# Developmental cytokine response profiles and the clinical and immunologic expression of atopy during the first year of life

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Mechanisms of allergy

**Background: Allergic diseases have been linked to abnormal patterns of immune development, and this has stimulated efforts to define the precise patterns of cytokine dysregulation that are associated with specific atopic phenotypes.**
**Objective: Cytokine-response profiles were prospectively analyzed over the first year of life and compared with the clinical and immunologic expressions of atopy.**
**Methods: Umbilical cord and 1-year PBMCs were obtained from 285 subjects from allergic families. PHA-stimulated cytokine-response profiles (IL-5, IL-10, IL-13, and IFN-γ) were compared with blood eosinophil counts and total and specific IgE levels (dust mites, cat, egg, *Alternaria* species, peanut, milk, and dog) at age 1 year and at the development of atopic dermatitis and food allergy.**
**Results: For the cohort as a whole, cytokine responses did not evolve according to a strict $T_H1$ or $T_H2$ polarization pattern. PHA-stimulated cord blood cells secreted low levels of IL-5 (2.1 pg/mL), moderate levels of IFN-γ (57.4 pg/mL), and greater amounts of IL-13 (281.8 pg/mL). From birth to 1 year, IL-5 responses dramatically increased, whereas IL-13 and IFN-γ responses significantly decreased. Reduced cord blood secretion of IL-10 and IFN-γ was associated with subsequent sensitization to egg. In addition, there was evidence of $T_H2$ polarization (increased IL-5 and IL-13 levels) associated with blood eosinophilia and increased total IgE levels by age 1 year.**
**Conclusion: These findings demonstrate that cytokine responses change markedly during the first year of life and provide further evidence of a close relationship between $T_H2$ skewing of immune responses and the incidence of atopic manifestations in children. (J Allergy Clin Immunol 2003;112:740-6.)**

***Key words:*** *Cytokines, $T_H2$, immune development, allergy, IgE*

Recent observations have stimulated research efforts to further define the relative importance and pathophysiologic contributions of cytokine dysregulation (so-called $T_H1/T_H2$ imbalance) to the development of various atopic phenotypes.[1] Although questions remain regarding the full effect of a $T_H1/T_H2$ dysregulation in established atopic diseases, the contribution of cytokine polarization to the inception and evolution of various atopic diseases, including asthma, has received more uniform support. For example, there is evidence to suggest that at birth, possibly because of placentally derived $T_H2$ trophic factors, allergen-induced mononuclear cell (MNC) responses are skewed toward a $T_H2$-like phenotype,[2] and IFN-γ responses are particularly low.[3,4] Furthermore, IFN-γ responses gradually increase in magnitude in normal infants, whereas in allergic infants IFN-γ responses are reduced at birth, and their development is delayed.[2,5,6] As a result, this reduced IFN-γ generation causes a $T_H1/T_H2$ imbalance that might be a risk factor for the subsequent development of allergic disease, asthma, or both.[2,3,7,8] Although the concept of a $T_H1/T_H2$ imbalance is an attractive theory, cord blood IL-13 responses also appear to be suppressed in children who go on to have atopic disease.[9] Collectively, these data suggest a complex interplay of $T_H1/T_H2$ responses in relationship to immune maturation and the development of allergic disease.

Although these observations are of considerable interest, it is important to note that they were made in a small number of patients who displayed a variety of different atopic phenotypes. To extend these observations, the present study, Childhood Origins of Asthma, is designed to analyze the development of cytokine-response profiles beginning at birth in a large cohort of high-risk children and to determine their relationships with clinical and biologic markers of atopy and eventually asthma. To test the hypothesis that different atopic phenotypes will be asso-

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*Abbreviations used*
AD: Atopic dermatitis
FEIA: Fluoroenzyme immunoassay
MNC: Mononuclear cell

ciated with distinct $T_H2$ patterns of cytokine development, subjects were prospectively evaluated for the first year of life with historical questionnaires, physical examinations, and laboratory studies to compare cytokine responses with the development of total and allergen-specific IgE, blood eosinophilia, atopic dermatitis (AD), and food allergy.

