

Low IFN- γ production in the first year of life as a predictor of wheeze during childhood

Debra A. Stern, MS,^a Stefano Guerra, MD, PhD,^{a,b} Marilyn Halonen, PhD,^{a,c}
Anne L. Wright, PhD,^{a,d} and Fernando D. Martinez, MD^{a,d} Tucson, Ariz

Background: Diminished cytokine production in infancy has been associated with an increased risk for allergen sensitization and early-life wheeze.

Objective: We sought to assess the effect of low cytokine production in the first year of life on the development of wheeze through age 13 years.

Methods: Cytokine production (IFN- γ and IL-2) by mitogen-stimulated mononuclear cells was determined from peripheral blood samples (9.4 months, $n = 118$) in a subset of healthy infants enrolled in the Tucson Children's Respiratory Study. The occurrence of wheeze during the previous year was ascertained at ages 2, 3, 6, 8, 11, and 13 years by means of questionnaire. Relative risk for wheeze was computed with generalized estimating equations.

Results: The risk of wheezing between 2 and 13 years was significantly higher for subjects with low 9-month IFN- γ production (relative risk, 2.29; 95% CI, 1.35-3.89) and borderline significant for those with intermediate IFN- γ production (relative risk, 1.59; 95% CI, 0.95-2.68) compared with those who produced high levels of IFN- γ (P value for linear association = .002). Nine-month IL-2 production was unrelated to wheeze. In relation to complex wheezing phenotypes, 9-month IFN- γ production was inversely related to toddler wheeze (occurring only before age 6 years, $P = .03$) and chronic wheeze (occurring before and after age 6 years, $P = .007$) but not school-age wheeze (occurring only after age 6 years, $P = .06$).

Conclusion: The results suggest that characteristics of the immune system present during the first year of life can anticipate the likelihood of development of episodes of airway obstruction characterized by wheezing.

Clinical implications: Immune susceptibility to asthma is established very early during postnatal life. (*J Allergy Clin Immunol* 2007;120:835-41.)

Key words: Asthma, wheeze, IFN- γ , IL-2, cytokine, infancy

Recent evidence suggests that events occurring during the first years of life have an important role in the inception of many cases of childhood asthma.¹ Longitudinal studies

Abbreviations used

GEE: Generalized estimating equation

PMA: Phorbol 12-myristate 13-acetate

RR: Relative risk

RSV-LRI: Respiratory syncytial virus-induced lower respiratory tract illness

sqrt unit: Square root of the unit

in which follow-up was started at birth have suggested that children who will go on to have asthma have alterations in the developmental processes that characterize the maturation of the immune system and the airways.² These processes appear to be regulated both by environmental exposures³ and hereditary factors.⁴⁻⁷

Production of IFN- γ by PBMCs has been used as a potential marker for the postnatal immune maturation processes that are associated with the subsequent risk for development of asthma and allergies.⁸ Macaubas et al⁹ reported an association of low levels of IFN- γ production in cord blood and increased risk of allergic outcomes at age 6 years in high-risk children. Neaville et al¹⁰ reported decreased IFN- γ levels in cord blood associated with increased sensitization to egg whites at 1 year in high-risk children. In addition, Guerra et al,¹¹ using data from the Infant Immune Study, reported that low IFN- γ production at 3 months of age was associated with recurrent wheeze in the first year of life in a sample of children representative of the general population. Using the same data as the current analyses, we previously reported that low IFN- γ production at 9 months was associated with a decreased risk of atopic sensitization at age 6 years,¹² but the association between IFN- γ responses and wheezing was not assessed.

It has been shown that exposure to bacterial products, such as endotoxin, stimulates the development of IFN- γ responses in infants,³ and thus it has been speculated that IFN- γ responses could be one pathway by which exposure to other children in day care, to pets, and to a farming environment might alter risk for development of asthma and allergies.¹³⁻¹⁵ However, it is at present not known whether production of IFN- γ in early life is associated with subsequent development of asthma and asthma-like symptoms after the first years of life.

The purpose of this study was to assess the association between mitogen-stimulated IFN- γ and IL-2 production by PBMCs at 9 months of age and the subsequent development of wheeze up to the age of 13 years.

From ^aThe Arizona Respiratory Center, Departments of ^cPharmacology and ^dPediatrics, College of Medicine, and ^bthe Mel and Enid Zuckerman College of Public Health, University of Arizona.

Supported by National Heart, Lung, and Blood Institute grants HL-14136 and HL-56177.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication August 17, 2006; revised May 18, 2007; accepted for publication May 25, 2007.

Available online August 10, 2007.

Reprint requests: Fernando D. Martinez, MD, Arizona Respiratory Center, University of Arizona HSC, Tucson, AZ 85724. E-mail: fernando@arc.arizona.edu.

0091-6749/\$32.00

© 2007 American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2007.05.050

METHODS

Subjects

Parents of 1246 infants who were members of a health maintenance organization in Tucson, Arizona, agreed to have their healthy newborns participate in the Tucson Children's Respiratory Study, a longitudinal prospective study of respiratory illness.¹⁶ Tucson has a population of about 500,000 (Census 2000) and is located in the desert southwest, and study subjects lived primarily in suburban neighborhoods. Infants were enrolled between May 1980 and October 1984. After February 1983, studies of blood mononuclear cell function were added to the data-acquisition protocols, and 296 infants subsequently enrolled in the CRS were eligible for these immune studies.¹² Informed consent was obtained from the parents of participating subjects, and the Institutional Review Board of the University of Arizona approved the study.

