

Reduced Interferon γ Production and Soluble CD14 Levels in Early Life Predict Recurrent Wheezing by 1 Year of Age

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It is unknown whether reduced production of IFN γ in early life, before any lower respiratory tract illness, is a risk factor for recurrent wheezing in infancy. We followed 238 infants prospectively from birth to 1 year of age. At birth and at 3 months of age, IFN γ production from polyclonally stimulated peripheral blood mononuclear cells and soluble CD14 (sCD14) levels in plasma were measured. The odds of developing recurrent wheezing (assessed by questionnaire) in the first year of life were up to 4.5 times higher for children in the lowest quartile of IFN γ production at 3 months ($p = 0.0005$) and 3.2 times higher for children in the lowest quartile of sCD14 levels at birth ($p = 0.004$) as compared with children in the other 3 combined quartiles of IFN γ and sCD14, respectively. Findings were confirmed in the multivariate analysis. IFN γ production at 3 months and sCD14 levels at birth were correlated ($r = 0.188$, $p = 0.031$). Our findings from a longitudinal cohort suggest that impaired IFN γ production at 3 months and reduced plasma-sCD14 levels at birth significantly increase the risk of developing recurrent wheezing in the first year of life.

Keywords: wheezing; asthma; IFN γ ; CD14 antigen; LPS receptor

Approximately one out of three children wheezes in the first year of life, usually in association with viral infections (1). In most cases, wheezing in infancy represents a transient condition that has no apparent effect on later health (2). However, for a subgroup of infants who wheeze repeatedly, this condition is associated with the subsequent development of asthma (3) and with long-term, possibly permanent reductions in lung function. In fact, for children with the most severe forms of asthma, the onset of respiratory symptoms usually occurs very early in life (4). Therefore, understanding the risk factors for early recurrent wheezing may have important implications for both researchers and clinicians.

Although environmental risk factors for infection, such as exposure to other children, are primary risks for early wheezing, alterations in the maturation of the immune system also appear to influence host susceptibility. When measured apart from acute infectious episodes, production of IFN γ has been consistently found to be reduced among children with both atopic (5) and nonatopic (6, 7) wheezing. Studies of infants during the acute phase of respiratory syncytial virus infection, the most common cause of wheezing in the first year of life, also reported suppressed production of IFN γ from stimulated peripheral blood mononuclear cells (8). In addition, an inverse relation between IFN γ expression, as measured by the levels of messenger RNA,

and the severity of the disease was found (9). However, given the cross-sectional nature of all these studies, it is unclear whether the altered production of IFN γ is a predisposing factor for wheezing or is simply a consequence of the accompanying inflammatory process (10).

If a relation between low IFN γ production and early wheezing exists, the factors that affect the maturation of IFN γ responses early in life might play an important role in the development of this condition as well. CD14, a sentinel molecule of the constitutive innate immune system that acts as a pattern recognition receptor for endotoxin (LPS, derived from cell walls of gram-negative bacteria), is one such factor. IFN γ production has been shown to correlate positively with the level of exposure to endotoxin among infants (11) and with plasma-soluble CD14 levels themselves among 11-year-old children (12). Taken together, this evidence suggests that early in life, the CD14-mediated response to LPS exposure may play an important role in enhancing the maturation of IFN γ production (13), and, consequently, preventing the inception of recurrent wheezing.

The aim of our study was to determine, in a birth cohort followed longitudinally, whether impaired IFN γ production and/or reduced plasma-soluble CD14 levels in early life are significant risk factors for the development of recurrent wheezing in the first year of life. Some of the results of this study have been previously reported in the form of an abstract (14).

METHODS

The Infant Immune Study is a prospective study of the development of immunologic markers of asthma risk in childhood. Pregnant women obtaining care for their newborns through collaborating pediatricians were contacted at 32 to 35 weeks gestation. Questionnaires regarding health during pregnancy, parental respiratory health, and demographic characteristics were obtained before the infant's birth. At 1, 2, 3, 6, 9, and 12 months of age, parents completed questionnaires assessing the child's passive smoke exposure, breastfeeding status, and exposure to other children (defined as either the presence of $\geq$ one older sibling or day care attendance with other children in the first three months of life).

