

Association of interleukin-2 and interferon- γ production by blood mononuclear cells in infancy with parental allergy skin tests and with subsequent development of atopy

Fernando D. Martinez, MD, Debra A. Stern, MS, Anne L. Wright, PhD, Catharine J. Holberg, MSc, Lynn M. Taussig, MD, and Marilyn Halonen, PhD Tucson, Ariz.

The mechanisms regulating the onset of atopic sensitization in human beings are not yet fully clarified. We assessed the capacity of mitogen-stimulated umbilical and peripheral blood mononuclear cells to produce interferon- γ (IFN- γ) and interleukin-2 (IL-2) at birth and at 9 months of age in 159 infants. Mononuclear cell production of both IFN- γ and IL-2 at 9 months, but not at birth, was found to be inversely related to parental immediate skin test reactivity to seven local aeroallergens. Skin test reactivity at the age of 6 years was also inversely related to IFN- γ and IL-2 production at 9 months of age. However, no relationship was evident between total serum IgE levels at 6 years and production of these cytokines at 9 months. The proportions of circulating lymphocytes and CD4+ or CD8+ cells were also unrelated to skin test reactivity at the age of 6 years. These data suggest that mechanisms regulating skin test reactivity to inhaled allergens may involve deficient IFN- γ production, deficient IL-2 production, or both during or preceding the time of initial sensitization and that additional mechanisms are involved in regulating total serum IgE level. (J ALLERGY CLIN IMMUNOL 1995;96:652-60.)

Key words: IgE, IFN- γ , IL-2, cytokines, immediate hypersensitivity, allergy skin test, T_{H1} lymphocytes, T_{H2} lymphocytes, infancy

Recent epidemiologic evidence has suggested that most cases of asthma ascertained through surveys of large community samples may be IgE-mediated.¹ Moreover, most children with asthma have positive immediate hypersensitivity skin test reactions to common inhaled allergens, and it has been shown that the risk of having asthma is directly proportional to the number of positive reactions to a battery of allergens found in the sampled community.² The severity of asthma also shows a direct correlation to the number of skin

Abbreviations used

CI:	Confidence interval
IFN- γ :	Interferon- γ
IL-2:	Interleukin-2
PBMCs:	Peripheral blood mononuclear cells
ρ :	Spearman rank correlation coefficient
rho	

test reactions.³ The associative links between IgE level and skin test reactivity are very strong,⁴ and total IgE levels are thought to be reached in part as a sum of specific IgE responses.⁵ These IgE responses are influenced by processes regulating both the degree of commitment to IgE synthesis for each antigen and the maintenance of IgE synthesis, processes that are currently under much scrutiny and likely under separate regulation.^{6,7}

Based on studies of IgE regulation in mice,^{8,9} considerable progress has been made in the understanding of the molecular signals responsible for

From the Respiratory Sciences Center (Westend Laboratories) and the Departments of Pediatrics and Pharmacology, University of Arizona HSC, Tucson.

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Reprint requests: Fernando D. Martinez, MD, Respiratory Sciences Center, University of Arizona HSC, Tucson, AZ 85724.

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the regulation of human IgE synthesis.¹⁰ Interleukin-4 (IL-4) is known to promote the synthesis of IgE in human peripheral blood mononuclear cells (PBMCs), and this effect is inhibited in vitro by interferon- γ (IFN- γ).¹¹ IL-4 and IFN- γ are major products of T helper cell subgroups T_{H2} and T_{H1}, respectively.¹⁰ Recent studies suggest that T helper cell clones specific for sensitizing allergens secrete IL-4 but little to no IFN- γ . These cells can be identified in the peripheral blood and accumulate in target organs of atopic patients.¹²⁻¹⁷ These findings support the suggestion that IL-4 and IFN- γ may be the main cytokines involved in the control of IgE production and that atopy is associated with an enhanced susceptibility for B-cell commitment to IgE brought about by abnormal regulation of the differential development of IL-4-producing versus IFN- γ -producing helper T cells.⁶

Most of the evidence supporting abnormal T cell regulation of IgE is based on in vitro studies of IgE synthesis or on in vivo demonstration of cells in panels of highly selected atopic subjects. It is not known whether the T cell-related abnormalities described previously are present only in subjects with symptoms or whether they precede allergic sensitization and may thus be predisposing factors for the development of atopy. Moreover, little information exists on whether T-cell regulatory processes are involved in the complex genetic mechanisms involved in inheritance of total IgE levels.¹⁸ To begin to address these issues, we present the results of a longitudinal study relating PBMC IFN- γ and IL-2 production at birth and at 9 months to the development of eczema and rhinitis in the first year of life and to the subsequent development of skin test reactivity to common inhalant allergens at the age of 6 years and to the atopic status of the subjects' parents.

