

CD4+IL13+ T Lymphocytes at Birth and the Development of Wheezing and/or Asthma during the 1st Year of Life

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Key Words

Cord blood · T lymphocytes · Cytokines · Th2 cells ·
Asthma · Newborns

Abstract

Background: Despite our knowledge that maternal inheritance influences the development of asthma in childhood, attempts to identify a clear-cut Th2-oriented cytokine production by T lymphocytes at birth have given conflicting results. The prognostic significance of these cells for asthma development later in life remains to be determined. **Methods:** We evaluated at the single cell level Th1- and Th2-type cytokines in 208 randomly selected cord blood mononuclear cell (CBMC) samples obtained from pregnant women (group A, n = 68 with diagnosed respiratory allergic disease; group B, n = 140, with no evidence of atopy), and prospectively followed newborns for 1 year. **Results:** There was no difference in IFN- γ , IL-4 and IL-5 production at birth between both groups, whereas a correlation between CD4+IL13+ lymphocytes from CBMC samples derived from atopic moth-

ers and the occurrence of wheezing and/or asthma during the 1st year of life was found. **Conclusions:** Our observations suggest that the intracellular cytokine profile of cord blood CD4+ cells, in terms of IL-13 production, could be considered a useful tool for a more accurate identification of newborns from atopic mothers who are at high risk of developing asthma.

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Introduction

A Th2-biased cytokine production pattern is characteristic of CD4+ T lymphocytes of asthmatic subjects [1, 2]. Genetic and epidemiological studies have suggested an important role for maternal inheritance, but failed to identify risk factors indicating future development of respiratory allergy in children [3]. Indeed, uncontrolled observations have suggested that cord blood mononuclear cells (CBMC) of newborns from atopic mothers may produce more IL-4 and IL-5 than normal controls [4, 5]. To what extent a Th2-type cytokine production pattern by CBMC may correlate with the future development of an allergic respiratory disease has been the subject of debate [6, 7] and still represents an unresolved matter [8]. Recently, IL-13, a Th2-type cytokine that shares the same

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α -chain receptor as IL-4 on the surface of CD4+ cells, has been shown to be fundamental in the pathogenesis of experimental asthma [9–12].

For these reasons, we evaluated the cytokine profile of CBMC from a large series of pregnant women, and correlated it with the occurrence of wheezing and/or asthma in their babies in a 1-year prospective study.

Material and Methods

Study Design

A cohort of 2,503 unselected pregnant women living in the comune of Perugia (Central Italy), who were referred to six different hospitals for delivery, were recruited during a 1-year period. A modified self-administered-questionnaire of the European Community Respiratory Health Survey [13] was given to each subject and cumulative data were analyzed to evaluate the prevalence of asthma and/or rhinitis in the past or present. All subjects underwent skin prick testing to six major allergens (*Graminaceae*, *Olea europea*, *Parietaria officinalis*, *Dermatophagoides pteronyssinus*, *Cupressus sempervirens* and molds; Bracco-Allergopharma, Milano, Italy). 251 CBMC samples were randomly (1:10) collected at the time of delivery. Two main groups were identified: group A ($n = 68$), mothers with clinical symptoms of asthma and/or rhinitis in accordance with positive skin prick tests, and group B ($n = 140$), mothers who were completely asymptomatic. A third group ($n = 43$) was not included in the analysis due to non-concordance between clinical symptoms and skin prick test results.

After 1 year, the questionnaire of the International Study of Asthma and Allergies in Childhood [14] was administered to mothers to evaluate the number of asthma attacks and/or nights with wheezing per week in their babies. For a more accurate data analysis, family pediatrics were subsequently interviewed to confirm the diagnosis of wheezing and/or asthma in infants.

The study was approved by the regional ethic committee and informed consent was obtained from parents for cord blood collection.

Intracellular Th2 Cytokines

Intracellular cytokines were detected by flow cytometry using the method of Jung et al. [15] with partial modifications. Briefly, 10^6 CBMC/ml were stimulated with PMA plus ionomycin (Sigma, St. Louis, Mo., USA). After 5 h of activation, $10 \mu\text{g/ml}$ brefeldine A (Sigma) were added to the cultures and the cells were cultured for 3 additional hours. After a total 8 h of activation, CBMC were stained with either Cy-chrome-conjugated anti-CD4 (PharMingen, San Diego, Calif., USA), DX1 (NKR-P1A Ag, kindly supplied by Dr. Lewis L. Lanier, DNAX Research Institute, Palo Alto, Calif., USA), anti-CD8 (PharMingen); anti-Fc ϵ RI (kindly supplied by Dr. Akihiro Yachie, Juntendo University, Tokyo, Japan), anti-CD3 (Serotec), anti- $\gamma\delta$ (Serotec) and anti-CD57 (HNK1 Ag, Serotec) mAbs followed by a further incubation with an isotype-specific fluorescein-conjugated goat anti-mouse IgG (Southern Biotechnology, Birmingham, Ala., USA). Thereafter, cells were fixed by incubation for 20 min with 2% formaldehyde (Sigma) and permeabilized by incubation for 10 min in PBS supplemented with 2% FCS, 2 mM NaN₃ and 0.5% saponin (Sigma). Permeabilized CBMC were then incubated with PE-labeled anti-hIL-