Sample collection

Blood samples for immune studies were collected from eligible infants at a 9-month well-baby checkup. For this report, the 9-month samples were limited to those drawn at or before 1 year of age (mean age, 9.4 months [SD = 1.1], n = 118; earliest sample collected at 7 months of age).

IFN- γ and IL-2 production by blood mononuclear cells

Methods describing the IFN- γ and IL-2 production by mitogen-stimulated blood mononuclear cells have been previously published.¹² Briefly, blood was collected in heparin (5 μ L/mL), processed within 12 hours of collection, and layered on Lymphocyte Separation Medium (Litton Bionetics, Kensington, Md) after diluting 1:2 with RPMI 1640 medium.¹⁷ The cells at the blood/medium interface were collected and washed twice with Ca⁺⁺- and Mg⁺⁺-free PBS, and cells were resuspended in RPMI 1640 medium. A hemocytometer total cell count and chamber differential were performed to estimate the percentage of mononuclear cells. Immediately after separation, 1×10^6 mononuclear cells were stimulated with 10 μ g/mL concanavalin A (Pharmacia, Piscataway, NJ) and 10 ng/mL phorbol 12-myristate 13-acetate (PMA; Sigma Chemical Co, St Louis, Mo). Supernatant fluids from these cells and from unstimulated cells were harvested after 20 hours and stored at -70°C or 4°C for IFN- γ and IL-2 measurement, respectively.

IFN- γ and IL-2 measurement

The IL-2 bioassay has been described in detail previously.¹⁸ Murine IL-2-dependent CTLL-2 cells were added to serial log₂ dilutions of supernatants in triplicate, with final supernatant concentrations ranging from 25% to 0.5%. Cells were incubated for 40 hours and were pulsed with tritiated thymidine. Thymidine incorporation was measured and converted to units of IL-2 activity by means of probit analysis based on a standard for IL-2 prepared from rat spleen cells¹⁷ containing 1 unit of activity per 0.1 mL. IFN- γ measurements were carried out on the supernatant fluids by using a Sindbis virus-WISH cell system bioassay.¹⁷ Triplicate log₃ serial dilutions of the fluids were incubated with the WISH-cell monolayers in flat-bottom microtiter wells for 8 hours before the addition of a previously titrated amount of Sindbis virus. The mean the highest log₃ dilution of supernatant fluid causing a 50% reduction in cytopathic effect was determined as the end point, and the reciprocal of this dilution was recorded as the IFN- γ titer. Each assay included a leukocyte IFN standard (National Institutes of Allergy and Infectious Diseases, Bethesda, Md) to control for reproducibility of the test system. Virus titrations in supernatant fluids from unstimulated cells, medium containing concanavalin A and PMA, and medium only were used as negative controls.

Phenotypic and socioeconomic data

Respiratory health questionnaires were completed for all children enrolled in the Children's Respiratory Study by an adult caregiver (usually a parent). For the children with 9-month immune studies (n = 118), questionnaires were completed at ages 2 (mean, 1.5 years [SD = 0.3]; n = 114), 3 (mean; 3.0 years [SD = 0.4]; n = 100), 6 (mean, 6.3 years [SD = 0.7]; n = 112), 8 (mean, 8.1 years [SD = 0.4]; n = 91), 11 (mean, 10.8 years [SD = 0.5]; n = 104), and 13 (mean, 12.9 years [SD = 0.3]; n = 65) years. On the questionnaires, the adult caregiver was asked whether the child's chest had ever sounded wheezy or whistling during the previous year and how often this had occurred. At each age, children were then classified as wheezing if the parent reported 1 or more episode of wheeze during the previous year or as nonwheezing if no episodes of wheeze were reported for the previous year. In addition, physician-diagnosed asthma with active symptoms reported during the previous year was defined at each age.

Using the wheezing information from the questionnaires, 3 variables were computed to describe wheezing phenotypes between 2 and 13 years (schema presented in Table I). *Toddler wheeze positive* was defined as reported wheeze on the 2-year questionnaire, the 3-year questionnaire, or both (n = 31), and *toddler wheeze negative* was defined as negative for wheeze on both questionnaires (n = 75). Children who had missing questionnaires at age 2 years, 3 years, or both (n = 12) were excluded from analyses. Children were classified separately with regard to school-age wheeze based on reports of wheezing at ages 6, 8, 11, or 13 years. Children were categorized as *school-age wheeze positive* (n = 29) if wheezing was reported on at least 2 questionnaires, as *episodic school-age wheeze positive* if wheezing was reported on 1 questionnaire (n = 17), and as *school-age wheeze negative* if wheeze was never reported on available questionnaires at those same ages (n = 51). For the latter 2 groups only, children who had at least 3 available questionnaires at age 6 years or older were included in the categorizations. The remaining children with 2 or fewer completed questionnaires between the ages of 6 and 13 years (n = 21) were excluded from analyses. Finally, toddler wheeze and school-age wheeze were combined to compute a third long-term wheezing phenotype composed of 4 groups: no wheeze (n = 56), toddler wheeze only (before age 6 years, n = 7), school-age wheeze only (after age 6 years, n = 12), and chronic wheeze (before and after age 6 years, n = 17). Of the immune study children, 22% (n = 26/118) did not have enough questionnaire data to fully characterize their long-term wheezing phenotype.