Data about the presence of a physician-confirmed diagnosis of eczema and the infant's respiratory health were obtained from parents in a questionnaire completed at 12 months of age (mean age $\pm$ SD: 1.06 ± 0.13 years). Parents were asked if the infant's chest had ever sounded wheezy or whistling and, if yes, how often on a five-point scale from (1) "Very rarely" to (5) "On most days." A ranking of "1" on this scale was defined as infrequent wheezing, whereas a ranking of "2" or more was defined as recurrent wheezing. Age at the first episode of wheeze was also obtained.

Blood specimens were obtained twice in the infant's first year of life: at birth (from the umbilical cord), and by venipuncture at 3 months of age (mean age: 2.9 ± 1.3 months). Soluble CD14 (sCD14) levels were measured using a commercially available ELISA kit supplied by R&D Systems Inc. (Minneapolis, MN). Total serum IgE were measured with the Pharmacia & Upjohn (Kalamazoo, MI) AutoCAP FEIA assay.

Lymphocyte stimulations were performed on peripheral or cord

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blood mononuclear cells separated by standard density gradient centrifugation. Cells were stimulated with 10 μ g/ml Concanavalin A (Pharmacia, Piscataway, NJ) and 10 ng/ml phorbol myristate acetate (Sigma Chemical Co., St. Louis, MO). Supernatant fluids from these cultures and unstimulated control tubes were harvested after 18 to 24 hours and stored at -70°C for later testing for cytokines. The supernatants were assayed for IFN γ production using a commercially available kit (Genzyme, Minneapolis MN).

Statistical Analyses

χ^2 test and standard parametric procedures (*t* test, analysis of variance, Pearson correlation coefficient) were used. Values of IgE, sCD14, and IFN γ production at birth and 3 months were log-transformed due to the right-skewness of their distributions. To adjust for the correlation between measures obtained at birth and 3 months in the same subjects, random effects models (15) were used to compare IgE levels between children with and without wheezing.

On the basis of our working hypothesis, levels of IFN γ and sCD14 were categorized into the lowest quartile versus the other three quartiles combined (medium-high levels). Log-rank tests were used to compare the survival curves (time to the first episode of wheezing) between children with low- and medium-high levels of IFN γ and sCD14 at birth and 3 months. The relation of low IFN γ production and low sCD14 levels to wheezing was tested for potential confounding in multinomial logistic regression models, which determined odds ratios (OR) for both infrequent and recurrent wheezing in the same multivariate model after adjusting for the other variables examined. To include individuals with missing data, a missing category was created for categorical variables, whereas missing IgE values ($n = 48$) were replaced with the mean value. The study was approved by the University of Arizona Institutional Review Board. Informed consent was obtained from the mothers at enrollment.

RESULTS

To date, 894 women have been contacted for potential enrollment in the Infant Immune Study study, of whom 444 (49.7%) agreed to participate. There was no difference between participants and nonparticipants in relation to ethnicity, maternal and paternal age, and paternal education. However, as compared with mothers who refused to participate, those who enrolled in the study were more likely to report a positive history for asthma (20.6 vs. 10.8%, $p < 0.005$) and had on average more years of education (mean $\pm$ SD: 15.0 ± 2.8 vs. 14.4 ± 2.6 , $p < 0.05$).

As of the time of this report, 256 children had completed 1-year questionnaires. Among them, 238 children also had sCD14 levels and/or IFN γ production measured, and they represent our study population. Of these infants, 94 (39.5%) experienced wheezing episodes during the first year of life, and 41 of these (17.2%) experienced recurrent wheezing.

We compared the characteristics of infants with no wheezing, infrequent wheezing, and recurrent wheezing (Table 1). The distribution by sex and ethnicity was similar in the three groups. Both young maternal age and low parental education level tended to increase the risk for wheezing, but these associations did not reach statistical significance. Risk factors for wheezing included breastfeeding for less than 3 months, early exposure to other children, and physician-confirmed diagnosis of eczema. As expected, a physician diagnosis of lower respiratory tract illness was much more common among children in the wheezing groups than among children with no wheezing (31.9 vs. 7.6%, $p < 0.0001$). IgE levels at 3 months but not at birth were significantly higher in children with wheezing than in those with no wheezing ($p < 0.05$).

