

In Vitro IgE Synthesis in Neonates with or without a Family History of Atopy

STEFANO MICELI SOPO, M.D.,¹ GILDA FEDERICO, M.D.,¹
DANTE PARENTI, Ph.D.,² MARIA CONCETTA ALECCI, M.D.,²
GIULIA ZORZI, M.D., and ACHILLE STABILE, Ph.D.¹

ABSTRACT

One highly desirable objective of preventive medicine is to find predictive markers that could identify neonates who are at risk of developing atopic diseases. However, no one laboratory test is sufficiently sensitive and specific to be used for this purpose. The objective of this study was to evaluate the use of an *in vitro* IgE synthesis by cord blood lymphocytes of neonates with or without a family history of atopy. Cord blood mononuclear cells (CBMC) of 11 neonates with a family history of atopy and 9 neonates without a family history of atopy were incubated for 48 hr with human recombinant IL4 (hrIL4) in Petri plates. Cord blood lymphocytes (nonadherent cells) were then washed and incubated for an additional 9 days with various stimuli. hrIL4, hrIL4 + human recombinant interferon-gamma (hrIFN γ), hrIL4 + anti-IL4 antibody (ab), hrIL4 + anti-CD40 ab, hrIL4 + anti-CD40 ab + betalactoglobulin (BLG) hrIL4 + anti-IFN γ ab. Unstimulated cultures were included as negative controls. IgE concentrations in sera were measured by a fluorimmunoenzymatic method. Concentration of supernatants IgE ≥ 2.8 kU/L and serum IgE ≥ 0.7 kU/L were considered to be a positive test. Statistical analyses were performed using a Wilcoxon rank test, Mann-Whitney test for the sum of ranks, chi-square test, and/or Fisher's Exact test. Differences were considered significant when $p < 0.05$. The stimulatory effects of hr IL4 alone resulted in the highest production of IgE, which was significantly higher than the effects of hrIL4 + antiCD40 ab + BLG (medians: 2.9 vs 2.2 kU/L) or with hrIL4 + anti IL4 ab (medians: 2.9 vs. 2.4 kU/L) or the absence of any stimulus (medians of 2.9 vs. 0.5 kU/L). All stimuli resulted in production of Ig E, which was significantly higher than the IgE serum level alone (median of 0.17 kU/L). No significant differences were observed between the neonates with a family history of atopy versus those without a family history of atopy, neither with regard to median values of IgE nor with regard to the percentages of infants having IgE levels ≥ 2.8 kU/L in the culture's supernatants or ≥ 0.7 kU/L in the serum. Our results confirm that the neonatal B lymphocytes have the capacity to synthesize normal levels of IgE, if placed under adequate conditions. Low serum neonatal IgE levels are therefore the result of a deficit of the T-lymphocyte cooperation. However, *in vitro* IgE synthesis after exposure to a nonspecific stimulus (IL 4, anti-CD40 ab) is not a reliable predictive marker of atopy. (Pediatr Asthma Allergy Immunol 2001; 15[2]:97-105.)

¹Department of Pediatrics, Catholic University of Rome, Italy.

²Neonatology Clinic, Cristo Re Hospital, Rome, Italy.

