Taylor & Francis healthsciences

Mediators of Inflammation, **13**(2), 89–92 (April 2004)

Age-related differentiations of Th1/Th2 cytokines in newborn infants

Efthimia Protonotariou[1], Ariadne Malamitsi-Puchner[2], Demetrios Rizos[1], Basiliki Papagianni [3], Evangelia Moira[1], Angeliki Sarandakou[1,CA] and Demetrios Botsis[3]

[1]Hormonological Laboratory; [2]Neonatal Unit and [3]2nd Department of Obstetrics and Gynecology of University of Athens, 'Aretaieion' University Hospital, Ivis 101, Paleon Phaliro 17561, Greece

[CA]Corresponding author
Tel: +32 1 098 43942
Fax: +32 1 072 86229
E-mail: dsaran@tee.gr

***Objective*: To evaluate age-related differentiation of immune response in newborns by measuring serum concentrations of interleukin-2 (IL-2), interleukin-4 (IL-4) and interferon-γ (IFN-γ) during the perinatal period.**
***Subjects and methods*: Fifty-seven healthy term neonates, their mothers and 25 healthy adults (controls) age-matched to the mothers were included in the study. Cytokine concentrations were measured in the umbilical cord (UC), and in first-day (1N) and fifth-day (5N) neonatal samples, compared with those in maternal serum (MS) and control serum samples.**
***Results*: Serum IL-2 concentrations in the UC were markedly elevated compared with those in MS and controls ($p < 0.0001$), decreasing significantly thereafter up to 5N ($p < 0.001$). IL-4 serum concentrations did not differ significantly between the UC, 1N and 5N samples; they were, however, markedly elevated compared with those in MS ($p < 0.001$, $p < 0.0007$ and $p < 0.0001$, respectively) and controls ($p < 0.05$, $p < 0.01$ and $p < 0.006$, respectively). IFN-γ serum concentrations were significantly lower in the UC compared with those in controls ($p < 0.04$), increasing significantly up to 5N ($p < 0.03$). Both IFN-γ/IL-2 and IFN-γ/IL-4 ratios increased significantly in 5N, compared with those in the UC ($p < 0.001$ and $p < 0.03$).**
***Conclusion*: Our findings indicate a differential cytokine balance at birth with enhanced expression of IL-2 and IL-4 against IFN-γ. However, a regularization of immune response seems to proceed quickly during the early neonatal life.**

Key words: Interleukin-2, Interleukin-4, Interferon-γ, Developmental characteristics

Introduction

Cytokines are the hormonal messengers responsible for most of the biological effects in the immune system, such as cell-mediated immunity and allergic type responses. Based on their preferential functional capacity, cytokines can be divided into two families: type 1 (Th1) cytokines, such as interleukin-2 (IL-2) and interferon-γ (IFN-γ), which mainly stimulate cell-mediated immunity; and type-2 (Th2) cytokines, the main representative of which is interleukin-4 (IL-4), which primarily induce B-cell differentiation and are associated with allergic responses.[1]

In the mouse, a $CD4^+$ cell that produces IL-4 but not IFN-γ, or IL-2, is termed Th2, and a cell that produces IFN-γ but not IL-4 is termed Th1.[2] In humans, such a distinction between cell types is less clear because, depending on the stimulus, cells can produce both IFN-γ and IL-4. Therefore, it is better to use a ratio of IFN-γ/IL-4 to define the phenotype of the cell.[3]

However, although IFN-γ and IL-4 are useful markers for Th1 versus Th2 responses, they do not give definitive descriptions of the responses. On the other hand, in newborns, IFN-γ production by cord and peripheral neonatal cells does not correlate with IL-2 production.[1] Thus, the safest way to describe the cytokine response patterns is to determine both the ratios and the absolute amounts of cytokines.[4]

For this reason, we focused to study serum concentrations of IL-2, IL-4 and IFN-γ, as well as their ratios IFN-γ/IL-2 and IFN-γ/IL-4, during the perinatal period, in order to evaluate the age-related differentiation of immune response in newborns.