In Figure 1, the distributions for IFN γ production and sCD14 levels are shown in box plots for birth and 3 months. All these variables were skewed toward higher values. After log-transformation, their distributions showed satisfactory normalization. There were no differences between subjects with missing and

nonmissing measurements of either IFN γ or sCD14 at any point in time in relation to ethnicity, parental asthma status, parental age or parental education. Male infants were, however, more likely to have missing measurements of IFN γ production at 3 months than female infants (38.8 vs. 24.6%, respectively; $p = 0.02$).

The proportion of children who did and did not develop infrequent and recurrent wheezing within the groups of low and medium-high IFN γ production and sCD14 levels are shown in Table 2. The odds of developing recurrent (but not infrequent) wheezing were up to 4.5 times higher for children in the lowest quartile of IFN γ production at 3 months ($p = 0.0005$) and 3.2 times higher for children in the lowest quartile of sCD14 levels at birth ($p = 0.004$) as compared with children with medium-high levels of IFN γ and sCD14, respectively. The effects of reduced IFN γ and sCD14 levels on the risk of wheezing appeared to be additive because up to 90% (10 out of 11) of children with both low IFN γ at 3 months and low sCD14 at birth experienced infrequent or recurrent wheezing in the first year of life. In contrast, low IFN γ production at birth and low sCD14 levels at 3 months showed no relation with wheezing in the first year. The increased risk for wheezing appeared to be specific for individuals in the lowest quartiles of IFN γ production at 3 months and sCD14 levels at birth, as the proportion of subjects with wheezing neither differed significantly among the intermediate quartiles and the highest quartile for IFN γ at 3 months nor for sCD14 at birth (data not shown). We also found no differences in wheezing rates between infants with missing and nonmissing measurements of either IFN γ or sCD14 at any point in time. When the analysis relating IFN γ to wheezing was redone after removing 23 children who had the onset of the first wheezing episode by 3 months of age, low IFN γ production at 3 months was still a significant predictor of the risk for recurrent wheezing (3.6, 1.3–9.8).

In Figure 2, the time to the first wheezing episode associated with low versus medium-high levels of IFN γ at 3 months and sCD14 at birth are shown in separate survival curves. Wheezing occurred significantly earlier for subjects with low IFN γ production at 3 months as compared with subjects with medium-high levels ($p = 0.0007$). Survival curves of these two groups diverged. Similarly, children with low sCD14 levels at birth showed a survival curve significantly steeper than that of children with medium-high sCD14 levels ($p = 0.019$). After removing the 23 children with onset of wheezing by 3 months of age, these findings were confirmed for children with low IFN γ production at 3 months (data not shown). Neither sCD14 levels at 3 months nor IFN γ production at birth had any predictive value for the age of onset of wheezing (data not shown).

We found a significant increase from birth to 3 months of both IFN γ production (geometric means: 336–588 pg/ml) and sCD14 levels (0.73–1.52 μ g/ml). Of note, a correlation matrix including IFN γ and sCD14 at birth and 3 months showed that only IFN γ production at 3 months and sCD14 levels at birth were significantly correlated ($r = 0.188$, $p = 0.031$). IFN γ production at birth and 3 months also tended to correlate ($r = 0.224$, $p = 0.073$), but there was no correlation between sCD14 levels at birth and 3 months ($r = -0.010$, $p = \text{not significant}$). Infants with low sCD14 levels at birth showed a reduced maturation of IFN γ production as compared with infants with medium-high sCD14 levels, although the association did not reach statistical significance. Geometric means for IFN γ production increased from 338 pg/ml at birth to 667 pg/ml at 3 months in the latter group but only from 360 to 461 pg/ml for those in the lowest quartile of sCD14 levels at birth.

After adjusting for sex, ethnicity, eczema, early exposure to other children, maternal age, IgE levels, and sCD14 levels at