INTRODUCTION

OVER THE PAST 30 YEARS, an increasing prevalence of atopic disease has been observed in children with no family history of atopy.⁽¹⁾ Because preventive measures such as the substitution of cow's milk formulas have shown uncertain efficacy, but may have significant costs, they are advisable only for neonates with a high risk of developing allergy. Early and reliable identification of such neonates is needed. Currently, the most reliable marker is a positive family history of atopy. However, 40% of neonates with biparental family history of atopy and 70% with monoparental history of atopy do not develop atopic diseases. In contrast, 50% of atopic children have an absent family history of atopy.⁽²⁾ Therefore, more sensitive and specific markers of atopy in neonates are needed. Over the past several years, studies have examined the neonatal lymphocyte activity and the production of cytokines such as interferon-gamma (IFN γ) and interleukin-4 (IL4) as a possible predictors of atopy. A reduction *in vitro* production of IFN γ at birth in neonates with a family history of atopy^(3,4) was found to be associated with development of atopic diseases.^(5,6) Other studies did not confirm these findings.⁽⁷⁻¹⁰⁾ Some authors have observed a persistent TH2 phenotype in the first 2 years of life in infants who develop atopy, while the switch to the TH1 phenotype was observed in non atopic infants.^(11,12) Thus, the *in vitro* measurement of cytokines, particularly IFN γ , cannot be considered as a useful screening test for atopy because of the inconsistent data in the literature. There is no laboratory marker available at the present time that can significantly improve the predictability of the family history of atopy and help identify neonates that will be at risk of atopic diseases in childhood. High elevated cord blood levels of IgE have been confirmed to be an important risk factor for allergic sensitization, but they have a very low level of sensitivity.⁽¹³⁾ We have hypothesized that the sensitivity of the test may be improved by measuring the cord blood lymphocytes (CBL) *in vitro* synthesis of IgE by stimulation. Bergmann et al.⁽¹⁴⁾ pointed out the utility of evaluating IgE *in vitro* production by neonatal mononuclear cells after an adequate stimulus as a marker of atopic prediction. In fact, it has been demonstrated that the B lymphocyte of an allergic subject have an enhanced capacity to produce IgE, if compared with a normal subject.⁽¹⁵⁾ The aim of this research was to evaluate any significant differences between neonates with or without a family history of atopy with regard to *in vitro* IgE synthesis by CBL after adequate stimuli.

METHODS

Study population

Twenty caucasian parents of neonates (8 males and 12 females) who were born at term by natural delivery were contacted to participate in this study. Informed consent and approval by the ethics committee was obtained for this study. The parents were evaluated for evidence of history of atopy. The finding of at least one parent affected by atopic disease was considered as a positive family history of atopy. The following findings were regarded as evidence of atopic disease in parents: (1) asthma: recurrent episodes of wheezing or dyspnea or medically confirmed asthma; (2) food allergy: weeping, erythematous itching, papular and vesicular lesions involving primarily the face and the flexural folds, recurrent vomiting, diarrhea, or crampy abdominal pain and/or urticarial skin reaction a few minutes after the ingestion of a specific food, that is, milk, eggs, fish, etc; (3) rhinitis: episodes of nose rubbing, fits of sneezing, sniffing with nasal discharge and/or blockage excluding obvious infectious rhinitis. The allergic diagnosis of the symptoms was confirmed by skin prick tests and double-blind, placebo-controlled food challenge (DBPCFC)⁽¹⁶⁾: 9 neonates had a negative family history of atopy (atopic risk = 0) and 11 had a positive history (9 uniparental, atopic risk = 1; 2 biparental, atopic risk = 2) of atopy. The mothers did not take any special precaution regarding their own diet during the pregnancy.

Blood sampling

Approximately 12–14 mL of cord blood samples of all neonates were aseptically collected in heparinized tubes immediately after delivery with the usual procedures, including careful cleaning of the cord and stick puncture of the umbilical vein, to avoid maternal contamination. Moreover, total serum IgA concentrations were measured in all cord samples by a standard nephelometric technique (Beckman IgA test kit; Beckman

Instruments Inc., Brea, CA). If IgA levels were >1.8 mg/dL the sample was considered contaminated and not used.⁽¹⁷⁾ An aliquot (4 mL) of every blood sample was centrifuged to obtain autologous serum.

Mononuclear cell isolation

The remaining aliquots (8–10 mL) of blood were placed into heparinized sterile tubes for leukocyte isolation. Cord blood mononuclear cells (CBMC) were obtained by Ficoll–Hypaque gradient centrifugation and then were washed three times with Ca^{++} and Mg^{++} free phosphate-buffered saline (PBS). Thereafter, CBMC were resuspended at a concentration of 1×10^6 cells/mL in culture medium (RPMI 1640; Flow Laboratories, Irvine, Scotland) supplemented with antibiotics (100 U/mL of penicillin and 100 mg/mL of streptomycin; Flow Laboratories, Irvine, Scotland), L-glutamine (2 mmol/L; Gibco Laboratories, Grand Island, NY) and 10% autologous human serum.