Material and methods

The study was approved by the Ethics Committee of Aretaieion Hospital and informed written consent was acquired from the participating mothers and controls on admission in the Clinic. The study

ISSN 0962-9351 print/ISSN 1466-1861 online/04/20089-04
DOI: 10.1080/09629350410001688468

included 57 healthy, appropriate for gestational age term neonates, delivered after a singleton uncomplicated pregnancy from healthy, non-smoking mothers (mean age, 23.8 ± 4.4 years; range, 20–39 years). In all cases, Apgar scores were ≥ 8 in the first and fifth minutes, and placentas were normal in appearance and weight (mean weight ± SD, 449 ± 16 g). The demographic data of participating neonates are presented in Table 1. The mothers of the aforementioned neonates also participated in the study, as well as 25 healthy, non-pregnant, non-smoking women acting as controls (mean age ± SD, 23.0 ± 4.8 years; range, 21–40 years), with regular menstrual cycles, all in the periovulation period of their cycle.

Blood samples were collected from controls, mothers before delivery (first stage of labor), the doubly clamped umbilical cord (UC) at delivery (mixed arteriovenous blood), and the neonates in the first and fifth day postpartum. Blood was collected in pyrogen-free tubes, was immediately centrifuged after clotting; and the supernatant serum was kept frozen at −30°C until assay.

Cytokines were measured using highly sensitive immunoenzyme techniques, with commercially available enzyme-linked immunosorbent assays (EASIA; Medgenix, Fleurus, Belgium for IL-2; Quantikine® hIL-4; HS R&D Systems, MN, USA for IL-4; and Quantikine® IFN-γ; R&D Systems for IFN-γ). With an extension of the standard curve at very low concentrations and the application of a logistic program for the best fitting, very low sensitivities were achieved. Thus, the new performance characteristics were as follows: sensitivity, intra-assay and inter-assay coefficients of variation were, respectively: 0.1 IU/ml, 5.7% and 7.5% for IL-2; 0.11 pg/ml, 4.6% and 5.8% for IL-4; and 3 pg/ml, 2.6% and 6.4% for IFN-γ.

Statistics

Statistical analysis involved parametric tests for IL-2 (*t*-test, one-way analysis of variance) and non-parametric tests for IL-4 and IFN-γ (Wilcoxon test, and Kruskal–Wallis analysis of variance), since data presented normal and abnormal distributions, respectively. Comparisons between IL-2 and IFN-γ or IL-4 were performed with non-parametric tests. The level $p < 0.05$ was considered statistically significant.

Table 1. Clinical characteristics of neonates

Sex	
Girls (*n*)	29
Boys (*n*)	28
Birth weight (g) (mean ± SD, range)	3382 ± 360 (2530–4180)
Weeks of pregnancy (mean ± SD, range)	39.8 ± 0.95 (38–41)
Mode of delivery	
Elective cesarean section (*n*)	17
Vaginal delivery (*n*)	40

SD, standard deviation.

Results

Summarized data are presented in Tables 2 and 3:

- Serum IL-2 concentrations in the UC were markedly elevated, compared with those in maternal serum (MS) samples and controls ($p < 0.0001$), decreasing significantly up to the fifth neonatal day (5N) ($p < 0.001$).
- IL-4 and IFN-γ serum concentrations presented a great variation, especially in cord and neonatal samples, with a high percentage of zero values.
- IL-4 serum concentrations did not differ significantly between the UC, first neonatal day (1N) and 5N samples; however, they were markedly elevated compared with those in MS ($p < 0.001$, $p < 0.0007$ and $p < 0.0001$, respectively) and controls ($p < 0.05$, $p < 0.01$ and $p < 0.006$, respectively).
- Serum IFN-γ concentrations were significantly lower in the UC compared with those in adult controls ($p < 0.04$) showing, however, a significant increase in the fifth day of life ($p < 0.03$).
- IFN-γ concentrations in the MS and UC samples were dependent significantly on the mode of delivery, being significantly higher in vaginal delivery [4.70 pg/ml (1.05–11.17) and 3.95 pg/ml (0.18–10.46), respectively] compared with those in cesarean section [3.85 pg/ml (0.0–8.5), $p < 0.005$ and 3.43 pg/ml (0.0–6.8), $p < 0.006$, respectively].
- Both IFN-γ/IL-2 and IFN-γ/IL-4 ratios in the UC samples were significantly lower compared with those in MS samples and controls ($p < 0.001$ and $p < 0.03$, respectively). On 5N, however, cytokine ratios showed a significant increase, compared with UC values ($p < 0.001$ and $p < 0.03$, respectively).