TABLE 1. COMPARISON BETWEEN INFANTS WITH NO WHEEZING, INFREQUENT WHEEZING, AND RECURRENT WHEEZING

	No Wheezing (n = 144)	Any Wheezing	
		Infrequent (n = 53)	Recurrent (n = 41)
Sex, male, %	48.60	45.30	53.70
Parental ethnicity, %			
Both parents non-Hispanic white	60.40	60.40	48.80
White/Hispanic	13.20	17.00	12.20
Hispanic/Hispanic	13.20	9.40	17.10
Other	13.20	13.20	22.00
Maternal age, yr; mean $\pm$ SEM	29.5 $\pm$ 0.5	27.4 $\pm$ 0.7	28.7 $\pm$ 1.1
Paternal age, yr; mean $\pm$ SEM	31.6 $\pm$ 0.6	29.9 $\pm$ 0.8	31.0 $\pm$ 1.1
Maternal education, yr; mean $\pm$ SEM	15.2 $\pm$ 0.2	14.9 $\pm$ 0.4	14.7 $\pm$ 0.3
Paternal education, yr; mean $\pm$ SEM	15.0 $\pm$ 0.3	14.8 $\pm$ 0.3	14.5 $\pm$ 0.4
Ever maternal asthma, %	20.90	17.60	25.00
Ever paternal asthma, %	15.90	12.80	25.70
Breastfed at least 3 mo, %*	66.20	51.90	53.80
At least one parent smoker, %	22.90	28.30	31.70
Early exposure to other children, %†	61.70	84.30	75.70
Lower respiratory illness in the first yr, %‡	7.60	28.30	36.60
Physician-confirmed eczema, %§**	13.20	32.10	31.70
IgE levels in IU/ml, geometric mean (95% CI)			
At birth, n = 206	0.21 (0.17, 0.26)	0.21 (0.16, 0.28)	0.32 (0.20, 0.53)
At 3 mo, n = 190*	1.04 (0.84, 1.28)	1.54 (1.13, 2.10)	1.54 (0.91, 2.60)

Definition of abbreviation: CI = confidence interval.

Statistical comparisons were performed both across the three groups and between children with no wheezing and any wheezing.

* No wheezing versus any wheezing; $p < 0.05$.

† No wheezing versus any wheezing; $p < 0.005$.

‡ χ^2 test across the three groups; $p < 0.01$.

§ No wheezing versus any wheezing; $p < 0.0001$.

|| χ^2 test across the three groups; $p < 0.0001$.

¶ χ^2 test across the three groups; $p < 0.005$.

** No wheezing versus any wheezing; $p < 0.0005$.

birth, the risk for recurrent wheezing among infants with low IFN γ production at 3 months remained significant (adjusted odds ratio 4.7, $p = 0.002$) (Table 3). Similarly, having low sCD14 levels at birth increased by almost 3 times the odds of developing recurrent (but not infrequent) wheezing. There was no significant interaction between low IFN γ and low sCD14 levels in predicting infrequent or recurrent wheezing, although statistical power might have been insufficient for detecting any effect modification. Eczema was the only factor significantly associated with both infrequent and recurrent wheezing. In contrast, early exposure to other children, young maternal age, and high IgE levels at 3 months were significant risk factors for infrequent but not recurrent wheezing. All the associations, with the exception of

eczema and infrequent wheezing, remained significant when the model was retested without substituting missing IgE levels. In this model, IgE levels showed even higher adjusted odds ratios for infrequent wheezing (2.7, $p = 0.021$).

DISCUSSION

This study provides the first evidence that reduced IFN γ production in early life is associated with an increased risk of developing recurrent wheezing by 1 year of age. This observation was specific for recurrent wheezing. Both low IFN γ production at 3 months and low sCD14 levels at birth were associated with early onset of wheeze, and they correlated significantly and directly with