CBL stimulation for IgE synthesis

CBMC were then incubated at 37°C in a humidified atmosphere with 5% CO_2 for 48 hr in Petri dishes at 1×10^6 cells/mL concentration in medium supplemented with 200 U/mL of human recombinant IL4 (hrIL4, cod. 2181-01, Genzyme). After 48 hr of incubation, CBL (the nonadherent cells) were gently removed. CBL were then washed three times in PBS, counted, and tested for viability by Trypan blue dye exclusion and resuspended at concentrations of 10^6 per mL of medium. Then, several kinds of cell culture were prepared containing the following components:

1. hrIL4 (500 U/mL);
2. hrIL4 (500 U/mL) + hrIFN γ [250 U/mL of human recombinant IFN γ , cod. 80-3348-01, Genzyme, in accordance to Rousset et al.⁽¹⁸⁾];
3. hrIL4 (500 U/mL) + anti-IL4 receptor antibody (anti-IL4R ab) [10 $\mu\text{g/mL}$ of monoclonal mouse anti-human IL4 receptor (CDw124) IgG1, cod. 80-3285-01, Genzyme, in accordance to Snapper et al.⁽¹⁹⁾];
4. hrIL4 (500 U/mL) + anti-IFN γ receptor (anti-IFN γ R) ab (10 $\mu\text{g/mL}$ of monoclonal mouse anti-human IFN γ receptor ab, cod. 1223-00, Genzyme, in accordance to the instructions of the producer);
5. hrIL4 (500 U/mL) + anti-CD40 ab (5 $\mu\text{g/mL}$ of monoclonal anti-CD40 ab, cod. CDP40, Ylem, in accordance to the instructions of the producer);
6. hrIL4 (500 U/mL) + anti-CD40 ab (5 $\mu\text{g/mL}$) plus betalactoglobulin [100 $\mu\text{g/mL}$ of betalactoglobulin (BLG) (SARM Allergeni, Pomezia, Italy) in accordance to our previous experience⁽⁹⁾ and to other authors⁽²⁰⁾];
7. A culture without any added stimulus, apart from the initial incubation with hrIL4, was included as a negative control.

All cell cultures were then incubated for 9 days and supernatants were collected and stored at -70°C until testing for IgE concentrations.

IgE measurement

Total IgE was measured in cell culture supernatants and in sera of each subject using a fluoroimmunoassay procedure kit (Pharmacia CAP IgE FEIA, cod. 10921301, Pharmacia & Upjohn diagnostic AB; Pharmacia, Uppsala, Sweden) capable of detecting total IgE in various fluids, including cell cultures supernatants and serum, within a range of 0.35 to 100 kU/L. We considered the test positive when IgE in the supernatant samples was ≥ 2.8 kU/L. This cut-off was chosen on the basis of a previously used cut-off for cord serum IgE of 0.7 kU/L, which was quadrupled when we used IL4, the cytokine recognized to be the principal inducer of IgE synthesis as a stimulus.

Statistical analyses

For a correct analysis of the results, the values under the sensitivity level of the method were considered as half of the sensitivity level as suggested by Khalak et al.,⁽²¹⁾ which means in our case 0.17 kU/L. Nonparametric tests were used as the data did not follow the normal distribution. Wilcoxon rank test was adopted to

compare the different stimuli among each other and with the serum, considering the whole group of subjects, without distinguishing the neonates with and without a family history of atopy. Mann–Whitney sum ranks test was adopted to compare the neonates with a family history of atopy versus those without a family history of atopy with regard to each stimulus and to the serum. Finally, chi-square test or Fisher’s Exact test when indicated, were used to compare the percentages of neonates with a family history of atopy and those without a family history of atopy that had $\text{IgE} \geq 2.8 \text{ kU/L}$ in the supernatants or $\geq 0.7 \text{ kU/L}$ in the serum. The data were analyzed by the means of a program “Primer of biostatistics” of Stanton A. Glanz, version 4.02i.