METHODS

Subjects

The subjects of this report are participants in the Tucson Children's Respiratory Study, a longitudinal study of risk factors for and consequences of acute and chronic lower respiratory tract illnesses in children.¹⁹ A total of 1246 infants whose families used a health maintenance organization were enrolled at birth between May 1980 and October 1984. After February 1983, studies of mononuclear cell numbers and function were added to the data acquisition protocols, and all 296 infants subsequently enrolled in the study were eligible.

Parents of enrolled subjects completed detailed questionnaires at regular intervals during the study. Parental history of atopy, maternal smoking, and ethnicity were determined from responses on the enrollment question-

naire. Information on physician-diagnosed eczema and rhinitis occurring during the first year of life was obtained from the questionnaire completed during the infants' second year of life (mean age, 1.6 years; SD, 0.35 years).

When the subgroup of subjects sampled and studied for PBMC function ($n = 159$) was compared with subjects enrolled during the same period but not included in the PBMC studies ($n = 137$), no significant differences were seen in maternal or paternal history of allergy, maternal smoking history, sex, or ethnicity. Of the 159 infants with immune studies, 47.2% were girls, 69.6% were non-Hispanic whites (both parents), 18.4% were Hispanic, and 12% were of other or mixed ethnicities.

During the first year infants enrolled in the Tucson Children's Respiratory Study regularly visited the health maintenance organization clinic for well-baby checkups. PBMC sampling was scheduled for cord blood at enrollment and for peripheral blood samples obtained at a checkup visit between 6.6 and 12 months of age (mean age 9.4 months $\pm$ 1.1 SD and henceforth referred to as the "9-month" sample). Blood samples were obtained from 72 infants at birth (cord blood) and 119 infants at 9 months for studies of PBMC function, including 32 cord and 9-month samples from the same individuals. The main factors determining exclusion of eligible infants from the PBMC studies were parental refusal to give consent for phlebotomy, restrictions in the timely availability of trained personnel to perform the analyses, and technical factors such as insufficient amount or quality of the sample.

All enrolled children and their families living in Tucson (and still participating in the study) were eligible for total serum IgE measurements and allergy skin tests when the children were 6 years old (mean age 6.4 years $\pm$ 0.5 SD). Of the 159 infants with cord studies, 9-month PBMC studies, or both, immediate skin prick tests to inhaled allergens were performed on 113 children, and assays were performed for serum IgE levels in 76. Allergy skin tests were also concomitantly performed on 115 mothers and 89 fathers of these children.

Informed consent was obtained from parents of participating subjects, and the study was approved by the College of Medicine Human Subjects Committee of the University of Arizona.

IFN- γ and IL-2 production by PBMCs

Blood was collected in heparin (5 μ l/ml) and processed within 12 hours of collection. Blood was layered on Lymphocyte Separation Medium (Litton Bionetics, Kensington, Md.) after dilution of 1:2 was performed with RPMI-1640 medium (Gibco, Grand Island, NY).²⁰ The cells at the blood-medium interface were collected, washed twice with Ca⁺⁺- and Mg⁺⁺-free phosphate-buffered saline solution, and resuspended in RPMI-1640 medium. A hemocytometer total cell count and chamber

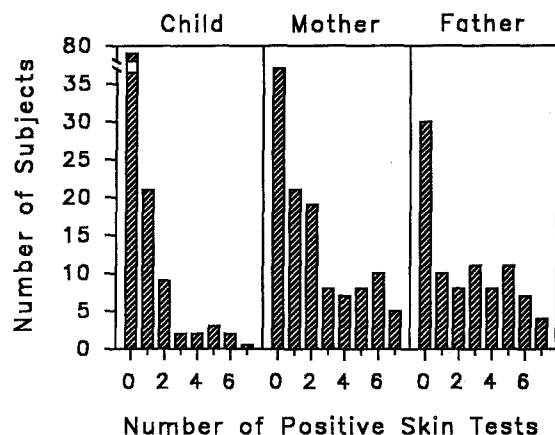


FIG. 1. Frequency distribution of individuals with zero to seven positive skin test results among the children, mothers, and fathers of this study. Ordinate is discontinuous for children with zero positive skin test results ($n = 74$).

differential were performed to estimate the percent of mononuclear cells.