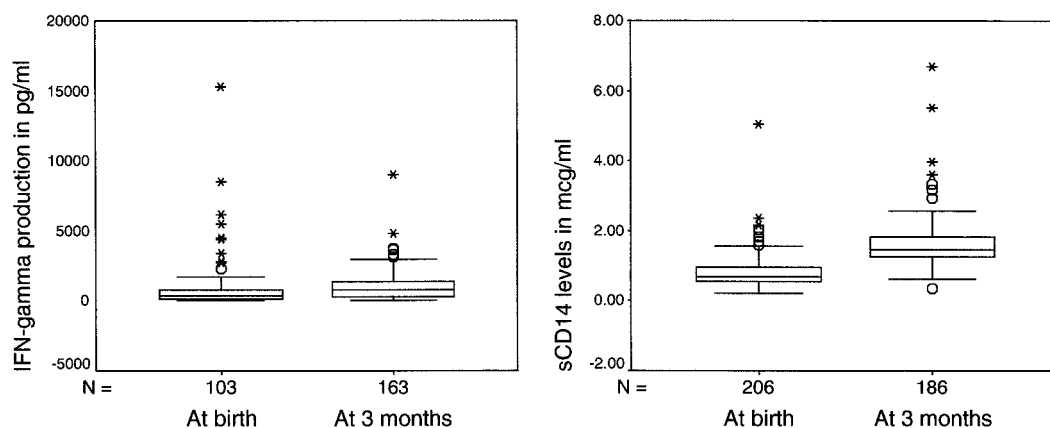


Figure 1. Distribution of IFN γ production and soluble CD14 (sCD14) levels at birth and 3 months. Box plots show the median, interquartile range, adjacent values (lines projecting from the box), outliers (circles), and extreme cases (stars). Adjacent values are observations not more than 1.5 times, outliers observations between 1.5 and 3 times, and extreme cases observations more than three times the height of the box beyond either quartile. One extreme observation of

IFN γ at 3 months higher than 20,000 pg/ml is not shown for graphic reasons. All the four distributions were skewed toward higher values. Skewness statistics: 4.9 for IFN γ at birth, 10.2 for IFN γ at 3 months, 4.1 for sCD14 at birth, and 3.3 for sCD14 at 3 months.

TABLE 2. PROPORTION OF CHILDREN WITH NO WHEEZING, INFREQUENT WHEEZING, AND RECURRENT WHEEZING IN THE GROUPS WITH LOW AND MEDIUM-HIGH IFN γ PRODUCTION AND SCD14 LEVELS

	No Wheezing % (n)	Any wheezing	
		Infrequent % (n)	Recurrent % (n)
IFN γ production at birth, n = 103			
Low	72.0 (18)	12.0 (3)	16.0 (4)
Medium-high	57.7 (45)	23.1 (18)	19.2 (15)
OR, 95% CI	1.00	0.42 (0.11–1.59)	0.67 (0.20–2.28)
IFN γ production at 3 mo, n = 163*			
Low	40.0 (16)	25.0 (10)	35.0 (14)
Medium-high	67.5 (83)	19.5 (24)	13.0 (16)
OR (95% CI)	1.00	2.16 (0.87–5.38)	4.54 (1.85–11.1) [†]
SCD14 levels at birth, n = 206 [‡]			
Low	47.1 (24)	23.5 (12)	29.4 (15)
Medium-high	65.2 (101)	21.9 (34)	12.9 (20)
OR (95% CI)	1.00	1.48 (0.67–3.29)	3.16 (1.41–7.05) [§]
SCD14 levels at 3 mo, n = 186			
Low	69.6 (32)	13.0 (6)	17.4 (8)
Medium-high	58.6 (82)	22.1 (31)	19.3 (27)
OR (95% CI)	1.00	0.50 (0.19–1.30)	0.76 (0.31–1.85)

Definition of abbreviations: CI = confidence interval; OR = odds ratio; sCD14 = soluble sCD14.

Low vs. medium-high groups had the following medians (interquartile ranges): 68 (41–102) vs. 540 (265–1,215) pg/ml for IFN γ at birth; 156 (55–211) vs. 1,020 (650–1,613) pg/ml for IFN γ at 3 mo; 0.46 (0.37–0.51) vs. 0.77 (0.65–1.11) μ g/ml for sCD14 at birth; 1.06 (0.97–1.16) vs. 1.63 (1.40–1.99) μ g/ml for sCD14 at 3 months.

* χ^2 test across the three wheezing categories: $p < 0.005$.

[†] $p = 0.0005$.

[‡] χ^2 test across the three wheezing categories: $p < 0.05$.

[§] $p = 0.004$.

each other. After removing the children with onset of wheezing by 3 months of age, low IFN γ production at 3 months was still a significant predictor of wheezing in the first year of life.

Several previous studies have shown that altered IFN γ responses are associated with wheezing during childhood. Among 5- to 15-year-old children, Leech and coworkers (7) found that both CD4- and CD8-positive T cells from children

with nonatopic wheezing produced significantly less IFN γ than those from nonwheezing control subjects. Similarly, Koning and coworkers (6) reported reduced IFN γ responses by nonspecifically stimulated T cells in preschool children without atopy but with recurrent wheezing as compared with healthy subjects.