## METHODS

### Experimental design

Study subjects included 285 children with at least one parent with respiratory allergies (defined as ≥1 positive aeroallergen skin test responses), asthma (defined historically), or both (Table I). After obtaining informed consent, subjects were enrolled at birth (from November 1998 through March 2000) and followed prospectively for at least 1 year. Data collected included parent and child questionnaires regarding health histories, with a specific focus on atopic diseases, behaviors affecting health, and environmental exposures. Physical examinations were performed at regularly scheduled times (generally 2, 4, 6, 9, and 12 months) by the subjects' primary physicians, and these records were evaluated to document the development of AD and food allergy. Blood samples were collected at birth (cord blood) and at age 1 year. Laboratory studies included cytokine-response profiles from stimulated cord blood MNCs and PBMCs at age 1 year. Additional studies performed at age 1 year included RAST testing, total IgE level measurement, and peripheral blood eosinophil count measurement. This study was approved by the University of Wisconsin Human Subjects Committee.

### Collection of blood samples

Cord blood samples were collected on each of the study participants. After the birth of the child, the umbilical cord was ligated before delivery of the placenta. The cord blood was then collected into sterile heparinized tubes that were labeled with the subject name and time of collection and kept at room temperature. Cord blood specimens were processed within 16 hours of collection. Peripheral blood samples (2.5-10 mL) were collected at age 1 year by means of venipuncture at the antecubital site of the arm. Lidocaine 2.5% and prilocaine 2.5% (EMLA, AstraZeneca) was applied to the area 45 to 60 minutes before phlebotomy. The blood was collected in sterile heparinized tubes, kept at room temperature, and then processed on the day of collection.

### MNC stimulation

MNCs were separated by means of density centrifugation (LSM Lymphocyte Separation Medium; ICN Biomedicals Inc, Aurora, Ohio), and plasma was removed and stored at –80°C in labeled microcentrifuge tubes. The MNCs were suspended ($10^6$ cells/mL) in RPMI-1640 supplemented with 10% FBS (Hyclone), L-glutamine (2 mmol/L), penicillin (100 U/mL), and streptomycin (100 μg/mL) and incubated (24 hours at 37°C and 5% $CO_2$) in a 24-well, flat-bottom, cell-culture plate (1 mL/well; Corning Inc, Corning, NY) with PHA (5 μg/mL; Sigma, St Louis, Mo) or medium alone. The PHA was placed in aliquots in single-use portions and frozen at –80°C at the beginning of the study so that the same stimulant was used throughout the study. After the culture supernatants were collected, they were frozen (–80°C) in aliquots pending analysis for cytokines.

### Cytokine ELISA

Levels of IFN-γ, IL-5, IL-10, and IL-13 in culture supernatants were evaluated by means of ELISA (Pharmingen, San Diego, Calif). The manufacturer's protocol was followed except that the sample volume was reduced to 50 μL. The sensitivities of the ELISAs are as follows: IFN-γ, 3.1 pg/mL; IL-5, 1.9 pg/mL; IL-10, 7.8 pg/mL; and IL-13, 3.1 pg/mL. Duplicate wells were run for each sample, and mean values are reported.

### Total and allergen-specific IgE

Fluoroenzyme immunoassays (FEIAs) were performed on plasma obtained from 1-year peripheral blood samples by using an automated instrument (Unicap 100; Pharmacia and Upjohn Diagnostics, Kalamazoo, Mich) to determine total and specific IgE levels. Specific IgE levels were determined for 2 species of dust mite (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), cat dander, egg white, *Alternaria alternata*, peanut, milk, and dog. The sensitivity for detection of specific IgE was 0.35 kU/L, and values greater than or equal to 0.35 kU/L were considered positive. The sensitivity for detection of total IgE was 2 kU/L.

### Measurement of blood eosinophils

Total white blood cell counts were performed by using an automated instrument. Blood smears from peripheral blood samples were stained (Wright-Giemsa stain), and a 200-count white blood cell differential was performed. The absolute eosinophil counts were then calculated for all peripheral blood samples.

### AD and food allergy definitions

AD was defined as physician diagnosed either by means of documentation by a health care provider on the medical record or by means of parental report of physician-diagnosed AD on the historical questionnaires. Three groups were defined and used for this study: (1) children with no AD anytime within the first year of life; (2) children with AD documented at any point within the first year; and (3) children with active disease at age 1 year.

Food allergy was defined by using allergen-specific IgE test results (FEIA) and historical reports (parental reports, physician documentation, or both). Three groups were defined: (1) *probable food allergy* includes a positive FEIA and a convincing history, defined as a reproducible adverse response that is temporally related to a specific food and involves typical organ systems[10]; (2) *questionable food allergy* includes a positive FEIA result with an indeterminate history or a negative FEIA result with a suggestive history; and (3) *no food allergy* includes a negative FEIA result with a negative history or a positive FEIA result with no adverse reactions to the food or foods in question.

