antagonistic relationship. Scand J Immunol 1998;48:459-68.
5. Gabrielsson S, Soderlund A, Nilsson C, Lilja G, Nordlund M, Troye-Blomberg M. Influence of atopic heredity on IL-4-, IL-12- and IFN-gamma-producing cells in in vitro activated cord blood mononuclear cells. Clin Exp Immunol 2001;126: 390-6.
6. Halonen M, Lohman IC, Stern DA, Spangenberg A, Anderson D, Mobley S, et al. Th1/Th2 patterns and balance in cytokine production in the parents and infants of a large birth cohort. J Immunol 2009;182:3285-93.
7. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. N Engl J Med 1989;320:271-7.
8. Burrows B, Martinez FD, Cline MG, Lebowitz MD. The relationship between parental and children's serum IgE and asthma. Am J Respir Crit Care Med 1995;152: 1497-500.
9. Halonen M, Stern DA, Lohman C, Wright AL, Brown MA, Martinez FD. Two subphenotypes of childhood asthma that differ in maternal and paternal influences on asthma risk. Am J Respir Crit Care Med 1999;160:564-70.
10. Su Y, Rothers J, Stern DA, Halonen M, Wright AL. Relation of early antibiotic use to childhood asthma: confounding by indication? Clin Exp Allergy 2010;40: 1222-9.
11. Crestani E, Lohman IC, Guerra S, Wright AL, Halonen M. Association of IL-5 cytokine production and in vivo IgE levels in infants and parents. J Allergy Clin Immunol 2007;120:820-6.
12. Duramad P, Harley K, Lipsett M, Bradman A, Eskenazi B, Holland NT, et al. Early environmental exposures and intracellular Th1/Th2 cytokine profiles in 24-month-old children living in an agricultural area. Environ Health Perspect 2006;114: 1916-22.
13. Fitzpatrick AM, Higgins M, Holguin F, Brown LA, Teague WG. The molecular phenotype of severe asthma in children. J Allergy Clin Immunol 2010;125: 851-7, e18.
14. Heaton T, Rowe J, Turner S, Aalberse RC, de Klerk N, Suriyaarachchi D, et al. An immunoepidemiological approach to asthma: identification of in-vitro T-cell response patterns associated with different wheezing phenotypes in children. Lancet 2005;365:142-9.
15. Hoekstra MO, Hoekstra Y, De Reus D, Rutgers B, Gerritsen J, Kauffman HF. Interleukin-4, interferon-gamma and interleukin-5 in peripheral blood of children with moderate atopic asthma. Clin Exp Allergy 1997;27:1254-60.
16. Macaubas C, Sly PD, Burton P, Tiller K, Yabuhara A, Holt BJ, et al. Regulation of T-helper cell responses to inhalant allergen during early childhood. Clin Exp Allergy 1999;29:1223-31.
17. Neaville WA, Tisler C, Bhattacharya A, Anklam K, Gilbertson-White S, Hamilton R, et al. Developmental cytokine response profiles and the clinical and immunologic expression of atopy during the first year of life. J Allergy Clin Immunol 2003;112:740-6.
18. Ohshima Y, Yasutomi M, Omata N, Yamada A, Fujisawa K, Kasuga K, et al. Dysregulation of IL-13 production by cord blood CD4+ T cells is associated with the subsequent development of atopic disease in infants. Pediatr Res 2002;51: 195-200.
19. Rowe J, Kusel M, Holt BJ, Suriyaarachchi D, Serralha M, Hollams E, et al. Prenatal versus postnatal sensitization to environmental allergens in a high-risk birth cohort. J Allergy Clin Immunol 2007;119:1164-73.
20. Smart JM, Kemp AS. Increased Th1 and Th2 allergen-induced cytokine responses in children with atopic disease. Clin Exp Allergy 2002;32:796-802.
21. Smart JM, Tang ML, Kemp AS. Polyclonal and allergen-induced cytokine responses in children with elevated immunoglobulin E but no atopic disease. Clin Exp Allergy 2002;32:1552-7.
22. Tang ML, Coleman J, Kemp AS. Interleukin-4 and interferon-gamma production in atopic and non-atopic children with asthma. Clin Exp Allergy 1995;25:515-21.
23. Uekert SJ, Akan G, Evns MD, Li Z, Roberg K, Tisler C, et al. Sex-related differences in immune development and the expression of atopy in early childhood. J Allergy Clin Immunol 2006;118:1375-81.
24. Guerra S, Lohman IC, Halonen M, Martinez FD, Wright AL. Reduced interferon gamma production and soluble CD14 levels in early life predict recurrent wheezing by 1 year of age. Am J Respir Crit Care Med 2004;169:70-6.
25. Kim JH, Kim KH, Woo HY, Shim JY. Maternal cytokine production during pregnancy and the development of childhood wheezing and allergic disease in offspring three years of age. J Asthma 2008;45:948-52.
26. Nurse B, Haus M, Puterman AS, Weinberg EG, Potter PC. Reduced interferon-gamma but normal IL-4 and IL-5 release by peripheral blood mononuclear cells from Xhosa children with atopic asthma. J Allergy Clin Immunol 1997;100:662-8.
27. Stern DA, Guerra S, Halonen M, Wright AL, Martinez FD. Low IFN-gamma production in the first year of life as a predictor of wheeze during childhood. J Allergy Clin Immunol 2007;120:835-41.
28. Poulsen LK, Hummelshoj L. Triggers of IgE class switching and allergy development. Ann Med 2007;39:440-56.
29. Robinson DS, Durham SR, Kay AB. Cytokines. 3. Cytokines in asthma. Thorax 1993;48:845-53.
30. Punnonen J, Aversa G, Cocks BG, McKenzie AN, Menon S, Zurawski G, et al. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. Proc Natl Acad Sci U S A 1993;90:3730-4.
31. Vercelli D, Jabara HH, Arai K, Yokota T, Geha RS. Endogenous interleukin 6 plays an obligatory role in interleukin 4-dependent human IgE synthesis. Eur J Immunol 1989;19:1419-24.
32. Vercelli D. Genetics of IL-13 and functional relevance of IL-13 variants. Curr Opin Allergy Clin Immunol 2002;2:389-93.
33. Humbert M, Durham SR, Kimmitt P, Powell N, Assoufi B, Pfister R, et al. Elevated expression of messenger ribonucleic acid encoding IL-13 in the bronchial mucosa of atopic and nonatopic subjects with asthma. J Allergy Clin Immunol 1997;99: 657-65.
34. Walker C, Bode E, Boer L, Hansel TT, Blaser K, Virchow JC Jr. Allergic and nonallergic asthmatics have distinct patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage. Am Rev Respir Dis 1992;146: 109-15.
35. Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. J Clin Invest 1999;103: 779-88.
36. Kronmal RA. Spurious correlation and the fallacy of the ratio standard revisited. J R Stat Soc A 1993;156:379-92.
37. Williams TJ, Jones CA, Miles EA, Warner JO, Warner JA. Fetal and neonatal IL-13 production during pregnancy and at birth and subsequent development of atopic symptoms. J Allergy Clin Immunol 2000;105:951-9.
38. Tadaki H, Arakawa H, Sugiyama M, Ozawa K, Mizuno T, Mochizuki H, et al. Association of cord blood cytokine levels with wheezy infants in the first year of life. Pediatr Allergy Immunol 2009;20:227-33.