Immediately after cell separation was performed, 10^6 PBMCs were incubated in 1 ml aliquots of RPMI-1640 medium supplemented with 5% heat-inactivated fetal calf serum, 300 μ g/ml glutamine, 220 μ g/ml pyruvic acid, and 50 μ g/ml gentamicin at 37°C in a 5% CO₂ air atmosphere. Cells were stimulated with 10 μ g/ml concanavalin A (Pharmacia, Piscataway, N.J.) and 10 ng/ml phorbol myristate acetate (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 and IL-2 testing, respectively.

IL-2 bioassay

The IL-2 bioassay has been described in detail.²¹ Briefly, murine IL-2-dependent cytotoxic lymphoid line-2 cells were added to serial log₂ dilutions of supernatant 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 probit analysis based on a standard for IL-2 prepared from rat spleen cells²⁰ containing 1 unit of activity per 0.1 ml.

IFN- γ bioassay

IFN- γ titer determinations were carried out on the supernatant fluids with a Sindbis virus-human amnion (WISH) cell system.²⁰ Triplicate log₃ serial dilutions of the fluids were incubated with the WISH-cell monolayers in flat-bottom microtainer wells for 8 hours before a previously titrated amount of Sindbis virus was added.²⁰ The mean 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 phorbol myristate acetate, and medium only were used as negative controls.

In a subset of 16 blood samples (randomly selected), the type of IFN produced under the test conditions was determined. Each sample was aliquoted and incubated for 30 minutes at room temperature with pretitrated antibody to IFN- α , β , or γ . The polyclonal sheep anti-human IFN- α and sheep anti-human IFN- β antibodies and the IFN α , β , and γ standards were obtained from the National Institutes of Health. The anti-human IFN- γ was a monoclonal antibody obtained from Interferon Sciences, Inc. (New Brunswick, N.J.). An untreated aliquot was mixed with an equal volume of diluent. After incubation was performed, the samples were added to the WISH-cell monolayers and were titrated as previously described for quantitating IFN. A fourfold or greater decrease in IFN- γ titer, but not in IFN- α or IFN- β , was obtained in all cases.

T-cell subsets

T-cell subsets were determined as previously described²⁰ from the PBMC suspension. An indirect fluorescent antibody assay was standardized for use with 2×10^5 mononuclear cells in 0.2 ml. Cells were incubated on ice with 4 μ l of monoclonal antibodies (Ortho Diagnostics, Raritan, N.J.) specific for T cells (CD3), helper T cells (CD4), and cytotoxic/suppressor T cells (CD8). After incubation was performed for 30 minutes and 0.6 ml of neonatal calf serum was added, the excess antibody was removed by centrifugation at 50g for 5 minutes. The cells were then resuspended in fluorescein-conjugated F(ab')₂ fragments of goat anti-mouse IgG (heavy and light chain-specific; Cappel Laboratories, Cochranville, Pa.) for 30 minutes on ice and were washed three times in fetal calf serum. Slides were prepared by fixing the cells in 95% ethanol and mounting in gelvatol and were read for membrane fluorescence.²⁰

Serum IgE levels

Serum IgE levels were assayed by paper radioimmunosorbent test (PRIST) with commercially available kits (Pharmacia Diagnostics, Piscataway, N.J.) as previously described.²² All samples were assayed in duplicate, and results were expressed as international units per milliliter.