Parental smoking, education level, ethnicity, and history of physician-diagnosed asthma were determined by questionnaire at enrollment. The child's ethnicity was determined from the parental report and was categorized into 2 groups: at least 1 non-Hispanic white parent (75%) and Hispanic white parents combined with children of other ethnicities (mixed ethnic background, 25%). Dog ownership was determined at enrollment,¹⁴ and exposure to day care and siblings was determined for the first 6 months of life.¹³ The occurrence of respiratory syncytial virus-associated lower respiratory tract illnesses was assessed prospectively through 3 years of age.¹⁹ Skin prick tests to 7 local aeroallergens (Bermuda grass, *Alternaria alternata*, careless weed, house dust mix, and mesquite, mulberry, and olive tree pollens) were performed on the parents and children at the 6-year survey. Tests were read at 20 minutes, and the sizes of the wheals elicited by each allergen were recorded as the sum of the 2 diameters at right angles to each other (in millimeters). Test responses with wheal sizes of 3 mm or larger were considered positive.

Statistics

IFN- γ titers were normally distributed and IL-2 units were normally distributed after a square-root transformation. When analyzed as continuous variables, IFN- γ results are expressed as titers

TABLE I. Schema for wheezing phenotypes

Long-term wheeze phenotypes	n*	Questionnaire-assessed wheeze during the previous year at ages:					
		2 y	3 y	6 y	8 y	11 y	13 y
		Toddler wheeze†		School-age wheeze†			
No wheeze	56	No	No	No	No	No	No
Toddler wheeze only	7	Yes	Yes	No	No	No	No
School-age wheeze only	12	No	No	Yes	Yes	Yes	Yes
Chronic wheeze	17	Yes	Yes	Yes	Yes	Yes	Yes

*Twenty-six participants had insufficient information to be categorized for long-term wheeze phenotypes.

†Toddler wheeze was defined as wheeze on the 2-year questionnaire, the 3-year questionnaire, or both. School-age wheeze was defined as wheezing on at least 2 questionnaires between ages 6 and 13.

and IL-2 results are expressed as the square root of the units (sqrt units). IFN- γ titers and IL-2 values were categorized into low, intermediate, and high cytokine production groups, as previously published (9-month IFN- γ groups: <2 , $2 < 4$, and ≥ 4 titer; 9-month IL-2 groups: <1 , $1 < 1.4$, and ≥ 1.4 sqrt units), and used as ordinal predictor variables (see Fig E1 in the Online Repository at www.jacionline.org).¹² The Mantel-Haenszel test for linear association was used to assess univariate relations between ordinal cytokine variables and wheeze prevalence at each age. Logistic regression was used to adjust for covariates in analyses at each age. The relation between cytokine production and longitudinal wheeze between ages 2 and 13 years was assessed by using generalized estimating equations (GEEs), which are an extension of the generalized linear mixed models.²⁰⁻²² GEE allows for subjects to have missing data at any time point, accommodates correlated data by including an appropriate within-subject covariance structure, and can adjust for covariates. Selection of the best-fitting intrasubject correlation structure was made by using the quasilielihood or QIC approach.^{23,24} Results were reported by using robust SE estimates. Potential confounding factors tested in the models included parental skin test, education, smoking, and history of physician-diagnosed asthma and child skin test reactivity, ethnicity, dog ownership, day care/sibling exposure, and sex. Atopy was defined as at least 1 positive skin prick test response (binary); the number of positive skin test responses was used as an indicator of the severity of atopy and was used as an ordinal variable (0, 1-3, and 4-7 positive tests). Covariates were tested in marginal analysis, and any factors with significance of less than 0.1 were retained for a final model, and then the best-fit model retained only covariates having a *P* value of less than .1. Subjects missing covariate data (paternal asthma and age 6 skin test) were grouped as a separate category for that variable so that all subjects could be included in the model. Two-sided *P* values of less than .05 were considered significant for all tests. Statistical analyses were performed with SPSS for Windows 14.0 (SPSS, Inc, Chicago, Ill) and STATA 9.0 (StataCorp, College Station, Tex) software.

RESULTS

Immune study subject characteristics

The infants with immune studies were not significantly different from those without immune studies regarding the distribution of sex, ethnicity, and parental characteristics (Table II). In addition, the prevalence of atopy at age 6 years and wheeze during the previous year at ages 2 through 13 years was similar for both groups. Because the availability of data on current wheeze varied at each age, we assessed any potential bias among the participating children with and without follow-up data. Children

with follow-up wheeze data were more likely to have non-smoking, nonasthmatic, non-Hispanic white parents with more years of education compared with those without follow-up data, although these associations were not significant at all ages (see Table E1 in this article's Online Repository at www.jacionline.org).

Dog exposure from birth was associated with significantly higher IL-2 production at 9 months (mean $\pm$ SD: 1.3 ± 0.6 sqrt units [$n = 94$] and 1.7 ± 0.7 sqrt units [$n = 24$] for no dogs and dogs, respectively; *P* = .036) and no significant effect on IFN- γ titers (3.3 ± 1.4 sqrt units [$n = 93$] and 3.6 ± 1.1 sqrt units [$n = 24$] for no dogs and dogs, respectively; *P* = .4). Exposure by 6 months to day care, siblings, or both was unrelated to IL-2 production (1.5 ± 0.7 sqrt units [$n = 72$] and 1.3 ± 0.6 sqrt units [$n = 38$] for no and yes on day care/siblings, respectively; *P* = .2) or IFN- γ titers at 9 months (3.4 ± 1.3 sqrt units [$n = 71$] and 3.3 ± 1.4 sqrt units [$n = 38$] for no and yes on day care/siblings, respectively; *P* = .7).