RESULTS

Table 1 shows the results of IgE concentrations for each stimulus and for serum. The results are expressed as medians plus ranges, percentiles, and standard errors. In this table the neonates were not divided

TABLE 1. COMPARISON AMONG MEDIAN OF IgE LEVELS OF ALL 20 NEONATES, CONSIDERED AS AN ONLY GROUP

		<i>All neonates (20)</i>	<i>Significance</i>
hrIL4	median	2.9	a,b,c,d
	range	0.6–8.2	
	centiles	2.1 (25°)–3.8 (75°)	
	standard error	0.38	
Unstimulated	median	0.5	a
	range	0.17–6.3	
	centiles	0.17 (0°)–3.8 (75°)	
	standard error	0.48	
hrIL4 + hrIFNg	median	2.6	
	range	0.17–10.6	
	centiles	2.1 (25°)–3.3 (75°)	
	standard error	0.46	
Anti-CD40 ab + hr IL4	median	2.2	b
	range	0.7–3.6	
	centiles	2.1 (25°)–2.3 (75°)	
	standard error	0.18	
Anti-CD40 ab + hrIL4 + BLG	median	2.2	c
	range	0.4–3.7	
	centiles	2.1 (25°)–2.3 (75°)	
	standard error	0.17	
hrIL4 + Anti-IFNgR ab	median	2.5	
	range	1.2–4.2	
	centiles	2.2 (25°)–3.2 (75°)	
	standard error	0.19	
hrIL4 + Anti-IL4R ab	median	2.4	d
	range	0.17–3.9	
	centiles	2.3 (25°)–2.5 (75°)	
	standard error	0.18	
Serum	median	0.17	
	range	0.17–3.5	
	centiles	0.17 (0°)–0.7 (75°)	
	standard error	0.18	

a,b,c,d $p < 0.05$.

The level obtained from each stimulation was compared with the levels obtained from the other stimulations and from the serum, one by one.

Medians of the IgE levels of all cultures were significantly different from that of the serum (there are not symbols in the table for this comparison).

according to the family history and each stimulus was compared with other stimuli and with serum using Wilcoxon signed rank test. All stimuli induced an IgE level significantly higher than that measured in serum. The highest level of all was obtained after stimulus of the CBL with hrIL4 alone; significant differences were observed also among this culture and the cultures stimulated with hrIL4 + anti-CD40 ab + BLG, with hrIL4 + anti-IL4 ab and the unstimulated cultures.

In Table 2 we compare medians of IgE concentrations in the two groups of neonates with a family history of atopy or without a family history of atopy using the Mann-Whitney test. No significant difference was observed. Significant differences were observed comparing different stimuli among each other and with serum within each group of neonates, that is an analysis like that shown in Table 2. Significant differences are identified by a couple of symbols. So to simplify the table we did not add symbols for the differences among the different cultures and serum. Such differences were always significantly apart from the unstimulated culture in the group of neonates with a family history of atopy.

TABLE 2. COMPARISON OF MEDIANS OF IgE LEVELS OBTAINED FROM EACH STIMULATION AND FROM THE SERUM BETWEEN THE TWO GROUPS OF NEONATES AND INSIDE EACH GROUP OF NEONATES