Allergy skin tests

Allergy skin tests done with the prick technique were performed in the children and their parents when the children were 6 years old. Extracts of seven common aeroallergens in the Tucson area and diluent were obtained from Hollister-Stier Laboratories (Everett, Wash.): house dust mix (1:10), *Alternaria tenuis* (1:100), Bermuda grass (1:20), careless weed (1:20),

mesquite (1:20), mulberry (1:20), and olive (1:20). Test sites were examined after 20 minutes, and the sizes of the wheals were recorded as the sum of the two diameters measured at right angles (in millimeters). Wheal sizes of 3 mm or greater (after subtraction of diluent control value) were considered positive. The number of positive results was used as an estimate of sensitivity to aeroallergens.

Statistical analyses

Statistical analyses were performed with the Statistical Package for the Social Sciences for UNIX, version 4 (SPSS Inc., Chicago, Ill.). Total serum IgE values were log-normally distributed, and results were expressed as geometric mean and geometric SEM. IFN- γ titers were log₃-normally distributed, and results were expressed as the log₃ titer. IL-2 values were log₂-normally distributed and expressed as log₂ units. Statistical analyses involving these variables were performed with standard parametric techniques. The number of positive skin test results was used as an integer (values of 0 to 7 positive results), as a grouped variable (0, 1 to 3, or 4 to 7 positive results), or as the percent of group with one or more positive skin test results. The results were analyzed nonparametrically. A *p* value of less than 0.05 (two-tailed) was considered statistically significant for all tests.

RESULTS

The distribution of skin test reactivity for parents and children are shown in Fig. 1. Sensitization was lower in children compared with adults; 35% of children had positive skin test results compared with 68% of mothers and 66% of fathers. Although 38% of boys had a positive skin test result compared with 29% of girls, this difference was not statistically significant, nor were ethnic differences in skin test reactivity evident. Mean log₃ titers for IFN- γ production from PBMCs obtained at birth and at 9 months were 2.7 (*n* = 67, 95% confidence interval [CI]: 2.4 to 3.1) and 3.4 (*n* = 117, 95% CI: 3.2 to 3.6), respectively. Mean log₂ units for IL-2 production at birth and at 9 months were 1.8 (*n* = 72, 95% CI: 1.6 to 2.0) and 1.4 (*n* = 111, 95% CI: 1.3 to 1.5), respectively. Levels for these cytokines were not different in boys and girls, nor were any significant ethnic differences noted. Overall IL-2 production decreased (*p* < 0.01), and IFN- γ levels increased (*p* < 0.01) from birth to 9 months. The subgroup of matched samples (*n* = 28 for IL-2 and *n* = 31 for IFN- γ) showed the same trends with age but did not reach significance. IL-2 and IFN- γ production were significantly correlated at birth (*n* = 67, *r* = 0.29, *p* = 0.02) and even more so at 9 months (*n* = 109, *r* = 0.43, *p* < 0.0005).

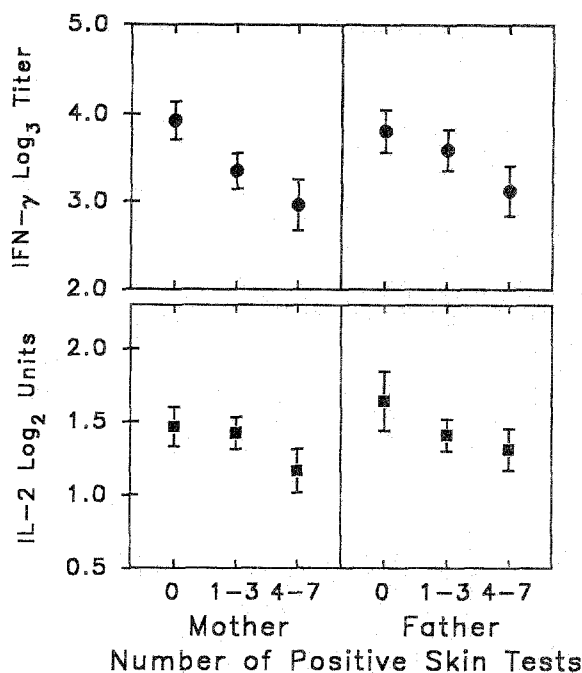


FIG. 2. Relation of IL-2 and IFN- γ production by PBMCs at 9 months of age to parental skin test reactivity. Maternal (*n* = 27, 40 and 26, ρ = -0.23, *p* = 0.01) and paternal (*n* = 26, 27 and 24, ρ = -0.21, *p* = 0.03) skin test reactivity were negatively correlated with 9-month IFN- γ production. Similarly, maternal (*n* = 27, 37 and 23, ρ = -0.25, *p* = 0.03) and paternal (*n* = 23, 26 and 23, ρ = -0.17, *p* = 0.07) skin test reactivity were negatively correlated with 9-month IL-2 production.