Early cytokine production and wheeze

The relation between the prevalence of wheeze during the previous year at each age and cytokine production at 9 months of age is presented in Table III. There was no significant relation between 9-month IL-2 production and subsequent wheeze. There was a significant inverse relation between IFN- γ production at 9 months and the prevalence of wheeze at ages 2, 11, and 13 years (Table III and see also Fig E2 in this article's Online Repository at www.jacionline.org). Similar trends (although nonsignificant) were observed at ages 3 and 8 years. These analyses were repeated in logistic regression models adjusting for sex, parental asthma, ethnicity, and skin tests, and there were no changes in the results (data not shown).

IFN- γ production at 9 months was unrelated to respiratory syncytial virus-induced lower respiratory tract illness (RSV-LRI) occurrence in the first 3 years of life (30.8% [4/13], 29.4% [10/34], and 29.8% [14/47] for low, intermediate, and high IFN- γ production, respectively; linear trend *P* = .9). In contrast, IL-2 production was directly related to RSV-LRI occurrence (10.0% [2/20], 26.5% [9/34], and 43.9% [18/41] for low, intermediate, and high production, respectively; linear trend *P* = .006).

When the wheezing phenotypes (Table I) were examined in relation to cytokine production, 9-month IFN- γ

TABLE II. Subject characteristics for eligible children (n = 296) with and without immune studies

Subject characteristics	With immune studies		Without immune studies		P value*
	n	Percent	n	Percent	
Sex (male)	118	51.7	178	51.1	.9
Ethnicity (Hispanic/mixed)	118	25.4	178	32.0	.2
Atopic at age 6 y (yes)†	95	31.6	103	35.0	.7
Active wheeze at age:					
2 y	114	23.7	140	22.9	.9
3 y	100	15.0	117	15.4	.9
6 y	112	30.4	133	28.6	.8
8 y	91	30.8	90	18.9	.09
11 y	104	28.8	124	18.5	.08
13 y	65	21.5	72	19.4	.8
Toddler wheeze‡	106	29.2	120	33.3	.6
School-age wheeze‡	97	29.9	101	20.8	.1
Chronic wheeze‡	92	18.5	90	10.0	.1
Day care/siblings	110	34.5	138	31.2	.6
Dogs at birth	118	20.3	174	29.3	.1
Maternal:					
Asthma (yes)	117	16.2	155	9.7	.1
Smoking (yes)	118	19.5	177	21.5	.8
Education (>12 y)	118	70.3	177	66.7	.5
Atopic (yes)†	96	70.8	115	58.3	.06
Paternal:					
Asthma (yes)	109	11.0	149	16.8	.2
Smoking (yes)	116	25.9	176	30.1	.5
Education (>12 y)	115	67.8	175	68.0	.9
Atopic (yes)†	86	66.3	86	62.8	.8

*P values are based on Pearson χ^2 or Fisher exact test statistics.

†At least 1 positive skin prick test response.

‡Toddler wheeze was defined as wheeze on the 2-year questionnaire, the 3-year questionnaire, or both. School-age wheeze was defined as wheezing on at least 2 questionnaires between ages 6 and 13 years. Chronic wheeze occurred before and after age 6 years.

TABLE III. Prevalence of wheeze by mitogen-stimulated 9-month peripheral blood IL-2 and IFN- γ production

	Wheeze during the previous year													
	Age 2 y		Age 3 y		Age 6 y		Age 8 y		Age 11 y		Age 13 y		Toddler wheeze*	School-age wheeze†
	n	Percent	n	Percent	n	Percent	n	Percent	n	Percent	n	Percent	n	Percent
9-mo IL-2 (sqrt units)‡														
<1.0	25	28.0	22	9.1	25	16.0	21	28.6	23	34.8	12	25.0	24	29.2
1 < 1.4	36	22.2	35	17.1	38	34.2	28	25.0	34	32.4	22	18.2	34	29.4
≥1.4	53	22.6	43	16.3	49	34.7	42	35.7	47	23.4	31	22.6	48	29.2
P value§		.7		.5		.14		.5		.3		.9		.9
9-mo IFN- γ (titer)‡														
<2	14	57.1	9	22.2	14	28.6	9	44.4	14	57.1	6	33.3	13	61.5
2 < 4	40	30.0	40	20.0	39	35.9	33	30.3	36	33.3	25	36.0	39	33.3
≥4	59	11.9	50	10.0	58	27.6	49	28.6	54	18.5	34	8.8	53	18.9
P value§		<.001		.17		.7		.4		.004		.02		.003

*Toddler wheeze positive was defined as wheeze on the 2-year questionnaire, the 3-year questionnaire, or both.

†School-age wheeze was defined as wheezing on at least 2 questionnaires between ages 6 and 13 years.

‡IL-2 is presented as square root transformed units. IFN- γ is presented as titers.

§P value for linear association based on the Mantel-Haenszel test.

production was inversely related to toddler wheeze, as well as school-age wheeze (Table III). The relation between 9-month IFN- γ and episodic school-age wheeze was not different from that with no childhood wheeze, and these 2 groups were combined for analyses (see Table E2

in this article's Online Repository at www.jacionline.org). IL-2 production was unrelated to either wheezing phenotype. When cytokine production was examined in relation to the long-term wheezing phenotypes by using the no-wheeze group for comparison, IFN- γ production at 9

TABLE IV. Long-term wheezing phenotypes in relation to mitogen-stimulated 9-month IFN- γ production

9-mo IFN- γ (titer)	N	Long-term wheeze phenotypes (% of IFN- γ group [n])			
		No wheeze	Toddler wheeze only*	School-age wheeze only*	Chronic wheeze*
<2, low	12	27.3 (3)	18.2 (2)	18.2 (2)	36.4 (4)
2 < 4, intermediate	36	52.8 (19)	8.3 (3)	16.7 (6)	22.2 (8)
≥ 4 , high	45	75.6 (34)	4.4 (2)	8.9 (4)	11.1 (5)
<i>P</i> value for linear association†			0.03	0.06	0.007

*Toddler wheeze only occurred before age 6 years. School-age wheeze only occurred after age 6 years. Chronic wheeze occurred before and after age 6 years.