Discussion

The fetal immune system tends to develop towards a Th2-type immune response, due to the production of the Th2 promoting factors, such as IL-4, IL-10 and prostaglandin E2 from the placenta.[1] Postnatally, newborn infants are selectively impaired regarding development of Th1 memory effector function and are biased to Th2 function in all phases of an immune response.[5]

Table 2. IL-2, IL-4 and IFN-γ concentrations in MS, UC, 1N and 5N samples and controls

Serum sample	IL-2 (IU/ml) (mean ± standard error)	IL-4 (pg/ml) (median, range)	IFN-γ (pg/ml) (median, range)
MS	0.125 ± 0.02*	0.110** (0.019–2.6)	4.04*** (0.0–11.17)
UC serum	0.48 ± 0.22*	0.195** (0.0–1.926)	3.72*** (0.18–10.46)
1N serum	0.32 ± 0.16	0.190 (0.0–1.965)	5.50 (0.0–17.66)
5N serum	0.23 ± 0.07*	0.204 (0.066–0.699)	7.19*** (0.0–23.2)
Adult controls	0.137 ± 0.06*	0.133** (0.0–0.410)	4.65*** (0.0–21.36)

* UC versus MS and controls, $p < 0.0001$; ** UC versus MS ($p < 0.001$) and controls, $p < 0.05$.
* UC versus 5N, $p < 0.001$; ** UC versus 1N and 5N, not significant.
*** UC versus MS and controls, $p < 0.04$; *** UC versus 5N, $p < 0.03$.

Consequently, umbilical cord white cells would be expected to produce a high level of IL-4, accompanied by a reduced production of IFN-γ, resulting in an initial dysregulation of IFN-γ and IL-4 in neonates.[6]

However, the proliferative response of T lymphocytes on their exposure to antigens is the result of two major events: the generation of the T-cell growth factor IL-2 and the induction of T-cell responsiveness to IL-2, controlled by the expression of an inducible cell surface IL-2 receptor (IL-2R).[7] Cord blood T cells synthesize sufficient amounts of IL-2, express IL-2R and are able to form a functional, intact, high-affinity receptor complex to support T-cell growth and proliferation. However, cord blood T cells lack the capability to induce the production of IFN-γ, regardless of IL-2 and IL-2R upregulation of its production.[8]

Indeed, IL-2 is a potent IFN-γ-inducing cytokine and is produced in nearly equal amounts by both memory and naive T cells from neonatal and adult blood. Therefore, the striking discrepancy between neonatal and adult IFN-γ production is not related to deficient IL-2 induction of IFN-γ.[9]

Consistent with these immunological demands, our findings demonstrate clearly elevated concentrations of IL-2 in umbilical cord and in the first-day samples, as well as significantly increased IL-4 levels in all three neonatal samples, compared with those in maternal and adult serum.

A central biological function of IL-4 is the induction of differentiation of $CD4^+$ Th precursor T lymphocytes into Th2 cells in both humans and mice.[10] An important finding of this study is that not only memory cells, but also naive human $CD4^+$ T cells can be an initial source of IL-4 during primary immune responses.

Table 3. Cytokine ratios during the perinatal and early neonatal period

Sample	IFN-γ/IL-4	IFN-γ/IL-2
MS	37.80*	32.32**
UC serum	18.69*	17.16**
1N serum	28.94	17.18
5N serum	34.24*	31.26**
Adult controls	36.96*	33.26**

* UC versus MS and controls, $p < 0.03$; ** UC versus MS and controls, $p < 0.001$.
* UC versus 5N, $p < 0.03$; ** UC versus 5N, $p < 0.001$.

Moreover, an essential biological activity of IL-4 in the development of allergic inflammation is the inhibition of T-cell apoptosis and the ability to drive the differentiation of Th0 into Th2 lymphocytes, which are able to secrete IL-4, IL-5 and IL-13, but not IFN-γ.[11]

The lack of sufficient IFN-γ production after birth may contribute to impaired neonatal antiviral responses and facilitate allergic sensitization.[12] Thus, the IL-4 levels in serum, but not these of IFN-γ, were associated with allergic disease in infancy. Elevated concentrations of IL-4 were recorded in atopic neonates, before the onset of clinical symptoms,[13] and the severity of the atopic state correlates with the degree of imbalance in IL-4 and IFN-γ production.[14]

In agreement with previous studies,[6,15] our findings indicate very low IFN-γ concentrations in the UC. IFN-γ deficiency in human neonates may represent a developmental phenomenon, possibly associated with cellular responsiveness of neonatal T cells to cytokines, which is crucial to Th1 differentiation as well as in translational and post-translational defects.[15,16]