Cytokine production in relation to parental skin test reactivity

The capacity of cord PBMCs to respond to mitogenic stimulation with IFN- γ production was unrelated to maternal or paternal skin test reactivity (*p* = 0.2 and 0.2, respectively). In contrast, IFN- γ production by PBMCs obtained at 9 months showed a significant inverse relationship to the number of positive maternal skin test results (Fig. 2; *n* = 93, Spearman ρ (ρ) = -0.23, *p* = 0.01) and paternal skin test results (*n* = 77, ρ = -0.21, *p* = 0.03). The lowest IFN- γ titers were observed in infants whose parents were both strongly sensitive to aeroallergens (Table I; maternal-paternal interaction *p* = 0.03).

Cord PBMC production of IL-2 was also unrelated to maternal or paternal skin test reactivity. Nine-month IL-2 production was significantly inversely related to maternal skin test reactivity (Fig. 2; *n* = 87, ρ = -0.25, *p* = 0.03); paternal skin test reactivity showed a similar trend but did not reach significance (Fig. 2; *n* = 72, ρ = -0.17, *p* = 0.07).

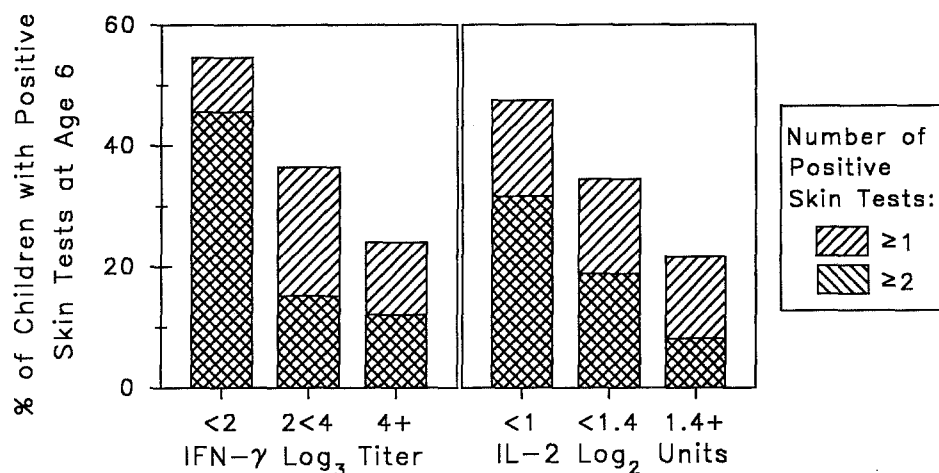


FIG. 3. Relation of skin test reactivity at age 6 years to PBMC IFN- γ and IL-2 production at 9 months of age. Percent of children with one or more positive skin test results was inversely related to 9-month IFN- γ ($n = 11, 33$, and 50 , trend chi square = 4.2 , $p < 0.04$) and IL-2 production ($n = 19, 32$, and 37 , trend chi square = 3.9 , $p < 0.05$). Percent of children with two or more positive skin test results was inversely related to IFN- γ (trend chi square = 5.0 , $p < 0.03$) and IL-2 production (trend chi square = 4.9 , $p < 0.03$).

TABLE I. Relation of IFN- γ and IL-2 production by PBMCs from infants at 9 months of age to parental skin test reactivity

No. of positive skin test results for mother	No. of positive skin test results for father (<i>n</i>)		
	0	1-3	4-7
IFN- γ log ₃ titer $\pm$ SEM			
0	3.8 $\pm$ 0.8 (4)	4.0 $\pm$ 0.5 (9)	4.4 $\pm$ 0.2 (7)
1-3	3.9 $\pm$ 0.4 (11)	3.8 $\pm$ 0.2 (10)	2.7 $\pm$ 0.4 (11)
4-7	3.9 $\pm$ 0.4 (9)	2.5 $\pm$ 0.5 (6)	2.2 $\pm$ 0.6 (5)
IL-2 log ₂ units mean $\pm$ SEM			
0	1.5 $\pm$ 0.7 (4)	1.6 $\pm$ 0.1 (9)	1.6 $\pm$ 0.2 (7)
1-3	1.7 $\pm$ 0.3 (9)	1.4 $\pm$ 0.1 (10)	1.4 $\pm$ 0.3 (10)
4-7	1.6 $\pm$ 0.4 (8)	0.8 $\pm$ 0.1 (5)	0.8 $\pm$ 0.2 (5)