Studies on cytokine production during the acute phase of lower respiratory illness have reported apparently conflicting

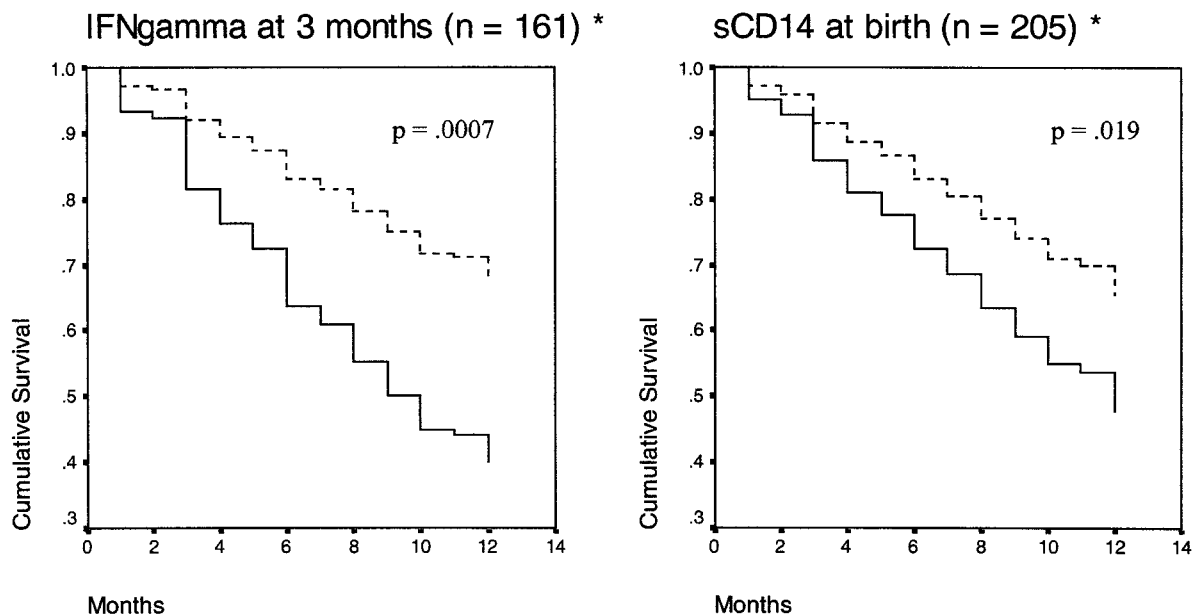


Figure 2. Survival curves for wheezing by IFN γ production at 3 months (solid line, low IFN γ , dashed line, medium-high IFN γ) and sCD14 levels at birth (solid line, low sCD14, dashed line, medium-high sCD14). p Values represent log-rank tests. *Numbers are slightly lower than those shown in Table 2 because of cases who failed to report age at first wheezing episode.

TABLE 3. ADJUSTED ODDS RATIO (WITH 95% CONFIDENCE INTERVAL AND P VALUES) ASSOCIATED WITH INFREQUENT AND RECURRENT WHEEZING FOUND IN MULTINOMIAL LOGISTIC REGRESSION

	Infrequent Wheezing		Recurrent Wheezing	
	Adjusted OR	95% CI	Adjusted OR	95% CI
Low IFN γ at 3 mo	1.31	0.46–3.73	4.27	1.57–11.6
Low sCD14 at birth	1.08	0.45–2.62	3.60	1.41–9.18
Sex, male	0.65	0.31–1.33	1.18	0.54–2.61
Parental ethnicity*				
White/Hispanic	1.31	0.48–3.55	1.41	0.43–4.65
Hispanic/Hispanic	0.34	0.10–1.15	2.38	0.72–7.90
Other	0.86	0.30–2.48	2.09	0.73–6.00
Physician-confirmed eczema	2.63	1.14–6.05	3.13	1.25–7.84
Early exposure to other children	4.76	1.87–12.1	1.36	0.52–3.52
Maternal age, yr	0.91	0.85–0.97	1.01	0.95–1.09
IgE levels at 3 mo†	2.27	1.03–5.02	1.31	0.56–3.05

Definition of abbreviations: CI = confidence interval; OR = odds ratio; sCD14 = soluble sCD14.

Boldface indicates significant predictors; n = 238.

* White/white is the reference group.