		<i>Neonates with family history of atopy (11)</i>	<i>Neonates without family history of atopy (9)</i>
hrIL4	median	2.7 ^a	3.5 ^{b,c}
	range	1.1–8.2	0.6–5.6
	25°–75° per centiles	1.9–3.8	2.1–3.7
	standard error	0.59	0.49
Unstimulated	median	0.17 ^a	0.8 ^b
	range	0.17–5.2	0.17–6.3
	0°–75° per centiles	0.17–3.99	0.17–3.83
	standard error	0.62	0.8
hrIL4 + hrIFNg	median	2.3	2.7
	range	1.3–10.6	0.17–4.03
	25°–75° per centiles	1.8–3.2	2.5–3.3
	standard error	0.8	0.37
Anti-CD40 ab + hr IL4	median	2.2	2.2 ^c
	range	1.05–3.6	0.7–3.5
	25°–75° per centiles	2.21–2.27	2.08–2.25
	standard error	0.26	0.26
Anti-CD40 ab + hrIL4 + BLG	median	2.2	2.2
	range	1.3–3.7	0.4–3.4
	25°–75° per centiles	2.15–2.22	2.21–3.04
	standard error	0.18	0.31
hrIL4 + Anti-IFNgR ab	median	2.5	2.5
	range	1.6–4.2	1.2–4.1
	25°–75° per centiles	2.28–3.02	1.5–3.4
	standard error	0.22	0.35
hrIL4 + Anti-IL4R ab	median	2.4	2.4
	range	0.17–3.4	1.2–3.9
	25°–75° per centiles	1.8–3.2	2.39–2.54
	standard error	0.26	0.26
Serum	median	0.17	0.17
	range	0.17–1.32	0.17–3.5
	0°–75° per centiles	0.17–1.23	0.17–0.7
	standard error	0.15	0.31

^{a,b,c} $p < 0.05$.

Difference between stimulation and serum is not shown with symbols: in fact, this difference was always significant except for the unstimulated culture in neonates with family history of atopy.

In Table 3 we compare the percentages of subjects with $\text{IgE} \geq 2.8$ in supernatants and $\text{IgE} \geq 0.7$ kU/L in serum between the neonates with a family history of atopy and those without a family history of atopy. Analysis was performed using chi-square and Fisher's Exact test. No significant differences were observed.

DISCUSSION

The aim of this study was to evaluate if the *in vitro* IgE synthesis by CBL, after exposure to various ad-equate stimuli was significantly different in neonates with and without a family history of atopy. The mol-ecular events at the basis of IgE synthesis have been widely studied.⁽²²⁾ The B lymphocyte requires at least two stimuli for the isotypic switching, the IL4 receptor (IL4R) stimulation by IL4⁽²³⁾ and the link between the CD 40 ligand, expressed on the cell surface of the activated T cells, and the CD40 receptor, constitu-tively expressed on the surface of the B lymphocytes.⁽²⁴⁾ CD40 ligand provides the initial signal for the IgE switching by the means of the RNA C ϵ transcription.⁽²³⁾ The monoclonal anti-CD40 ab has been demon-strated to be able to substitute the signal provided by the CD40L.⁽²⁵⁾ IFNg has a suppressive action,⁽²⁶⁾ and also the anti-IL4 ab inhibits IgE synthesis.⁽²³⁾ The inhibition of the IL4 induced IgE synthesis by IFNg does not take place on the B lymphocytes that already undergone the switch to IgE synthesis,⁽²⁷⁾ and is no longer IL-4-dependent.⁽²⁸⁾ IgE synthesis is a complex process; many factors interact by unknown mechanisms. Atopic subjects show an abnormal pathogenetic pathway for IgE synthesis. In general, the IgE synthesis is made easier than in nonatopic subjects.^(15,29-31)

Little is known about the IgE synthesis in the neonate. Two studies have previously evaluated the *in vitro* IgE synthesis in neonates.^(32,33) Pastorelli et al.⁽³²⁾ reported that, after the preincubation of CBL from in-cubation for 48 hr with hrIL4, the CBL stimulated with pokeweed (PWM) or unstimulated did not produce IgE, while the CBL incubated with hrIL4 produced levels of IgE not significantly different from the adult controls.⁽³²⁾ The addition of anti-IL4 ab inhibited the IgE synthesis. Fuleihan et al.⁽³³⁾ have stimulated the CBMC of 4 neonates for 9 days with 5 ng/mL of hrIL4 with or without the addition of 5 $\mu\text{g/mL}$ of anti-CD40 ab. These authors have observed the absence of IgE in the supernatant of the unstimulated cultures and a low or absent IgE production in the supernatant of cultures stimulated with hrIL4 alone. The addi-tion of anti-CD 40 ab to hrIL4 cultures significantly increased the IgE production in all samples. The adult controls showed a high IgE production after stimulus with hrIL4, with or without the addition of the anti-CD40 ab. The significant difference between the cultures stimulated with hrIL4 alone and the cultures stim-ulated with hrIL4 + anti-CD40 ab is an indirect demonstration that the neonatal T lymphocyte does not ex-press CD40L normally.