### Statistical analysis

Continuous parameters are summarized by using medians and interquartile ranges. Dichotomous parameters are summarized as percentages. Changes in the cytokine-response profile from cord blood to age 1 year were assessed by using the Wilcoxon signed-rank test. Associations between cytokine-response profiles (cord blood, age 1 year, or change from cord blood to age 1 year) and biologic markers of atopy (absolute eosinophil counts or total IgE levels) were assessed by using the Spearman rank correlation coefficient. Rates of allergic sensitization (≥1 positive FEIA result) were calculated for quartiles of cord blood cytokines. Differences in cytokine-response profiles on the basis of the presence or absence of allergic sensitization at age 1 year were assessed by using the Wilcoxon rank sum test. Similar analyses were conducted for outcomes of AD during the first year of life and active AD at the 1-year visit.

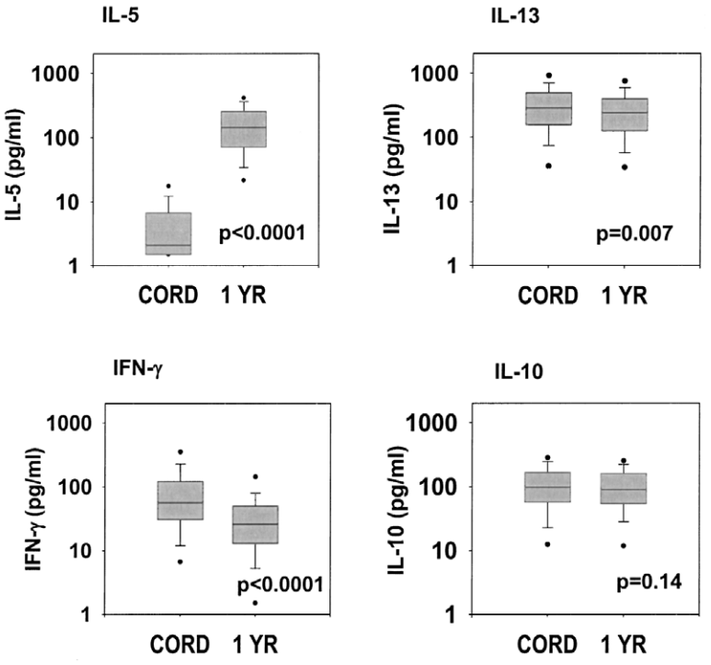


**FIG 1.** Interval changes for cytokine-response profiles from birth to 1 year for the entire cohort. The *box* represents the 25th and 75th percentiles, with medians indicated by *horizontal lines*, and the 5th and 95th percentiles are represented by *filled circles*.

**TABLE I.** Subject demographics

| | |
|---|---|
| Maternal characteristics | |
| Age (y) | 31.3 ± 4.8 |
| History | 43.7% |
| Allergy | |
| Asthma | 3.9% |
| Both | 36.2% |
| Ethnicity, white | 86.8% |
| Paternal characteristics | |
| Age (y) | 32.9 ± 5.3 |
| History | |
| Allergy | 48.6% |
| Asthma | 2.1% |
| Both | 24.6% |
| Ethnicity, white | 86.8% |
| Child characteristics | |
| Sex, male | 56.3% |
| Ethnicity, white | 86.8% |
| Birth weight (kg) | 3.54 ± 0.54 |
| Head circumference (cm) | 34.6 ± 2.4 |
| Apgar score | |
| 10 | 7.4% |
| 9 | 80.9% |
| 8 | 9.6% |
| 7 | 2.1% |

Values are presented as means ± SD for continuous variables and percentages for categoric variables.

## RESULTS

### Immunologic development in the first year of life

To begin to evaluate the immunologic development of this high-risk cohort, we compared the cytokine secretion patterns at birth (cord blood) with those obtained at age 1 year. Although the pattern of cytokine responses changed significantly, the changes did not follow either a $T_H1$ or $T_H2$ polarization pattern (Fig 1). PHA-stimulated cord blood cells secreted low levels of IL-5 (median, 2.1 pg/mL), moderate levels of IFN-γ (57.4 pg/mL) and IL-10 (100 pg/mL), and greater amounts of IL-13 (281.8 pg/mL). PHA-induced IL-5 responses markedly increased from birth to 1 year (Fig 1, *A*), whereas IL-13 and IFN-γ responses decreased by 16% and 54%, respectively (Fig 1, *B* and *C*). There was no significant change in IL-10 responses in the first year of life (Fig 1, *D*).