† Log-transformed IU/ml.

findings with respect to IFN γ . van Schaik and coworkers (16) found higher concentrations of IFN γ in the respiratory secretions of children with virus-induced wheezing than in those of children with uncomplicated upper respiratory infection. In contrast, Román and coworkers (8) reported suppressed IFN γ production from stimulated peripheral blood mononuclear cells of infants with acute respiratory syncytial virus infection. Although such differences might relate to tissue-specific responses, Aberle and coworkers (9) found higher IFN γ expression in peripheral blood mononuclear cells of infants with respiratory syncytial virus illness than in those of healthy control subjects but only in the presence of a moderate rather than severe form of the disease. In fact, within the group of diseased children, IFN γ expression correlated inversely with the severity of the disease. Apart from the methodological differences that make these findings difficult to compare, it is of note that these studies were all cross-sectional in nature, leaving the temporal relationship between the alteration of IFN γ responses and the onset of wheezing unknown.

Maturation of IFN γ production in early life is likely to be affected by both genetic and environmental factors. We hypothesized sCD14 to be among these factors. Given its pivotal role in innate immunity as a “sentinel molecule,” serum sCD14 levels have been investigated in several disease states, including septic shock (17), infectious and autoimmune diseases (18, 19), as well as asthma. Interestingly, elevated serum sCD14 levels have been postulated to play a predisposing role in asthma exacerbations (20) but, at the same time, a protective role in asthma inception (13). This protective effect is believed to be exerted during a critical period early in life, when the development of the immune system requires environmental signals that trigger precoded maturational mechanisms. Such environmental signals include exposure to microbial endotoxin (21) that, in turn, involves processes mediated in part by the receptor CD14. Therefore, it is plausible to hypothesize that elevated sCD14 levels might be associated with the postnatal maturation of IFN γ production.

Our findings support this scenario in several ways. They confirm that the maturation of IFN γ production begins very early in life, as we found mitogen-induced IFN γ expression much higher at 3 months than at birth. They also suggest that sCD14 levels may be able to affect this maturation as early as in the first 3 months of life because sCD14 levels at birth correlated positively and significantly with IFN γ production at 3 months. However, it should be acknowledged that this correlation cannot

represent proof that sCD14 levels and IFN γ production are in the same causal pathway to recurrent wheezing, as we cannot exclude the presence of potential confounders, such as unknown common risk factors for low levels of both sCD14 and IFN γ . Previous studies have shown that exposure to higher levels of house dust endotoxin is associated with lower prevalence of allergic sensitization in children (22) and that lifestyle factors that correlate with endotoxin exposure, such as farming (23) and having indoor dogs (24), may prevent asthma-like symptoms when these factors begin very early in life. In apparent contrast, Litonjua and coworkers found high concentrations of house dust endotoxin to be associated with an increased risk of wheezing among preschool age children, but this trend appeared to reverse as children got older (25). Whether these effects of endotoxin exposure are mediated by an enhanced maturation of IFN γ responses remains to be determined. The levels of endotoxin exposure might also be important, as suggested by a recently reported nonlinear U-shaped relationship between levels of house dust endotoxin exposure and risk of repeated wheezing among infants at risk for asthma and allergy (26).

sCD14 levels at birth and IFN γ production at 3 months correlated directly and significantly and, apart from the effect of other confounders, adjusting low sCD14 levels and IFN γ production for each other in multivariate analyses reduced the magnitude of their association with recurrent wheezing by approximately 10% (data not shown), possibly because of the existence of a common pathway. However, each still showed consistently high and significant odds ratios for recurrent wheezing in the multivariate analysis, suggesting the existence of additional independent pathways. This is not surprising because it is very likely that other genetic and environmental factors may influence the maturation of IFN γ responses, independent of sCD14 levels. Similarly, sCD14 levels themselves may affect the risk for wheezing through mechanisms that are independent of IFN γ maturation, e.g., by mediating the innate immune response to wheezing-inducing pathogens, as recently reported for respiratory syncytial virus (27).