**TABLE E1.** Regression coefficients and *P* values for log total IgE at 1, 2, 3, and 5 years for individual cytokines (unadjusted) and for $T_H2$ cytokines adjusted for IFN-γ levels (adjusted) at 3 and 12 months

| | | 1 y | | 2 y | | 3 y | | 5 y | |
|---|---|---|---|---|---|---|---|---|---|
| **Model** | | **Coefficient** | ***P* value** | **Coefficient** | ***P* value** | **Coefficient** | ***P* value** | **Coefficient** | ***P* value** |
| | 3 mo Cytokines | | | | | | | | |
| | Unadjusted | | | | | | | | |
| 1 | IL-4 | **0.08** | **.040** | 0.01 | .753 | 0.05 | .239 | **0.13** | **.002** |
| 2 | IL-5* | 0.11 | .154 | 0.08 | .335 | 0.06 | .435 | 0.16 | .053 |
| 3 | IL-13 | −0.02 | .690 | −0.06 | .177 | 0.01 | .823 | **0.09** | **.035** |
| 4 | IFN-γ | −0.02 | .610 | −0.06 | .145 | −0.04 | .350 | −0.02 | .586 |
| | Adjusted | | | | | | | | |
| 5 | IL-4 | **0.11** | **.009** | 0.05 | .255 | 0.09 | .061 | **0.18** | **<.001** |
| | IFN-γ | −0.07 | .092 | −0.09 | .064 | −0.08 | .084 | **−0.10** | **.025** |
| 6 | IL-5* | 0.12 | .124 | 0.10 | .213 | 0.08 | .335 | **0.17** | **.039** |
| | IFN-γ | −0.03 | .426 | −0.07 | .096 | −0.05 | .268 | −0.04 | .348 |
| 7 | IL-13 | 0.00 | .990 | −0.03 | .611 | 0.04 | .364 | **0.14** | **.007** |
| | IFN-γ | −0.02 | .531 | −0.06 | .248 | −0.07 | .248 | −0.08 | 0.086 |
| | 12-mo Cytokines | | | | | | | | |
| | Unadjusted | | | | | | | | |
| 8 | IL-4 | **0.10** | **.005** | **0.13** | **.001** | **0.17** | **<.001** | **0.17** | **<.001** |
| 9 | IL-5 | **0.13** | **<.001** | **0.12** | **.002** | **0.12** | **.001** | **0.13** | **.001** |
| 10 | IL-13 | 0.04 | .269 | 0.04 | .273 | **0.09** | **.022** | 0.07 | .074 |
| 11 | IFN-γ | −0.02 | .570 | −0.04 | .239 | −0.02 | .613 | 0.00 | .971 |
| | Adjusted | | | | | | | | |
| 12 | IL-4 | **0.13** | **.001** | **0.17** | **<.001** | **0.21** | **<.001** | **0.21** | **<.001** |
| | IFN-γ | −0.07 | .062 | **−0.11** | **.006** | **−0.10** | **.014** | −0.08 | .059 |
| 13 | IL-5 | **0.14** | **<.001** | **0.14** | **<.001** | **0.15** | **<.001** | **0.14** | **.001** |
| | IFN-γ | −0.06 | .085 | **−0.09** | **.028** | −0.06 | .118 | −0.04 | .329 |
| 14 | IL-13 | 0.07 | .111 | 0.09 | .055 | **0.13** | **.003** | **0.10** | **.046** |
| | IFN-γ | −0.06 | .184 | **−0.09** | **.044** | −0.08 | .056 | −0.05 | .309 |