A multiplicative interaction was significant between maternal and paternal skin test reactivity as determinants of IFN- γ production ($p = 0.03$) after which no significant independent parental effect remained. Similar trends were present for IL-2 production, but a multiplicative interaction did not reach statistical significance.

Infants whose parents both had strong positive skin test results tended to have the lowest values for IL-2 production, although a maternal-paternal interaction did not reach statistical significance (Table I).

Cytokine production in infancy in relation to subsequent skin test reactivity and IgE levels of the children at the age of 6 years

No relationship was evident between skin test reactivity of the children at the age of 6 years and either IL-2 or IFN- γ production at birth. In con-

trast, significant inverse relations were observed between the frequency of children with positive skin test results at the age of 6 years and both IFN- γ and IL-2 production at 9 months of age (Fig. 3; $p < 0.04$ and $p < 0.05$, respectively). These relationships were even more evident when the analyses were restricted to two or more positive skin test results (Fig. 3; $p < 0.03$ and $p < 0.03$, respectively).

No relationship could be discerned between total serum IgE levels at 6 years of age and either IL-2 or IFN- γ production by cord or 9-month PBMCs (Table II).

TABLE II. Relation of total serum IgE levels at 6 years of age to IFN- γ and IL-2 production by PBMCs at 9 months of age

Log ₃ IFN- γ		IgE (IU/ml)		Log ₂ IL-2		IgE (IU/ml)	
Titer	n	Geometric mean (SEM)		Units	n	Geometric mean (SEM)	
<2	8	46.5 (2.1)		<1	16	41.5 (1.5)	
2<4	21	27.7 (1.4)		1<1.4	20	26.1 (1.3)	
≥ 4	38	31.7 (1.3)		≥ 1.4	27	24.6 (1.4)	
Pearson		$r = -0.08$ $p = 0.5$				$r = -0.07$ $p = 0.6$	

TABLE III. Mean percent lymphocytes and T-cell subsets at 9 months of age by number of positive skin test results at 6 years of age

Cells at 9 months	No. of positive skin tests			ANOVA p Value
	0	1-3	4-7	
Mean $\pm$ SEM (n)				
Lymphocytes* (%)	71.4 $\pm$ 1.6 (52)	75.3 $\pm$ 2.3 (21)	67.5 $\pm$ 3.8 (6)	0.2
CD3 $\dagger$ (%)	72.1 $\pm$ 1.1 (52)	72.0 $\pm$ 1.6 (22)	78.8 $\pm$ 1.1 (4)	0.2
CD4 $\dagger$ (%)	52.3 $\pm$ 0.9 (51)	51.7 $\pm$ 1.6 (24)	55.5 $\pm$ 2.9 (4)	0.6
CD8 $\dagger$ (%)	18.7 $\pm$ 0.8 (50)	17.6 $\pm$ 0.9 (24)	20.0 $\pm$ 3.5 (4)	0.6
CD4/CD8 ratio	3.0 $\pm$ 0.1 (49)	3.0 $\pm$ 0.1 (24)	3.1 $\pm$ 0.6 (4)	0.9

ANOVA, Analysis of variance.

*Percent of total leukocytes.

$\dagger$ Percent of lymphocytes in PBMC preparation.

Cytokine production in relation to eczema and rhinitis in the first year of life

Physician-diagnosed eczema or rhinitis in the first year of life was unrelated to either IFN- γ or IL-2 production at birth or 9 months (data not shown).

T-cell subsets and subsequent skin test reactivity

No association was seen between the percent of circulating lymphocytes or T-cell subsets either at 9 months (Table III) or at birth (data not shown) and subsequent atopic status of the child at the age of 6 years.