### Cytokine responses and biologic markers of atopy

To compare cytokine-response profiles to biologic markers of atopy at age 1 year, we first evaluated relationships between peripheral blood absolute eosinophil counts and total IgE levels. Cord blood cytokine responses did not correlate with either of these atopic markers at age 1 year (Table II). In contrast, there were weak but

**TABLE II.** Spearman rank correlation coefficients between cytokine responses and biomarkers

| | Absolute eosinophil count | Total IgE |
|---|---|---|
| Cord blood | | |
| IFN-γ | 0.079 | 0.108 |
| IL-5 | 0.006 | –0.038 |
| IL-10 | –0.008 | 0.013 |
| IL-13 | –0.017 | –0.033 |
| 1 y | | |
| IFN-γ | –0.04 | –0.043 |
| IL-5 | 0.265‡ | 0.163† |
| IL-10 | 0.168* | 0.08 |
| IL-13 | 0.264‡ | 0.16† |
| Change (1 y, cord blood) | | |
| IFN-γ | –0.115 | –0.116 |
| IL-5 | 0.26† | 0.162† |
| IL-10 | 0.109 | 0.074 |
| IL-13 | 0.215† | 0.165† |

*$P < .05$.
†$P < .01$.
‡$P < .001$.

**TABLE III.** Cord blood cytokine responses and sensitization to egg

| Cytokine responses (quartiles, pg/mL) | n | Sensitization to egg (% positive) | *P* value |
|---|---|---|---|
| IFN-γ | | | .079 |
| <31.0 | 71 | 25.7% | |
| 31.0-57.3 | 71 | 14.3% | |
| 57.4-121.6 | 72 | 11.3% | |
| ≥121.7 | 70 | 16.2% | |
| IL-5 | | | .114 |
| <1.9 | 137 | 20.1% | |
| 1.9-2.0 | 7 | 14.3% | |
| 2.1-6.7 | 69 | 11.8% | |
| ≥6.8 | 71 | 15.7% | |
| IL-10 | | | .013 |
| <58.4 | 71 | 24.3% | |
| 58.4-99.4 | 70 | 17.6% | |
| 99.4-164.3 | 71 | 18.8% | |
| ≥164.4 | 70 | 7.1% | |
| IL-13 | | | .293 |
| <152.9 | 71 | 21.4% | |
| 152.9-281.7 | 71 | 15.7% | |
| 281.7-487.3 | 71 | 14.5% | |
| ≥487.4 | 70 | 15.9% | |

significant positive correlations between 1-year IL-5 and IL-13 responses and both markers of atopy and between 1-year IL-10 responses and the absolute eosinophil count (Table II). Positive correlations were also noted between changes in IL-5 and IL-13 responses from birth to 1 year and both markers of atopy (Table II). In contrast, there was no correlation between IFN-γ responses and either eosinophilia or total IgE.

Cord blood cytokine responses were grouped according to quartiles to determine whether cytokine responses at birth predicted the risk of allergen sensitization at age 1 year. Infants with high IL-10 responses at birth were significantly less likely to be sensitized to egg at age 1 year (Table III). There were no significant associations between egg sensitization and 1-year cytokine responses. Allergic sensitization in general, defined as detectable IgE for any one of the 8 allergens tested, was not associated with specific patterns of cytokine responses (data not shown).

### Cytokine responses, AD, and food allergy

Cytokine-response profiles were evaluated in children according to the presence or absence of AD or food allergy. AD was associated with increased total IgE (17 vs 11 IU/mL, $P = .02$), egg-specific IgE (29% vs 8%, $P < .001$), and a trend toward increased peripheral blood eosinophils (237 vs 194 cells/mL, $P = .07$); however, there were no significant associations between the presence of AD and cytokine-response profiles at birth or age 1 year (Table IV). Likewise, food allergy was not associated with significant alterations in polyclonal cytokine-response profiles (Table IV).