†Compared with the no-wheeze group.

months was inversely related to toddler wheeze only (linear trend $\chi^2 = 4.6$, $P = .03$) and chronic wheeze (linear trend $\chi^2 = 7.2$, $P = .007$) but was not significantly related to school-age wheeze only (linear trend $\chi^2 = 3.7$, $P = .06$; Table IV), although the group sizes for these comparisons were small.

Modeling the relation of early cytokine production and wheeze

When assessed in a longitudinal model (GEE), the likelihood of wheezing at any age between 2 and 13 years was significantly higher for infants with low 9-month IFN- γ production (relative risk [RR], 2.29; 95% CI, 1.35-3.89) and borderline significant for those with intermediate IFN- γ production (RR, 1.59; 95% CI, 0.95-2.68) compared with those who produced high levels of IFN- γ (P for linear association = .002, shown as model A in Table E3 in this article's Online Repository at www.jacionline.org). There was a significant inverse association between 9-month IFN- γ production (ordinal: ≥ 4 , 2 < 4, and <2) and physician-diagnosed asthma (RR, 1.51; 95% CI, 1.05-2.16; $P = .025$). In contrast, there was no significant relation between IL-2 production and subsequent wheeze.

Low IFN- γ production at 9 months has been shown in this group of children to be associated with an increased risk for the development of atopy at age 6 years.¹² Therefore the association between 9-month IFN- γ production and subsequent wheeze from age 2 to age 13 years was adjusted for skin test reactivity at age 6 years. Nine-month IFN- γ production was still significantly and inversely related to subsequent wheeze when skin test was included in the GEE models as an ordinal variable (0, 1-3, and 4-7 positive test results; shown as model B in Table E3 in the Online Repository at www.jacionline.org; P for linear association = .017). IFN- γ production at 9 months was also inversely related to chronic wheeze ($P = .01$) independent of skin test reactivity at age 6 years (data not shown).

We tested other covariates as potential confounders of the IFN- γ and wheeze relation and found that a paternal history of asthma, male sex, and a mixed ethnic background were all associated with an increased risk for wheeze between ages 2 and 13 years (data not shown). After adjusting for these factors in a GEE model, the inverse relation between 9-month IFN- γ production and wheeze was still significant (P for linear association = .004; shown as model C in Table E3 in the Online

Repository at www.jacionline.org). Parental smoking was included in the final model to adjust for any potential bias in follow-up data and did not change the results (data not shown).

DISCUSSION

The results of this study strongly suggest that characteristics of the immune system present during the first year of life can anticipate the likelihood of developing episodes of airway obstruction characterized by wheezing. We cannot conclude from these data whether IFN- γ production itself is responsible for our findings or whether IFN- γ production acts as a marker for a more global alteration of the immune system that is present in early life in these children. However, the lack of relation of wheeze to IL-2 production indicates that our findings do not represent a general alteration in T_H cell function.

We and others have previously shown that children of atopic parents are more likely to show a diminished IFN- γ response and those with diminished IFN- γ production are more likely to go on to have skin test reactivity to local aeroallergens.^{8,9,12} These findings have suggested the hypothesis that an imbalance between T_H1 and T_H2 responses in early life might create a window of opportunity for the development of T_H2 responses against local aeroallergens in children predisposed to asthma and allergies.³ However, increased likelihood of development of allergic responses cannot be the only mechanism that explains the findings in the current study. In a different population enrolled in Tucson, Arizona, we showed that children with impaired IFN- γ production as early as 3 months were more likely to have wheezing episodes during the first year of life,¹¹ at a time at which allergic sensitization is unlikely to play a major role in determining these wheezing episodes. Moreover, in this study we found that the association between 9-month IFN- γ responses and chronic wheezing was independent of sensitization by age 6 years. It is thus possible that IFN- γ responses during the first year of life might be a common link that explains the strong association between wheezing in early life and the subsequent development of allergy-related asthma. In this scenario children who have impaired IFN- γ production in early life would be more susceptible first to certain viral illnesses in infancy and later in childhood to the development of atopic asthma as a result of the known association

between low IFN- γ production and the likelihood of development of atopic sensitization later in life.

In support of this scenario, we observed that low 9-month IFN- γ production was a feature common to both chronic and toddler-only wheezing but not to school-age-only wheezing. Thus our data support the hypothesis that factors that interact with low IFN- γ production in early life determine early allergic sensitization and the risk for persistent wheezing. These factors, we postulate, might be absent among toddler-only wheezers, and this could explain the remission of their condition after IFN- γ responses have matured.² What other factors might be involved is currently unknown, but it is reasonable to postulate that environmental exposure interacting with the genetic background of the individual play a crucial role.²⁵

Environmental factors might also influence IFN- γ responses in early life. For example, Gereda et al³ have shown that exposure to endotoxin present in house dust is associated with increased IFN- γ responses in infants and young children. In our study exposure to dogs and day care/siblings in the first year of life was not significantly related to IFN- γ production at 9 months. However, because of the small number of subjects involved in this study, these results should be interpreted cautiously. IL-2 production at 9 months was related to dog exposure: children exposed to dogs from birth produced more IL-2. Although there was no relation between 9-month IFN- γ production and RSV-LRIs, 9-month IL-2 production was directly related to the occurrence of RSV-LRIs. This result suggests that the immune factors that determine acute lower respiratory tract illness during viral infection might be different from those that determine wheezing, as reported by parents in questionnaires.