DISCUSSION

That IFN- γ plays a crucial role in the control of human IgE production against aeroallergens is a prevailing hypothesis,¹⁰ supported namely by in vitro studies, which have demonstrated that exogenously provided IFN- γ can inhibit the commitment to IgE production by IL-4-stimulated mononuclear cells.¹¹ In addition to inhibition by exogenous IFN- γ , recent studies have shown that endogenously produced IFN- γ plays a critical role

in preventing IgE synthesis after activation with the T-cell-dependent B-cell mitogen pokeweed mitogen with cells from either atopic or normal subjects.^{23, 24} Nevertheless, a role for IFN- γ as an important physiologic regulator of IgE synthesis in vivo remains conjectural.

Most in vivo studies examining the role of IFN- γ in human allergy have been performed in patients with allergies who have symptoms. Although a significant relative deficit of IFN- γ production by PBMCs has been reported in atopic patients compared with nonatopic subjects,^{12, 25} it is not possible to determine from these studies whether the reported deficit was a cause, a consequence, or simply a marker of the allergic status.⁷ Moreover, in vivo studies, in which high doses of IFN- γ were injected into human subjects with clinical manifestations of allergy, yielded conflicting and inconclusive results regarding the capacity of IFN- γ to decrease IgE levels.^{26, 27}

In this study IFN- γ and IL-2 production by PBMCs was examined in infancy, and the amounts produced were assessed for an association with the development of eczema and rhinitis during the first year of life and of skin test sensitivity to inhaled

allergens later in childhood. Studies by others have shown that in children with parental history of atopy, levels of specific IgE against inhaled allergens were usually very low or undetectable before the age of 1 year, even for those children who later showed high levels of specific IgE against the same allergens.^{28, 29} In a small pilot study we have obtained similar results among 22 children tested for serum levels of specific IgE to the seven skin test allergens by RAST: each of the eight children with positive skin test results at the age of 6 years also had positive RAST results in serum obtained at 6 years, whereas none had positive RAST results for these aeroallergens in sera obtained at 9 months of age (unpublished results). If indeed infants at 9 months have not yet begun producing IgE to aeroallergens, then decreased PBMC production of IFN- γ and IL-2 may be an inherent characteristic of individuals antedating and subsequently contributing to the development of atopy. The lack of any association between eczema or rhinitis during the first year of life and IFN- γ or IL-2 production by PBMCs supports this hypothesis. Nonetheless, we cannot say with certainty that children who had positive test responses to aeroallergens at the age of 6 years had not already been sensitized to these allergens at the age of 9 months, with specific IgE levels present but below detectability by RAST. In either case, however, a decreased capability of PBMCs to produce IFN- γ and IL-2 at 9 months of age shows a significant link to specific IgE production assessed by skin test reactivity several years later.

It is not possible to determine from this study the type of cell(s) responsible for the production of IFN- γ , IL-2, or both in the PBMC samples. In vitro studies have convincingly shown that IFN- γ and IL-2 are produced by the T_{H1} subclass of T helper cells and not by T_{H2} cells.⁶ It has been suggested, however, that the bulk of circulating T helper cells in early life are immature cells that appear to be multipotential for cytokine production when stimulated.³⁰ The close association between IL-2 and IFN- γ production at birth in the infants of this study provides support that these cytokines may be derived from the same cell type. Compared with adult PBMC production of cytokines, IFN- γ was decreased and IL-2 was increased at birth;²⁰ these findings are in agreement with previous reports and the suggested cell types described previously.^{31, 32} The increase in IFN- γ and the decrease in IL-2 production by 9 months (with cytokine production still showing correlation) supports

the possibility that the producing cell matures during this time period.