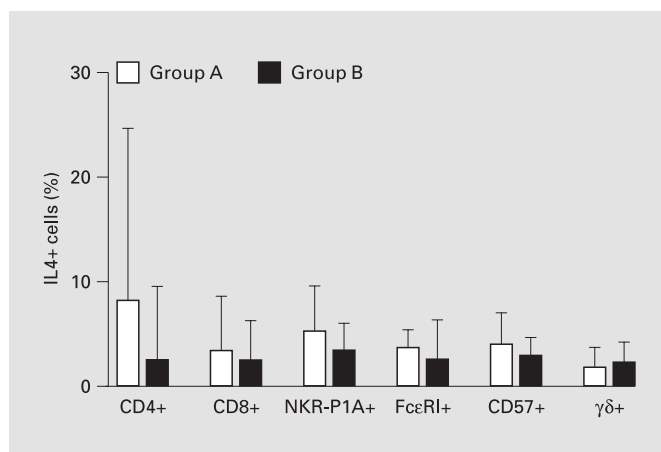


Fig. 1. Distribution of IL-4-producing cells among mononuclear cells of cord blood samples from the two study groups. There was no clear-cut subset distribution in IL-4+ cells among the two groups (group A: 68 samples; group B: 140 samples).

4, IL-5, IL-13 and IFN- γ mAbs (Becton Dickinson, San Jose, Calif., USA). Two-color immunofluorescent staining was used to enumerate cytokine-positive cells in lymphocyte, basophil and NK cell subpopulations (FACScan flow cytometer, Becton Dickinson). In some experiments, three-color immunofluorescence data were analyzed on gated Cy-chrome-CD4+ cells.

Cord Blood Total IgE

Total IgE in cord blood samples as determined using a conventional assay with CAP System (Pharmacia, Uppsala, Sweden). Values <0.5 kU/l were considered negative or undetectable, values ≥ 0.5 kU/l detectable or high.

Statistical Analysis

Due to the non-normal distribution of samples, the Kruskal-Wallis one-way analysis of variance and multiple logistic regression analysis were adopted for statistical evaluation. Data were analyzed by means of the Prophet software (version 5.0, BBN Systems and Technologies).

Results and Discussion

Our study was performed to evaluate the presence of T helper cells with a Th2-oriented cytokine production pattern at birth. Group A CBMC showed a higher percentage of CD4+ lymphocytes ($31.8 \pm 11.9\%$) than group B ($23.6 \pm 10.8\%$; $p = 0.033$), and higher total IgE levels (samples with IgE ≥ 0.5 kU/l; group A, $18.1 \pm 3.5\%$, group B, $6.4 \pm 1.7\%$, $p < 0.05$). Significant differences in IL-4+ cells were found neither among CD4+, CD8+, NKR-P1A+, CD57+ nor $\gamma\delta$ lymphocytes (fig. 1), nor among CD4+NKR-P1A+ (data not shown). IL-4 production by

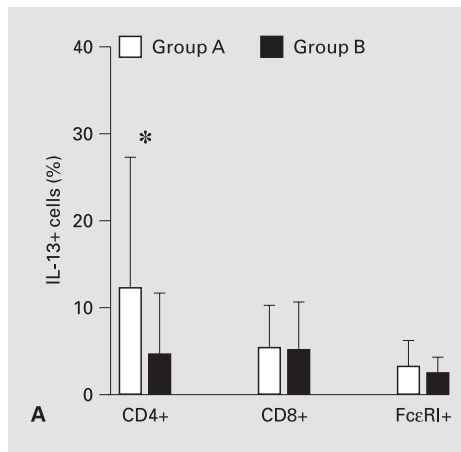
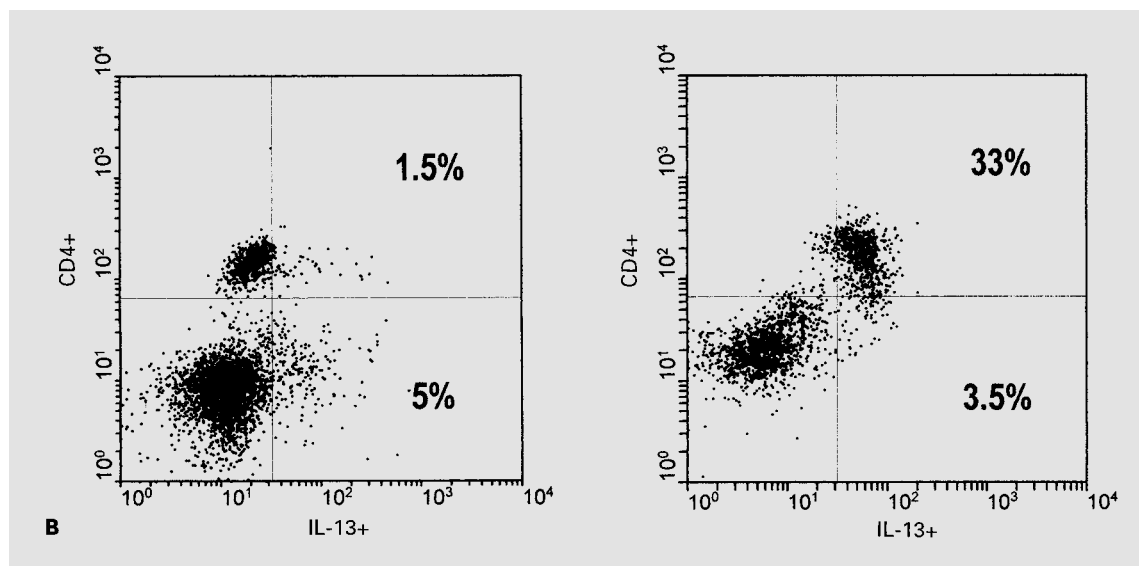