A limitation of our study was that, as is common in longitudinal cohorts, data became sparser once the enrolled children reached the teen years. In addition, IFN- γ responses in this report were measured by using a Sindbis virus-WISH cell system^{17,26} that only became available to our study at the end of the enrollment period for the Children's Respiratory Study (1983-1984). This method of detecting IFN- γ was the main technique available in the early 1980s, when commercial ELISA kits did not exist. Even though the method of detection was different from that in current use, each assay included a leukocyte IFN standard to control for reproducibility of the test system. Furthermore, virus titrations in supernatant fluids from unstimulated cells, from medium containing concanavalin A and PMA, and from medium only were used as negative controls.¹⁷

This work adds to the body of evidence that production of low levels of IFN- γ in early life provides risk for the development of asthma. This suggests that factors that could increase the production of IFN- γ in infancy might diminish this risk. It also suggests that measuring IFN- γ production in response to mitogen stimulation during the latter part of the first year of life might be an additional clinical guide to identification of children at increased risk for subsequent wheezing.

We thank Bruce Saul for data management, Dr Mary Jane Hicks and Dr Ruthann Kibler for performing the immune studies, Dr Duane Sherrill for guidance with the longitudinal modeling, and our study nurses, Marilyn Lindell and Lydia de la Ossa, for data collection and participant follow-up.

REFERENCES

- Martinez FD. Toward asthma prevention—does all that really matters happen before we learn to read? *N Engl J Med* 2003;349:1473-5.
- Holt PG, Upham JW, Sly PD. Contemporaneous maturation of immunologic and respiratory functions during early childhood: implications for development of asthma prevention strategies. *J Allergy Clin Immunol* 2005;116:16-25.
- Gereda JE, Leung DY, Thatayatikom A, Streib JE, Price MR, Klinnert MD, et al. Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet* 2000;355:1680-3.
- Rinas U, Horneff G, Wahn V. Interferon-gamma production by cord-blood mononuclear cells is reduced in newborns with a family history of atopic disease and is independent from cord blood IgE-levels. *Pediatr Allergy Immunol* 1993;4:60-4.
- Prescott SL, King B, Strong TL, Holt PG. The value of perinatal immune responses in predicting allergic disease at 6 years of age. *Allergy* 2003;58:1187-94.
- Lewis DB, Yu CC, Meyer J, English BK, Kahn SJ, Wilson CB. Cellular and molecular mechanisms for reduced interleukin 4 and interferon-gamma production by neonatal T cells. *J Clin Invest* 1991;87:194-202.
- Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Loh R, et al. Reciprocal age-related patterns of allergen-specific T-cell immunity in normal vs. atopic infants. *Clin Exp Allergy* 1998;28(suppl 5):39-44; discussion 50-1.
- Tang ML, Kemp AS, Thorburn J, Hill DJ. Reduced interferon-gamma secretion in neonates and subsequent atopy. *Lancet* 1994;344:983-5.
- Macaubas C, de Klerk NH, Holt BJ, Wee C, Kendall G, Firth M, et al. Association between antenatal cytokine production and the development of atopy and asthma at age 6 years. *Lancet* 2003;362:1192-7.
- 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.
- 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.
- Martinez FD, Stern DA, Wright AL, Holberg CJ, Taussig LM, Halonen M. Association of interleukin-2 and interferon-gamma production by blood mononuclear cells in infancy with parental allergy skin tests and with subsequent development of atopy. *J Allergy Clin Immunol* 1995;96:652-60.
- Ball TM, Castro-Rodriguez JA, Griffith KA, Holberg CJ, Martinez FD, Wright AL. Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. *N Engl J Med* 2000;343:538-43.
- Remes ST, Castro-Rodriguez JA, Holberg CJ, Martinez FD, Wright AL. Dog exposure in infancy decreases the subsequent risk of frequent wheeze but not of atopy. *J Allergy Clin Immunol* 2001;108:509-15.
- Oberle D, von Mutius E, von Kries R. Childhood asthma and continuous exposure to cats since the first year of life with cats allowed in the child's bedroom. *Allergy* 2003;58:1033-6.
- Taussig LM, Wright AL, Morgan WJ, Harrison HR, Ray CG, Associates GHM. The Tucson Children's Respiratory Study: I. Design and implementation of a prospective study of acute and chronic respiratory illness in children. *Am J Epidemiol* 1989;129:1219-31.
- Kibler R, Hicks MJ, Wright AL, Taussig LM. A comparative analysis of cord blood and adult lymphocytes: interleukin-2 and interferon production, natural killer cell activity, and lymphocyte populations. *Diagn Immunol* 1986;4:201-8.

18. Kibler R, Lucas DO, Hicks MJ, Poulos BT, Jones JF. Immune function in chronic active Epstein-Barr virus infection. *J Clin Immunol* 1985;5:46-54.
19. Stein RT, Sherrill D, Morgan WJ, Holberg CJ, Halonen M, Taussig LM, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet* 1999;354:541-5.
20. Ballinger GA. Using generalized estimating equations for longitudinal data analysis. *Organizational Research Methods* 2004;7:127-50.
21. Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. *Biometrika* 1986;73:13-22.
22. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. *Biometrics* 1986;42:121-30.
23. Hardin JW, Hilbe JM, Hilbe J. Generalized estimating equations. Chapman & Hall/CRC; 2002.
24. Pan W. Akaike's information criterion in generalized estimating equations. *Biometrics* 2001;57:120-5.
25. Hoffjan S, Nicolae D, Ostrovskaya I, Roberg K, Evans M, Mirel DB, et al. Gene-environment interaction effects on the development of immune responses in the 1st year of life. *Am J Hum Genet* 2005;76:696-704.
26. Jones JF, Minnich LM, Lucas DO, Fulginiti VA, Ingham Z, Langford MP, et al. Serum interferon in Navajo children with severe combined immunodeficiency disease inhibits lymphoblastogenesis. *J Clin Immunol* 1983;3:14-21.