We hypothesize that neonates with a familial atopic predisposition may show a dysregulation of IgE syn-thesis from birth. We reproduced *in vitro* the conditions for IgE synthesis; the addition *in vitro* of the main known stimuli,⁽³⁴⁾ such as hrIL4 and anti-CD40 ab, should take the place of the CD40L that is significantly reduced in neonates.⁽³⁵⁾ This was expected to demonstrate an increased synthesis of IgE in neonates that

TABLE 3. NUMBER OF SUBJECTS WITH IgE LEVELS ABOVE THE CUT-OFFS OF SUPERNATANT AND OF SERUM IN THE TWO GROUPS OF NEONATES (NO SIGNIFICANT DIFFERENCES WERE OBSERVED)

	Neonates with family history of atopy (11)	Neonates without family history of atopy (9)
hrIL4	5/11 (45%)	5/9 (55%)
Unstimulated	5/11 (45%)	4/9 (44%)
hrIL4 + hrIFNg	5/11 (45%)	4/9 (44%)
Anti-CD40 ab + hr IL4	3/11 (27%)	1/9 (11%)
Anti-CD40 ab + hrIL4 + BLG	1/11 (9%)	3/9 (33%)
hrIL4 + Anti-IFNgR ab	4/11 (36%)	4/9 (44%)
hrIL4 + Anti-IL4R ab	2/11 (18%)	2/9 (22%)
Serum	4/11 (36%)	2/9 (22%)

would develop atopic disease in childhood and were more likely to have a positive family history of atopy. We also added a common food allergen such as BLG to cultures of CBL with hrIL4 and anti-CD40 ab. Recent studies show a different cytokine pattern expression of CBL when stimulated with food allergens such as BLG⁽⁶⁾ or ovalbumin⁽¹²⁾ between neonates with a family history of atopy and without a family history of atopy. Our study confirms that the neonatal B lymphocyte are able to produce IgE if cultured in adequate conditions; the low capacity to produce IgE *in vivo* observed in the neonate is therefore due not to an intrinsic deficit of the B lymphocyte, but to an immature T-helper lymphocyte cooperation.⁽³³⁾ Also, in our samples hrIL4 induced the IgE synthesis, a significant difference was observed if compared with unstimulated culture or to the serum IgE levels. The significant reduction of IgE synthesis observed when anti-IL4 ab was added to the culture with hrIL4 demonstrate that IgE synthesis is IL4-dependent. We did not appreciate either a significant reduction of the IgE synthesis after the addition of hrIFNg or a relevant increase after the addition of anti-CD40 ab to hrIL4. The preincubation for 48 hr of CBMC with hrIL4 may have activated the switch of a great part of B lymphocytes towards the IgE synthesis. As we discussed previously, the B lymphocyte IgE⁺ is no longer responsive to the inhibiting action of IFNg⁽²⁷⁾ nor to the stimulus of IL4 towards the IgE synthesis.⁽²⁸⁾ Also the anti-CD40 ab may lose its action on the B lymphocyte IgE⁺.

CONCLUSIONS

Neonates with a family history of atopy and those without a family history of atopy were compared with regard to the IgE *in vitro* synthesis capability. If compared for each stimulus, the two groups did not show any significant difference with regard to the medians nor to the number of subjects with IgE $\geq < 2.8$ kU/L. It is also possible that different experimental conditions would show a significant difference between the two groups of neonates. For example, the elimination of the pre-incubation with hrIL4 and of the separation of nonadherent cells could be useful. Besides the use of nonspecific stimuli, such as hrIL4 and anti-CD40 ab, specific stimuli such as BLG or other foods or inhalant common allergens may be employed according to the studies on IFNg and IL4 *in vitro* production.^(6,8,10-12)