There are several potential mechanisms through which impaired IFN γ production might be associated with recurrent wheezing early in life. On the one hand, low IFN γ production may reflect an increased susceptibility to developing severe reactions to viral infections, the major trigger for wheezing episodes in the first year of life. Reduced IFN γ production may reflect

either decreased maturation of CD8⁺ T cells or decreased numbers of natural killer cells or Th1 cells. These deficits may, in turn, affect macrophage responses to viruses, leading to more invasive infections and decreased viral clearance from the lung. Recent findings (28) suggest that, at least in mice, IFN γ might play a role in limiting viral replication and inflammatory responses in respiratory syncytial virus infection. Alternatively, impaired IFN γ production may facilitate skewing toward Th2 differentiation of T cells, which may subsequently lead to atopic wheezing. Reduced production of IFN γ at birth has already been shown to be a hallmark of newborns at high risk of allergy (29) and a significant risk factor for the subsequent development of atopy (30). Consistent with this interpretation, we have already shown in a different birth cohort that IFN γ production at 9 months was inversely related to skin test reactivity at the age of 6 years (31).

Whether the mechanisms leading to low IFN γ production very early in life are different among subjects who will develop atopy- versus nonatopy-related wheezing is unknown. It is possible that an alteration of IFN γ production early in life may induce an increased susceptibility to viral wheezing through the same mechanisms, whether or not the infant is predisposed to becoming atopic. Consistently, reduced IFN γ remained a significant risk factor for recurrent wheezing also after adjusting for IgE levels and the presence of eczema. Additional genetic and developmental factors would then determine which subjects will go on to develop Th2-mediated responses, persistent wheezing and, in turn, atopic asthma. Although there is a lack of experimental and epidemiologic data that directly test this hypothesis, indirect evidence is provided by the fact that secular trends show a similar increase for both atopic and nonatopic wheezing, suggesting the existence of common underlying mechanisms (32).

Although the focus of this study was the relation of IFN γ production and sCD14 levels to wheezing, the multivariate analyses provide evidence of other risk factors. A physician-confirmed diagnosis of eczema was the only risk factor significantly associated with both recurrent and infrequent wheezing. However, the risk of a differential misclassification should be considered in interpreting this association because physicians might be more likely to label skin reactions as eczema in the presence of a positive history for another potential atopic symptom, such as wheezing. Early exposure to other children increased the odds for infrequent wheezing by almost five times. An increased risk for wheezing associated with day care attendance among preschool children, probably related to an increased exposure to infections, has been shown by previous studies (33, 34). In the multivariate analysis, we found young maternal age to be another significant risk factor for infrequent wheezing. This association has been already described (35, 36) and is likely to be related to both biological and socioeconomic factors. Finally, IgE levels at 3 months of age were also directly associated with the odds for infrequent but not recurrent wheezing. Interestingly, IgE levels were significantly higher only among subjects with the onset of wheezing after 6 months of age (data not shown), consistent with our previous report of an inverse relationship between cord IgE levels and lower respiratory tract illness events occurring before but not after 6 months of age (37). High IgE levels might be a marker for a process that delays the development of recurrent wheezing for infants, who may still be at increased risk for the development of repeated wheezing and asthma later in life.

There are some limitations to our study. Only approximately 50% of the women contacted for possible enrollment in the Tucson Infant Immune Study accepted to participate. Although these positive acceptance rates may not be surprising given the high level of commitment required by the participants in this

study (questionnaires completed on average on a bimonthly basis in the first year, blood samples drawn from infants frequently and as early as at 3 months of age, etc.), they might jeopardize the generalizability of our findings. However, it is noteworthy that mothers who participated in the study differed from those who refused participation only in their greater asthma prevalence and higher level of education. Among the 238 subjects included in the study, a variable proportion had missing measurements of sCD14 levels and IFN γ production either at birth or at 3 months, leading to some possible selection bias. Although we cannot exclude such a bias, it appears unlikely because the proportion of children with wheezing was very homogeneous ($\sim 40\%$) across all the groups regardless of missing values. Because we did not obtain environmental samples, we could not investigate whether the level of exposure to microbial burden might modulate the relation between sCD14, IFN γ responses, and wheezing. Furthermore, although the association between reduced IFN γ production at 3 months and recurrent wheezing was still present after removing children with onset of wheezing by 3 months of age, it should be acknowledged that the prospective nature of this relationship remains to a certain extent unclear as it is dependent on parental recall of the age of onset.

In conclusion, our findings from a longitudinal cohort suggest that impaired IFN γ production at 3 months and reduced plasma levels of sCD14 at birth increase significantly the risk of developing recurrent wheezing in the first year of life.

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