Fig. 2. Distribution of IL-13-producing cells in cord blood samples. **A** Percentages of IL-13+ cells in the two groups of subjects studied. □ = Group A; ■ = group B. There was a significant increase in double-stained CD4+IL-13+ T cells in group A as compared with group B. * $p = 0.034$. **B** Representative dot plots of two cord blood samples showing the different amounts of CD4+IL-13+ cells at birth among group B (left) and group A (right).



non-lymphoid cells, such as FcεRI+ cells, did not show significant differences between the two groups (fig. 1).

Our results argue against a critical role for early IL-4 production by either CD4+ [16] or NK-T lymphocyte subsets [17] in generating Th2 effector function characteristic of allergic diseases [18, 19]. Indeed, such a role has been questioned recently [20, 21]. Despite their ability to secrete Th2-type cytokines [22], there is no clear evidence from animal studies for a primary pathogenetic role of basophil/mast-cell-derived Th2-type cytokines in favoring the onset and persistence of the inflammatory late-phase allergic response. Indeed, IgE-deficient mice can develop antigen-induced airway hyperreactivity, which indicates that mechanisms other than IgE-mediated basophil/mast-cell degranulation are important for the development of airway allergic inflammation [23].

We did not find any difference in T lymphocytes producing IL-5 between the two groups. An extremely low number of T cells (below 1% in both groups, data not shown) stained positively with anti-IL-5 mAb. This finding is not surprising since IL-5+ circulating T lymphocytes are normally absent in adult and cord samples [24].

We observed low levels of IFN-γ production by cord blood CD3+ T cells in both groups (group A: $1.09 \pm 0.8\%$, group B: $1.85 \pm 0.9\%$, $p = \text{NS}$). The low IFN-γ production by cord blood T lymphocytes is a well-known phenomenon [25]. Recent data indicated the defective IFN-γ production during early life may favor the development of allergic diseases [26, 27]. Our results of samples collected at birth are not comparable with those of longitudinal studies performed with an in vitro stimulation of lymphocytes with allergen mixtures. In addition, we fol-

Table 1. Clinical manifestations of allergy at 1 year of age

Respiratory manifestations	Group A (n = 68)		Group B (n = 140)	
	n	%	n	%
Wheezing	14	20.58*	3	2.14
Asthma	9	13.23*	1	0.71

The questionnaire of the International Study of Asthma and Allergies in Childhood administered to mothers together with the final diagnosis of the family pediatrician were used to identify any symptomatic children.

* $p < 0.05$ compared to group B.

lowed our newborns for only 1 year, a time lapse in which Th1-type cytokine production still remains low [27].

We found a polarization in the production of IL-13 among group A CD4⁺ T lymphocytes (fig. 2), whereas no differences between groups were seen for IL-13 expression in other lymphocyte subsets or FcεRI⁺ cells.

A greater incidence of wheezing and/or asthma-equivalent symptoms was found in children from group A at the 1-year follow-up (table 1). Furthermore, the development of symptoms positively correlated with the highest per-

centages of CD4⁺IL-13⁺ cells at birth (logistic regression $\chi^2 = 4.792$, $p = 0.028$) in the same group.

Recently, an IL-4-independent pathway of Th2-mediated inflammation has been demonstrated in asthma [28], and a potential role for IL-13 is suggested by the observation that human T cells express a functional IL-13 receptor [10] able to transduce the Stat6-mediated signal, which is important for Th2 cell development [29]. Naive T cells from IL-13-deficient mice show impaired Th2 cytokine secretion when cultured in vitro under Th2-biasing conditions [9], and transgenic mice expressing IL-13 within the lung confirm the role of this cytokine in experimental asthma [30]. Our data point out to the correlation between the presence of CD4⁺IL-13⁺ T lymphocytes at birth and future development of asthma in a way strictly dependent on the atopic status of the mothers. The two human IL-13 receptors (Rα1 and Rα2) are single genes on chromosome Xq13 [31], and the recently described association between genetic variants of IL-13 Rα1 and atopy [32] supports, for the first time, an X-linked inheritance of atopic disorders.

In conclusion, we suggest that the intracellular cytokine profile of cord blood CD4⁺ cells, especially in terms of IL-13 production, could be considered a useful tool for a more accurate identification of newborns from atopic mothers, who are at high risk of developing asthma.

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