Additional findings of interest in the current study include the dependence of maternal and cord blood serum IFN-γ concentrations on the mode of delivery. Cytokine values were higher in mothers giving birth after vaginal delivery than in those delivering by elective cesarean section. Similarly, infants born after vaginal delivery had higher concentrations of IFN-γ compared with those born by elective cesarean section. Thus, labor possibly induces the production of such important modulator of immune response, both in mothers and their newborn infants, strengthening the maternal and neonatal defense against perinatal infections.[17]

The most important finding of this study was a clear increase of both IFN-γ/IL-2 and IFN-γ/IL-4 ratios from cord serum up to the fifth day of life. It seems that newborn infant cells can rapidly differentiate into cells with adult-like functional capacities. Thus, the relative immunodeficiency, immaturity, or lack of experience of the neonate may be an adaptive mechanism to optimize survival by balancing the

conflicting immunologic requirements of life *in utero* with those of the external environment.[18]

In conclusion, our findings indicate a differential cytokine balance at birth with enhanced expression of IL-2 and IL-4 against IFN-γ. However, a regularization of immune response seems to proceed quickly during the early neonatal life.

References

1. Vigano A, Esposito S, Arienti D, Zagliani A, Massironi E, Principi N, Clerici M. Differential development of type 1 and type 2 cytokines and β-chemokines in the ontogeny of healthy newborns. *Biol Neonate* 1999; **75**: 1–8.
2. Mosmann TL, Cherwinski H, Bond MW, Gieldlin MA, Coffman RL. Two types of murine helper T cell clones. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986; **136**: 2348–2357.
3. Cohen Sh. Cytokine profile data [letter]. *Immunol Today* 2000; **21**: 199.
4. Mosmann TL. Replay to Cohen [letter]. *Immunol Today* 2000; **21**: 199–200.
5. Adkins B, Bu Y, Guevara P. The generation of Th memory in neonates versus adults: prolonged primary Th2 effector function and impaired development of Th1 memory effector function in murine neonates. *J Immunol* 2001; **166**: 918–925.
6. Wakasugi N, Virelizier JL. Defective IFN-γ production in the human neonate. I. Dysregulation rather than intrinsic abnormality. *J Immnol* 1985; **134**: 167–171.
7. Robb RJ, Munck A, Smith KA. T cell growth factor receptors: quantitation, specificity and biological relevance. *J Exp Med* 1981; **154**: 1455–1474.
8. Von Freeden U, Zessack N, Van Valen F, Burdach S. Defective interferon gamma production in neonatal T cells is independent of interleukin-2 receptor binding. *Pediatr Res* 1991; **30**: 270–275.
9. Marsdi L. IL-12 and IFN-γ deficiencies in human neonates. *Pediatric Res* 2001; **49**: 316–317.
10. Noben-Trauth N, Hu-Li J, Paul WE. IL-4 secreted from individual naive CD4+ T cells acts in autocrine manner to induce Th2 differentiation. *Eur J Immunol* 2002; **32**: 1428–1433.
11. Jutel M, Pichler WJ, Skribic D. Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN-γ secretion in specific allergen stimulated T cell cultures. *J Immunol* 1995; **154**: 4187–4194.
12. Savelkoul HFJ. Immune parameters in high-risk atopic individuals during early childhood. *Am J Respir Crit Care Med* 2000; **162**: 5100–5104.
13. Bjorksten B, Borres MP, Einarsson R. Interleukin-4, soluble CD23 and interferon-gamma levels in serum during the first 18 months of life. *Int Arch Allergy Immunol* 1995; **107**: 34–36.
14. Tang M, Kemp A, Varigos G. IL-4 and interferon-gamma production in children with atopic disease. *Clin Exp Immunol* 1993; **92**: 120–124.
15. White GP, Watt PM, Holt BJ, Holt PG. Differential patterns of methylation of the IFN-gamma promoter at CpG and non-CpG sites underlie differences in IFN-gamma gene expression between human neonatal and adult CD45RO-T cells. *J Immunol* 2002; **168**: 2820–2827.
16. Frenkel L, Bryson Y. Ontogeny of phytohemagglutinin-induced gamma interferon by leukocytes of healthy infants and children: evidence of decreased production in infant younger than 2 months of age. *Clin Lab Observat* 1987; **111**: 97–100.
17. Buonocore G, De Filippo M, Gioia D, Picciolini E, Luzzi E, Bocci V, Bracci R. Maternal and neonatal plasma cytokine levels in relation to mode of delivery. *Biol Neonate* 1995; **68**: 104–110.
18. Selonka RL, Infante AJ. Neonatal immunology. *Semin Perinatol* 1998; **22**: 2–14.

Received 29 December 2003
Accepted 19 January 2004