## DISCUSSION

The increase in prevalence of allergic airway diseases over the last several years[11-13] has generated interest in gaining a better understanding of the pathogenesis of these disorders in infancy. Our findings extend the results of previous studies and provide new information regarding the development of polyclonal cytokine responses relative to the incidence of clinical and immunologic expression of atopy. For the group as a whole, polyclonal cytokine responses over the first year of life did not evolve according to a strict $T_H1$ or $T_H2$ polarization pattern. For example, there were opposite directional changes in IL-5 (increase) versus IL-13 (decrease). In contrast to the whole group, there was a $T_H2$ polarization in infants who had biologic markers of atopy, such as eosinophilia (increased IL-5, IL-10, and IL-13) and increased total IgE levels (increased IL-5 and IL-13). Furthermore, reduced secretion of IL-10 by mitogen-stimulated cord blood cells was identified as a risk factor for sensitization to egg, which was the most common sensitization noted in our cohort. Together, these findings provide further evidence of a close relationship between immune development ($T_H2$ skewing) and the incidence of atopic manifestations in children.

When evaluating the immune development of the entire cohort, the most dramatic change noted was the paucity of IL-5 secretion from cord blood cells and the marked increase by age 1 year. The relatively high IL-13 responses from the stimulated cord blood MNCs indicate that not all $T_H2$-like cytokines are suppressed at birth. Instead, this pattern suggests that IL-5 production might be selectively inhibited at birth and then developmentally cued by an as-yet-undefined postneonatal signal.

Other groups have also evaluated the development of cytokine responses in infancy. Prescott et al[2] demonstrated that allergen-specific cytokine responses at birth might be skewed toward a $T_H2$ phenotype. Specifically,

**TABLE IV.** Cord blood cytokine responses compared with AD and food allergy

| | Atopic dermatitis | | | Food allergy | | |
|---|---|---|---|---|---|---|
| | Never (n = 162) | Ever (n = 123) | *P* value (E vs N) | No (n = 251) | Yes (n = 15) | *P* value (N vs Y) |
| Cord blood | | | | | | |
| IFN-γ | 55.1 (32.9, 117.7) | 60 (30.7, 127.1) | .93 | 61.3 (33.8, 119.4) | 37.7 (14.7, 93.6) | .12 |
| IL-5 | 2.2 (1.5, 6.5) | 1.9 (1.5, 7.1) | .8 | 2.1 (1.5, 6.3) | 1.5 (1.5, 7.8) | .73 |
| IL-10 | 106.7 (66.5, 172.6) | 97.8 (49.5, 161.7) | .43 | 103.7 (59.6, 171.0) | 96.1 (35.1, 126.7) | .3 |
| IL-13 | 290.7 (170.2, 490.8) | 280.4 (145.6, 457.0) | .37 | 281.8 (152.9, 486.0) | 316.9 (102.7, 511.9) | .91 |
| Change (1 year, cord blood) | | | | | | |
| IFN-γ | –27.1 (–86.2, 2.1) | –34.8 (–94.1, –1.7) | .34 | –32.4 (–92.3, 0.0) | –15.7 (–52.7, 15.2) | .36 |
| IL-5 | 124.7 (62.1, 237.5) | 156.1 (69.4, 244.8) | .39 | 137.2 (69.8, 247.3) | 110.3 (52.3, 179.3) | .15 |
| IL-10 | –16.5 (–75.8, 56.9) | –5.2 (–51.7, 41.5) | .55 | –5.8 (–67.4, 49.4) | –24.3 (–60.7, 42.1) | .85 |
| IL-13 | –73.3 (–260.3, 116.0) | –6.7 (–219.9, 149.9) | .16 | –37.8 (–237.9, 122.6) | –55.9 (–319.8, 77.1) | .49 |

Values represent median (range).



allergen stimulation of cord blood cells upregulated IL-4, IL-5, and IL-9 but not IFN-γ mRNA. In addition, allergen-stimulated cord blood cells secreted high levels of IL-10 (approximately 3000 pg/mL) and secreted low levels (10 pg/mL) of IL-13. Additional studies by this group indicated that, during development, there is a decrease in $T_H2$ responses to inhalant allergens in nonatopic children, whereas there is an increased or persistent $T_H2$ response in atopic children.[14,15]

IL-13 is produced from $T_H2$ lymphocytes and plays a role in inhibiting pro-inflammatory cytokine production, in the induction of IgE isotype switching, and in vascular cell adhesion molecule 1 expression.[16-18] Paradoxically, studies have found that mitogen-stimulated[7] and allergen-stimulated[14,15] cord blood MNC production of IL-13 is reduced in atopic children. In infancy there appear to be age-dependent increases in allergen-specific IL-13 responses for the atopic children, whereas the responses wane in nonatopic children.[14,15] Data from the present study extend these findings by demonstrating significant correlations between the development of $T_H2$ skewing (increased polyclonal IL-13 and IL-5 responses) and specific biologic markers of atopy: total IgE and peripheral blood eosinophilia.