REFERENCES

1. Christie GL, McDougall CM, Helms PJ. Is the increase in asthma prevalence occurring in children without a family history of atopy? *Scot Med J* 1999; 43:180-182.
2. Nespoli L, Bottelli AM, Nosetti L. Prevenzione primaria delle malattie atopiche. *Riv Immunol Allergol Pediatr* 1995; 1:1-11.
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; 4:60-64.
4. Liao SY, Liao TN, Chiang BL, Huang MS, Chen CC, Chou CC, Hsieh KH. Decreased production of IFNg and increased production of IL-6 by cord blood mononuclear cells of newborns with a high risk of allergy. *Clin Exp Allergy* 1996; 26:397-405.
5. Tang MLK, Kemp AS, Thorburn J, Hill DJ. Reduced interferon-secretion in neonates and subsequent atopy. *Lancet* 1994; 344:983-985.
6. Warner JA, Miles EA, Jones AC, Quint 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; 24:423-430.
7. Martinez FD, Stern DA, Wright AL, Holberg CJ, Taussig LM, Halonen M. Association of interleukin-2 and interferon-production by blood mononuclear cells in infancy with parental allergy skin tests and with subsequent development of atopy. *J Allergy Clin Immunol* 1995; 96:652-660.
8. Szépfalusi Z, Nentwich I, Gerstmayr M, Jost E, Todoran L, Gratzl R, Herkner K, Urbanek R. Prenatal allergen contact with milk proteins. *Clin Exp Allergy* 1997; 27:28-35.

9. Stabile A, Miceli Sopo S, Guerrini B, Federico G, Maraglino V, Pesaresi MA. In vitro production of interleukin-4 and interferon-gamma after PHA & PMA stimulation, and mononuclear cell proliferative response to betalactoglobulin and ovalbumin in neonates with different family history of atopy. A follow-up of five years. *Pediatric Asthma Allergy Immunol* 1998; 12:259–270.
10. Piccinni MP, Beloni L, Giannarini L, Livi C, Scarselli G, Romagnani S, Maggi E. Abnormal production of T helper 2 cytokines interleukin-4 and interleukin-5 by T cells from newborns with atopic parents. *Eur J Immunol* 1996; 26:2293–2298.
11. Borres MP, Einarsson R, Björkstén B. Serum levels of interleukin 4, soluble CD23 and IFN- γ in relation to the development of allergic disease during the first 18 months of life. *Clin Exp Allergy* 1995; 25:543–548.
12. Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PJ. Development of allergen-specific cell memory in atopic and normal children. *Lancet* 1999; 353:196–200.
13. Edenharter G, Bergmann RL, Bergmann KE, Wahn V, Forster J, Zepp F, Wahn U. Cord blood IgE as risk factor and predictor for atopic disease. *Clin Exp Allergy* 1998; 28:671–678.
14. Bergmann KE, Bergmann RL, Schulz J, Grab T, Whan U. Prediction of atopic disease in the newborn: methodological aspects. *Clin Exp Allergy* 1990; 20(suppl 3):21–26.
15. Umetsu DT, Leung DY, Jabara HH, Siraganian R, Geha RS. Differential requirements of B cells from normal and allergic subjects for the induction of the IgE synthesis by an alloreactive T cell clone. *J Exp Med* 1985; 162:202–214.
16. Bock SA, Sampson HA, Atkins FM, Zeiger RS, Lehrer S, Sachs M, Bush RK, Metcalfe DD. Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. *J Allergy Clin Immunol* 1988; 82:986–997.
17. Halonen M, Stern D, Lyle S, Wright A, Taussig L, Martinez FD. Relationship of total serum IgE levels in cord and 9-month sera of infants. *Clin Exp Allergy* 1991; 21:235–241.
18. Rousset F, Robert J, Andary M. Shifts in interleukin 4 and interferon production by T cells of patients with elevated serum IgE levels and the modulatory effect of these lymphokines on spontaneous IgE synthesis. *J Allergy Clin Immunol* 1991; 87:58–69.
19. Snapper CM, Finkelman FD, Paul WE. Differential regulation of IgG1 and IgE synthesis by interleukin 4. *J Exp Med* 1988; 167:183–196.
20. Holt PG, O'Keefe P, Holt BJ, Upham JW, Baron-Hay MJ, Suphioglu C, Knox B, Stewart GA, Thomas WR, Sly PD. T-cell "priming" against environmental allergens in human neonates: sequential deletion of food antigen reactivity during infancy with concomitant expansion of responses to ubiquitous inhalant allergens. *Pediatr Allergy Immunol* 1995; 6:85–90.
21. Khalak R, Pichichero ME, D'Angio CT. Three-year follow-up of vaccine response in extremely preterm infants. *Pediatrics* 1998; 101:597–603.
22. Bacharier LB, Geha RS. Regulation of IgE synthesis: the molecular basis and implications for clinical modulation. *Allergy Asthma Proc* 1999; 20:1–8.
23. Del Prete G, Maggi E, Parronchi P, Chretien I, Tiri A, Macchia D, Ricci M, Banchereau J, De Vries J, Romagnani S. IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cells clones and their supernatants. *J Immunol* 1988; 140:4193–4198.
24. Banchereau J, Bazan F, Blanchard D, Briere F, Galizzi JP, van Kooten C, Liu YJ, Rousset F, Saeland S. The CD40 antigen and its ligand. *Annu Rev Immunol* 1994; 12:881–922.
25. Jabara HH, Fu SM, Geha RS, Vercelli D. CD40 and IgE: synergism between anti-CD40 monoclonal antibody and interleukin 4 in the induction of the IgE synthesis by highly purified human B cells. *J Exp Med* 1990; 172:1861–1864.
26. Xu L, Rothmann P. IFN- γ represses e-germline transcription and subsequently down-regulates switch recombination to e. *Int Immunol* 1994; 6:515–521.
27. Vercelli D, Jabara H, Cunningham-Rundles C, Abrams JS, Lewis DB, Meyer J, Schneider LC, Leung DY, Geha RS. Regulation of immunoglobulin E synthesis in the hyper-IgE syndrome. *J Clin Invest* 1990; 85:1666–1671.
28. Jabara HH, Vercelli D, Schneider L, Williams D, GenbauFFE F, Poisson L, Waters C, Geha R. Interleukin 4 receptor expression by human B cells: functional analysis with a human interleukin4 toxin, DAB 389 IL4. *J Allergy Clin Immunol* 1995; 95:893–900.