ON THE MOVE?

Send us your new address at least six weeks ahead

Don't miss a single issue of the journal! To ensure prompt service when you change your address, please photocopy and complete the form below.

Please send your change of address notification at least six weeks before your move to ensure continued service. We regret we cannot guarantee replacement of issues missed due to late notification.

JOURNAL TITLE:

Fill in the title of the journal here. _____

OLD ADDRESS:

Affix the address label from a recent issue of the journal here.

NEW ADDRESS:

Clearly print your new address here.

Name _____

Address _____

City/State/ZIP _____

COPY AND MAIL THIS FORM TO:

Elsevier Periodicals Customer Service
6277 Sea Harbor Dr
Orlando, FL 32887-4800

OR FAX TO:

800-225-6030
Outside the U.S.:
407-363-9661

OR PHONE:

800-654-2452
Outside the U.S.:
407-345-4000

OR E-MAIL:

elspcs@elsevier.com

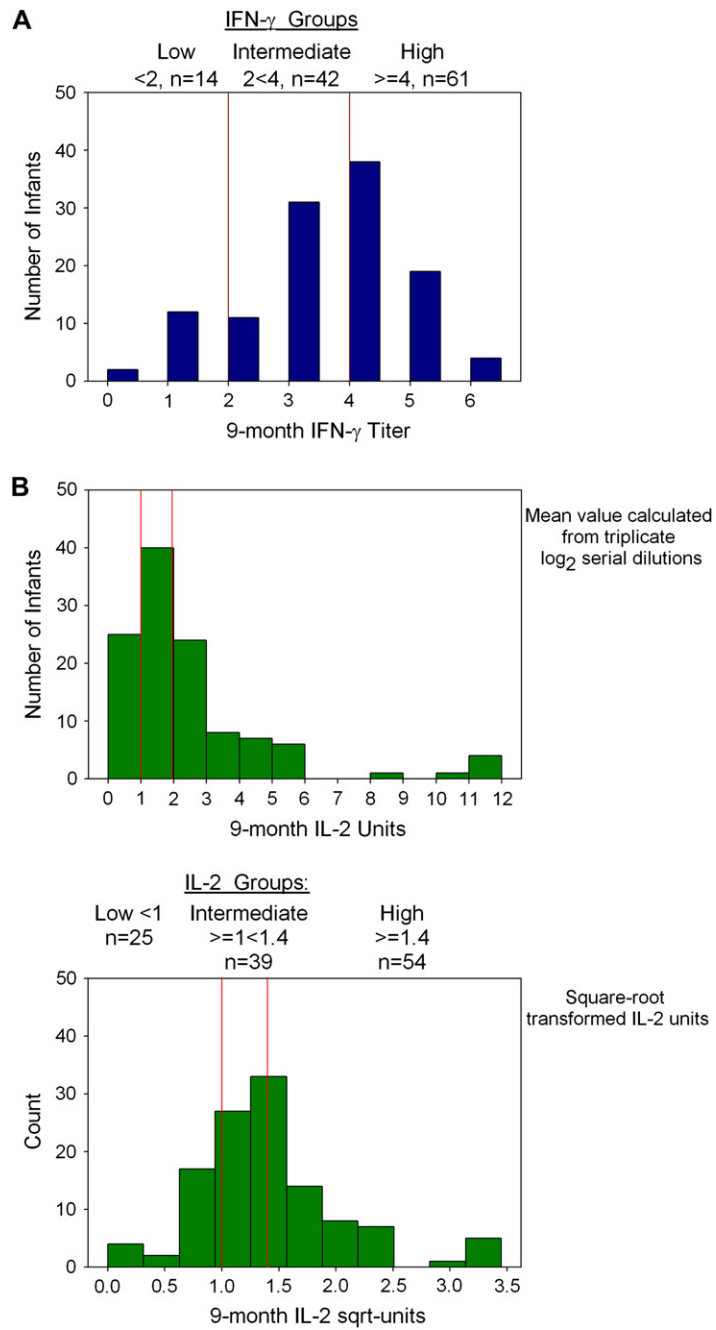


FIG E1. Distribution of 9-month IFN- γ (A) and IL-2 (B) values and group cutoff values.

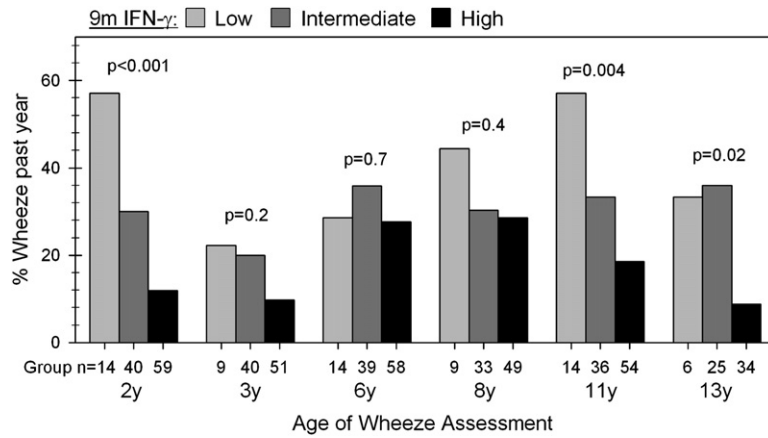


FIG E2. Prevalence of wheeze during the previous year at each survey age for the 9-month IFN- γ groups. *P* values for linear association are based on the Mantel-Haenszel test.