Although no statistically significant differences were noted between cord blood cytokine responses and allergen-specific IgE results grouped as a whole, reduced IL-10 responses in cord blood were associated with an increased frequency of egg allergen sensitization. In the first year of life, sensitization is most frequently seen with egg allergen, and this is a risk factor for the subsequent development of clinically apparent allergic diseases, such as AD, allergic rhinitis, and asthma.[19-22] Interestingly, IL-10 has been implicated in the development of tolerance through several potential mechanisms, including shifting antibody responses from IgE to IgG4[23,24] and promoting the development of T regulatory cells.[25,26]

Although patterns of cytokine responses were clearly associated with various immunologic aspects of atopy, we did not find a significant association with AD or food allergy. Previous studies of immune development in AD have yielded conflicting results: some studies have reported that AD is associated with reduced IFN-γ levels,[3,27] whereas Halonen and Martinez[8] found no such association. The divergent results of these studies are likely due to differences in the subject population, how AD was defined, and the length of follow-up. It is possible that a stronger relationship between AD and IFN-γ production exists with more severe disease: many of the children in our cohort had relatively mild disease compared with subjects in other studies.[3] Also, the design of the present study is a large prospective cohort, which inherently requires the clinical assessment of several investigators and historical data provided from the parents to make the diagnosis of AD. This differs from other studies in which the cohorts were smaller and a single investigator could make the diagnosis of AD on the basis of clinical findings.[27] We are continuing to monitor these relationships because the clinical expression of these atopic phenotypes appears de novo, regresses with time, or both. In addition, we will be evaluating these outcomes in relationship to the emergence of other atopic phenotypes (eg, asthma and rhinitis) as well.

There are several important differences between our study and previously published work. First, the cytokine-response profiles for this study were evaluated after mitogen stimulation rather than specific allergen stimulation. This enabled the measurement of cytokine protein secretion rather than relying on the measurement of mRNA, which has been reported for similar evaluations with allergen-evoked responses.[2,14,15] Because the use of allergen-specific stimulation would likely result in a significant proportion of the cohort having no response at birth, the polyclonal T-cell mitogen PHA was used to maximize our ability to investigate the developmental aspect of cytokine-response profiles beginning with cord blood in the entire cohort.

Second, this study is relatively unique in that it is a prospective evaluation of a large cohort of children who all have a parental history of allergy or asthma. Although this design could be criticized for the lack of inclusion of a low-risk (by parental history) control group, more than half of the study subjects have exhibited no biologic or clinical evidence of atopy. We are intrigued by the fact that the cytokine responses of the infants who are

expressing biologic markers of atopy (increased total IgE and absolute eosinophil counts) by the first year of life diverge from those of the children who are not and that cytokine-response profiles when evaluated during infancy are more likely to reflect the biologic rather than the clinical expression of atopy. As such, we would predict the continued study of this high-risk cohort will provide a unique opportunity to comprehensively evaluate gene-gene and gene-environment interactions that might facilitate not only the clinical expression of atopic phenotypes but also the downregulation of these traits.

In summary, the results of our study demonstrate the complex interplay of cytokine responses during infancy in relationship to age (development), biologic markers of atopy, and disease expression. Defining the immunologic characteristics of atopy in infancy and early childhood continues to be an important research goal. By comparing and contrasting the immunologic development for the different atopic phenotypes in infancy, it might be possible to identify early immunologic characteristics, clinical characteristics, or both that predispose to the development of allergic diseases, such as asthma.