29. Brodie C, Renz H, Bradley KL, Leung DYM, Gelfand EW. Anti-CD40 stimulates IgE production in peripheral blood mononuclear cell from patients with atopic dermatitis. *J Allergy Clin Immunol* 1992; 89:(abstract)324.
30. Ke Z, Clark EA, Saxon A. CD40 stimulation provides an IFN independent and IL4 dependent differentiation signal directly to human B cells for IgE production. *J Immunol* 1991; 146:1836–1842.
31. Vercelli D, Jabara HH, Lee BW, Woodland N, Geha RS, Leung DY. Human recombinant interleukin 4 induces Fc R2/CD23 on normal human monocytes. *J Exp Med* 1988; 167:1406–1416.
32. Pastorelli G, Rousset F, Pène J, Peronne C, Roncarolo MG, Tovo PA, De Fries JE. Cord blood B cells are mature in their capacity to switch to IgE-producing cells in response to interleukin-4 in vitro. *Clin Exp Immunol* 1990; 80:114–119.
33. Fuleihan R, Ahern B, Geha RS. Decreased expression of the ligand for CD40 in newborn lymphocytes. *Eur J Immunol* 1994; 24:1925–1928.
34. Koning H, Baert MRM, Oranje AP, Savelkoul HFJ, Neijens HJ. Development of immune functions related to allergic mechanisms in young children. *Pediatr Res* 1996; 40:363–375.
35. Brugnani D, Airò P, Graf D, et al. Ineffective expression of CD40 ligand on cord blood T cells may contribute to poor immunoglobulin production in the newborn. *Eur J Immunol* 1994; 24:1919–1924.

Address reprint requests to:
Stefano Miceli Sopo, M.D.
via Trionfale 8250
00135-Roma
Italy

E-mail: stefano.micelisopo@libero.it