TABLE E1. Characteristics of immune study children (n = 118) with and without wheeze data at each survey age

Immune study children (n = 118)		Male sex		≥1 Non-Hispanic white		Atopic age 6 y		Maternal Asthma		Maternal smoking		Maternal education ≥12 y		Paternal asthma		Paternal smoking		Paternal education ≥12 y	
Age	Wheeze data	N	Percent	N	Percent	N	Percent	N	Percent	N	Percent	N	Percent >12y	N	Percent	N	Percent	N	Percent
2 y	No	4	75.0	4	50.0	3	33.3	4	0.0	4	25.0	4	50.0	3	0.0	4	25.0	4	50.0
	Yes	114	50.9	114	75.4	92	31.5	113	16.8	114	19.3	114	71.1	106	11.3	112	25.9	76	68.5
3 y	No	18	55.6	18	66.7	11	27.3	18	38.9	18	33.3	18	50.0	16	6.3	17	47.1	17	58.8
	Yes	100	51.0	100	76.0	84	32.1	99	12.1	100	17.0	100	74.0	93	11.8	99	22.2	98	69.4
6 y	No	6	50.0	6	83.3	0	0.0	6	16.7	6	50.0	6	66.7	6	0.0	6	50.0	6	66.7
	Yes	112	51.8	112	74.1	95	31.6	111	16.2	112	17.9	112	70.5	103	11.7	110	24.5	109	67.9
8 y	No	27	66.7	27	51.9	18	44.4	27	25.9	27	40.7	27	55.6	25	16.0	26	46.2	26	46.2
	Yes	91	47.3	91	81.3	77	28.6	90	13.3	91	13.2	91	74.7	84	9.5	90	20.0	89	74.2
11 y	No	14	50.0	14	64.3	6	16.7	14	14.3	14	35.7	14	64.3	14	7.1	14	50.0	14	50.0
	Yes	104	51.9	104	76.0	89	32.6	103	16.5	104	17.3	104	71.2	95	11.6	104	22.5	101	70.3
13 y	No	53	49.1	53	64.2	38	26.3	53	24.5	53	22.6	53	56.6	48	10.4	52	34.6	51	60.8
	Yes	65	53.8	65	83.1	57	35.1	64	9.4	65	16.9	65	81.5	61	11.5	64	18.8	64	73.4
			0.7		0.02		0.5		0.04		0.5		0.004		0.9		0.06		0.2

TABLE E2. Relation of 9-month IFN- γ response to the school-age wheeze groups

9-mo IFN- γ (titer)	N	School-age wheezing phenotype (% of IFN- γ group [n])		
		No wheeze	Episodic school-age wheeze*	School-age wheeze*
<2, low	11	27.3 (3)	18.2 (2)	54.5 (6)
2 < 4, intermediate	36	52.8 (19)	8.3 (3)	38.9 (14)
$\geq$ 4, high	50	58.0 (29)	24.0 (12)	18.0 (9)
<i>P</i> value for linear association compared with no wheeze			.7	.01

**School-age wheeze* was defined as wheezing on at least 2 questionnaires between ages 6 and 13 years. *Episodic school-age wheeze* was defined as wheezing on 1 questionnaire between ages 6 and 13 years.

TABLE E3. Longitudinal models (GEE) for wheeze from age 2 to age 13 years

Factors	Wheeze from age 2 to 13 y					
	Model A: unadjusted*		Model B: adjusted for age 6 y skin test*		Model C: adjusted for covariates†	
	RR (95% CI)	P value	RR (95% CI)	P value	RR (95% CI)	P value
9-mo IFN- γ titer						
≥ 4 , high	Reference		Reference		Reference	
2 < 4, intermediate	1.59 (0.95-2.68)	.077	1.54 (0.92-2.59)	.103	1.48 (0.89-2.46)	.13
<2, low	2.29 (1.35-3.89)	.002	2.17 (1.25-3.79)	.006	2.00 (1.20-3.32)	.007
Sex (male)	—	—	—	—	1.60 (1.04-2.45)	.031
Ethnicity (mixed)	—	—	—	—	1.70 (1.15-2.50)	.007
Paternal asthma (yes)	—	—	—	—	2.07 (1.44-3.00)	<.001
Age 6 y skin test:						
1-3 positive test responses	—	—	1.42 (0.84-2.38)	.2	—	—
4-7 positive test responses	—	—	0.77 (0.38-1.57)	.5	—	—
Missing skin test data	—	—	1.33 (0.74-2.42)	.3	—	—

*Five hundred eighty-three questionnaire data points were included in the model, with a minimum of 1 and a maximum of 6 (average = 5.0), representing data from 116 individuals. A log link, an exchangeable correlation structure, and robust estimates were used.

†Five hundred thirty-nine questionnaire data points were included in the model, with a minimum of 1 and a maximum of 6 (average = 5.0), representing data from 109 individuals (9 children were missing paternal asthma information, and the GEE model would not converge if a dummy category of those with missing paternal asthma information was included in the model). We used a log link, an unstructured correlation structure, and robust estimates.