Boldfaced values indicate $P < .05$.

*Dichotomized: detectable/undetectable.

**TABLE E2.** ORs and *P* values for active asthma at 1, 2, 3, and 5 years for individual cytokines (unadjusted) and for $T_H2$ cytokines adjusted for IFN-γ levels (adjusted) at 3 and 12 months

| | | 1 y | | 2 y | | 3 y | | 5 y | |
|---|---|---|---|---|---|---|---|---|---|
| **Model** | | **OR** | ***P* value** | **OR** | ***P* value** | **OR** | ***P* value** | **OR** | ***P* value** |
| | 3-mo Cytokines | | | | | | | | |
| | Unadjusted | | | | | | | | |
| 1 | IL-4 | 1.1 | .863 | 1.6 | .163 | 1.3 | .366 | 1.2 | .423 |
| 2 | IL-5* | 0.9 | .899 | 1.8 | .254 | 1.2 | .729 | 1.0 | .924 |
| 3 | IL-13 | 1.6 | .333 | 1.7 | .092 | **1.9** | **.015** | **1.7** | **.005** |
| 4 | IFN-γ | 0.8 | .542 | 0.7 | .134 | 0.7 | .195 | 0.8 | .200 |
| | Adjusted | | | | | | | | |
| 5 | IL-4 | 1.2 | .675 | **2.3** | **.004** | 1.6 | .133 | 1.3 | .205 |
| | IFN-γ | 0.7 | .514 | **0.5** | **.005** | **0.6** | **.046** | 0.7 | .122 |
| 6 | IL-5* | 1.0 | .964 | 2.1 | .104 | 1.2 | .604 | 1.0 | .958 |
| | IFN-γ | 0.8 | .588 | 0.6 | .092 | 0.7 | .200 | 0.8 | .242 |
| 7 | IL-13 | 2.2 | .086 | **1.5** | **.003** | **3.0** | **<.001** | **2.3** | **<.001** |
| | IFN-γ | 0.5 | .068 | **0.5** | **.005** | **0.4** | **<.001** | **0.5** | **.009** |
| | 12-mo Cytokines | | | | | | | | |
| | Unadjusted | | | | | | | | |
| 8 | IL-4 | 1.1 | .750 | 1.1 | .676 | 1.1 | .810 | 1.3 | .147 |
| 9 | IL-5 | 1.0 | .989 | 1.1 | .840 | 1.0 | .985 | 0.9 | .721 |
| 10 | IL-13 | 1.0 | .950 | 1.0 | .865 | 0.9 | .705 | 1.1 | .687 |
| 11 | IFN-γ | 1.0 | .915 | 1.0 | .833 | 0.7 | .104 | 1.0 | .875 |
| | Adjusted | | | | | | | | |
| 12 | IL-4 | 1.1 | .737 | 1.2 | .440 | 1.2 | .472 | 1.4 | .129 |
| | IFN-γ | 1.0 | .904 | 0.9 | .510 | **0.7** | **.050** | 0.9 | .507 |
| 13 | IL-5 | 1.0 | .930 | 1.1 | .734 | 1.1 | .751 | 0.9 | .682 |
| | IFN-γ | 1.1 | .811 | 0.9 | .713 | 0.7 | .083 | 1.0 | .977 |
| 14 | IL-13 | 1.0 | .992 | 1.0 | .934 | 1.0 | .883 | 1.1 | .553 |
| | IFN-γ | 1.0 | .961 | 0.9 | .732 | 0.7 | .142 | 0.9 | .674 |

*OR*, Odds ratio. Boldfaced values indicate $P < .05$.
*Dichotomized: detectable/undetectable.