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## REFERENCES

1. Gern JE, Lemanske RF Jr, Busse WW. Early life origins of asthma. J Clin Invest 1999;104:837-43.
2. Prescott SL, Macaubas C, Holt BJ, Smallacombe TB, Loh R, Sly PD, et al. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. J Immunol 1998;160:4730-7.
3. Tang MLK, Kemp AS, Thorburn J, Hill DJ. Reduced interferon-gamma secretion in neonates and subsequent atopy. Lancet 1994;344:983-5.
4. Matsui E, Kaneko H, Teramoto T, Fukao T, Inoue R, Kasahara K, et al. Reduced IFN-gamma production in response to IL-12 stimulation and/or reduced IL-12 production in atopic patients. Clin Exp Allergy 2000;30:1250-6.
5. 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.
6. Hoekstra MO, Hoekstra Y, De Reus D, Rutger B, Gerritsen J, Kauffman HF. Interleukin-4, interferon-gamma and interleukin-5 in peripheral blood of children with moderate asthma. Clin Exp Allergy 1997;27:1254-60.
7. Prescott SL, Holt PG. Abnormalities in cord blood mononuclear cytokine production as a predictor of later atopic disease in childhood. Clin Exp Allergy 1998;28:1313-6.
8. Halonen M, Martinez FD. A deficient capacity to produce interferon-gamma: is it a risk for asthma and allergies? Clin Exp Allergy 1997;27:1234-6.
9. Williams TJ, Jones CA, Miles EA, Warner JO, Warner JA. Fetal and neonatal IL-13 production during pregnancy and at birth and subsequent development of atopic symptoms. J Allergy Clin Immunol 2000;105:951-9.
10. Sampson HA. Adverse reactions to food. In: Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, Busse WW, editors. Allergy: principles and practice. St. Louis: Mosby; 1998. p. 1162-82.
11. Shaw R, Crane J, O'Donnell T, Porteous L, Coleman E. Increasing asthma prevalence in a rural New Zealand adolescent population 1975-89. Arch Dis Child 1990;65:1319-23.
12. Nakagomi T, Itaya H, Tominaga T, Yamaki M, Hisamatsu S, Nagagomi O. Is atopy increasing? Lancet 1994;343:121-2.
13. von Mutius E, Weiland S, Fritzsch C, Duhme H, Keil U. Increasing prevalence of hayfever and atopy in Leipzig, East Germany. Lancet 1998;351:862-6.
14. Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG. Development of allergen-specific T-cell memory in atopic and normal children. Lancet 1999;353:196-200.
15. 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.
16. McKenzie AN, Culpepper JA, de Waal Malefyt R, Briere F, Punnonen J, Aversa G, et al. Interleukin 13, a T cell-derived cytokine that regulates human monocyte and B cell function. Proc Natl Acad Sci U S A 1993;90:3735-9.

17. Zurawski G, de Vries JE. Interleukin 13, an Interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. Immunol Today 1994;15:19-26.
18. Bochner BS, Klunk DA, Sterbinsky SA, Coffman RL, Schleimer RP. IL-13 selectively induces vascular cell adhesion molecule-1 expression in human endothelial cells. J Immunol 1995;154:799-803.
19. Sigurs N, Hattevig G, Kjellman B, et al. Appearance of atopic disease in relation to serum IgE antibodies in children followed up to 4 years. J Allergy Clin Immunol 1994;94:757-63.
20. Hattevig G. Kjellman B, Bjorksten B. Clinical symptoms and IgE responses to common food proteins and inhalants in the first 7 years of life. Clin Allergy 1987;17:571-8.
21. Hattevig G, Kjellman B, Johansson SG, Bjorksten B. Clinical symptoms and IgE responses to common food proteins in atopic and healthy children. Clin Allergy 1984;14:551-9.
22. Zeiger RS, Heller S. The development and prediction of atopy in high-risk children: follow-up at age seven years in a prospective randomized study of combined maternal and infant food allergy avoidance. J Allergy Clin Immunol 1995;95:1179-90.
23. Jeannin P, Lecoanet S, Delneste Y, Gauchat JF, Bonnefoy JY. IgE versus IgG4 production can be differentially regulated by IL-10. J Immunol 1998;160:3555-61.
24. Platts-Mills T, Vaughan J, Squillace S, Woodfolk J, Sporik R. Sensitization, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. Lancet 2001;357:752-6.
25. Akbari O, DeKruyff RH, Umetsu DT. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. Nat Immunol 2001;2:725-31.
26. Akbari O, Freeman GJ, Meyer EH, Greenfield GA, Chang TT, Sharpe AH, et al. Antigen-specific regulatory T cells develop via the ICOS#150;ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity. Nat Med 2002;8:1024-32.
27. Warner JA, Miles EA, Jones AC, Guint DJ, Colwell BM, Warner JO. Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic eczema? Clin Exp Allergy 1994;24:423-30.