A plausible explanation for the cytokine-atopy relationships shown in this study is that an IFN- γ - and IL-2-producing cell (most likely a T_{H1}-type cell or one of its precursor cells) is dysfunctional or underrepresented in PBMCs of 9-month-old subjects who later demonstrate specific sensitization to inhaled allergens. Whether the defect represents a delay in maturation or a more permanent alteration remains to be elucidated. A paucity of IFN- γ - and IL-2-producing cells was likely balanced by a relative predominance of IL-4-producing cells (T_{H2}- or T_{H2}-like cells), because the overall T helper cell percentages were not affected. Regrettably, at the time the assays of this study were performed, IL-4 had not yet been identified. We thus cannot exclude the possibility that other, more global alterations in cytokine production by PBMCs may be present in early life in subjects with atopic predisposition.³³

We found no significant relationship between IFN- γ or IL-2 production by cord or PBMCs in infancy and total IgE levels measured at the age of 6 years. Thus it appears that although a decreased capability of producing IFN- γ and IL-2 at 9 months is related to a proclivity to becoming sensitized to several allergens later in life, this proclivity is not the major factor controlling the total serum IgE level. These findings are in accord with data showing that IFN- γ suppresses the initial class switch of B cells into IgE-producing cells without effect on ongoing IgE synthesis.⁶ Thus we suggest that total IgE levels may be regulated by factors involved in maintaining (or suppressing) ongoing IgE synthesis in addition to factors regulating the initial commitment to IgE synthesis by B cells (such as IFN- γ and IL-2).

This study also demonstrates that IFN- γ and IL-2 production by PBMCs at 9 months was strongly associated with skin test reactivity of both parents. These results suggest that regulation of cytokine production by PBMCs may be part of the genetic mechanism(s) by which allergic susceptibility is inherited. The association with IFN- γ and IL-2 demonstrated a contribution from both parents: children of two highly atopic parents (≥ 3 positive skin test results) appeared to have the lowest IFN- γ and IL-2 production by PBMCs (Table I). One possible explanation for this apparent pattern of genetic transmission is recessive inheritance of a common gene responsible for low IFN- γ and IL-2 production by PBMCs. However, if the T-cell dysfunction should prove to be aller-

gen-driven, it is also possible that common environmental factors could explain the apparent transmission pattern observed in this study. The fact that an association was found between parental atopy and cytokine production at 9 months but not in cord blood suggests that if genetic influences are present, they require environmental exposure to express themselves. Clearly, additional analyses will be necessary to assess the inheritance patterns (if any) of IFN- γ and IL-2 production by PBMCs and of susceptibility to becoming sensitized to inhaled allergens.

Although to our knowledge no other studies have examined the relationship of infant IFN- γ and IL-2 production with an objective measure of parental atopy, two recent studies have attempted to determine whether infants with a family history of allergy have different patterns of cytokine production by PBMCs when compared with infants without such a history. Rinas et al.³⁴ examined IFN- γ and IL-2 production by cord PBMCs in 53 newborns with and without a family history of atopic disease. They reported no difference in mononuclear cell IL-2 production in infants with a family history of allergy compared with those without such a history when either phytohemagglutinin or OKT3 was used as the mitogen. In contrast, IFN- γ production was decreased with phytohemagglutinin but not OKT3 stimulation. This reduced IFN- γ production with phytohemagglutinin stimulation also differs from our results with concanavalin A stimulation and may be mitogen-specific. They also included all first-degree relatives for allergic history assessment and used a more subjective assessment of allergy (rather than skin test reactivity) for the independent variable. This is an important area for further study.

In a study of older infants, Holt et al.³³ assessed T-cell function in infants from a clinic population (7 to 35 months of age) whose first-degree relatives had ($n = 22$) or did not have ($n = 18$) a history of atopic disease. They found that those with a family history of allergy had decreased proliferative capacities of progenitor T cells and that individual T-cell clones from a few of these subjects produced less IFN- γ and IL-4 when stimulated. Whether such decreases result in a greater relative deficit of IFN- γ production (compared with IL-4 production) *in vivo* is not known. No follow-up data regarding the development of atopy in the children of either of the previously cited studies was provided.

In summary, our study documents that IFN- γ and IL-2 production by PBMCs at 9 months was

decreased in children of atopic parents. In addition, cytokine production by PBMCs at 9 months showed an inverse relationship with the subsequent degree of allergic sensitization assessed by the number of positive skin test results at the age of 6 years. The relationships with skin test reactivity were not evident for cord PBMCs obtained at birth. These data suggest that the degree of allergic sensitization may involve regulatory events that control the development of the number or function of IFN- γ - and IL-2-producing cells. Production of these cytokines appeared to be unrelated to total serum IgE levels at 6 years of age, suggesting that the total IgE level may be regulated by mechanisms in addition to those controlling the initiation of allergic sensitization.

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