Cytokine concentrations during the first days of life

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Received 16 December 2004; received in revised form 11 January 2006; accepted 10 April 2006

Abstract

Objective: To evaluate the cytokine concentration patterns during the first 5 days of life by measuring serum concentrations of type-1 cytokines, like interleukin-2 (IL-2) and interferon-γ (IFN-γ) and type-2 cytokines, like IL-4, as well as the receptors of IL-2 (sIL-2R) and IL-4 (sIL-4R) during the early neonatal period.

Subjects and methods: Forty-two healthy term neonates were included in the study. Cytokine concentrations were measured in umbilical cord, in the 1st and 5th day after birth and compared with those in serum of 30 healthy adults.

Results: IL-2 concentrations presented a decrease trend from umbilical cord to 5th day, while sIL-2R showed a significant elevation from umbilical cord to 5th day after birth. IL-4 concentrations did not differ significantly among umbilical cord, the 1st and the 5th day, while the sIL-4R showed the highest values in the 1st day after birth. Both IL-4 and sIL-4R concentrations in neonatal samples were elevated compared to adults. IFN-γ concentrations increased significantly from umbilical cord to 5th day of life.

Conclusion: Our findings indicate a dysregulation among IL-2, IL-4 and IFN-γ concentrations during the 1st day after birth, favoring a more precocious expression of IL-2 and IL-4 against IFN-γ that seems to be ameliorated in the end of the 1st week of life.

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Keywords: Interleukin-2 (IL-2); Interleukin-4 (IL-4); Interferon-γ (IFN-γ); sIL-2R; sIL-4R; Neonates

1. Introduction

Based on their preferential functional capacity, cytokines can be divided into two families: type-1 (Th1) cytokines interleukin-2 (IL-2) and interferon-γ (IFN-γ) that mainly stimulate cell-mediated immunity, and type-2 (Th2) cytokines, with main representative the interleukin-4 (IL-4), which primarily induce B cell differentiation and is associated with allergic responses [1].

Although IFN-γ and IL-4 are useful markers for Th1 versus Th2 responses, they do not give however, 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. Thus, the safest way to describe the cytokine response patterns is to determine both ratios and absolute amounts of cytokines [2].

For this reason, we focused on characteristic cytokines, such as IL-2, IL-4 and IFN-γ and their ratios, as well as the soluble receptors sIL-2R and sIL-4R during the first 5 days after birth, in order to estimate the maturation of immune response in very early life.

2. Material and methods

The Ethics Committee of our University Hospital approved this study and written informed consent was acquired from the mothers of the participating neonates and the healthy adults. The study comprised 42 healthy full-term, appropriate for gestational age neonates, born after single uncomplicated pregnancy and delivery from...
healthy, non-smoking mothers (mean age 23.5 ± 4.2, ranges 20–40) years. Apgar scores in the 1st and 5th minute were in all cases ≥8 and placenta were normal in appearance and weight. Demographic and clinical characteristics of participating neonates are shown in Table 1.

Blood was drawn from the doubly clamped umbilical cord (UC) at delivery (mixed arteriovenous blood), and from the neonates in the 1st (1N) and 5th (5N) day postpartum. Moreover, blood samples from 30 healthy adults (16 women and 14 men, mean age 24.0 ± 4.0, range 21–39 years), were also tested and compared with the results from infants. Samples were collected in pyrogen-free tubes and were immediately centrifuged after clotting. The supernatant serum was kept frozen at −30 °C until assay.

Cytokines were measured using highly sensitive immunoenzymatic techniques, with commercially available ELISA kits: (EASIA, Medgenix, Fleurus, Belgium for IL-2; Cellfree Il2R, T Cell Sciences for sIL-2R; Quantikine IL-4 HS for IL-4; Quantikine IFN-γ for IFN-γ and Quantikine hIL-4sR for sIL-4R, from R&D Systems, MN, 55413, USA). Performance characteristics (sensitivity, intra-assay and inter-assay CVs) for the assays were as follows: 0.1 IU/ml, 5.7% and 7.5% for IL-2; 0.11 pg/ml, 4.6% and 5.8% for IL-4; 3 pg/ml, 2.6% and 6.4% for IFN-γ; 50 U/ml, 5.2% and 6.5% for sIL-2R; 5 pg/ml, 2.6% and 5.2% for sIL-4R.

<table>
<thead>
<tr>
<th>Sex</th>
<th>N = 22</th>
<th>Boys</th>
<th>N = 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight (mean ± S.D., range)</td>
<td>3582 ± 360 g (2530–4180)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weeks of pregnancy (mean ± S.D., range)</td>
<td>39.8 ± 0.95 (38–41)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apgar score</td>
<td>8–10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placental weight (mean ± S.D.)</td>
<td>446 ± 13 g</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.1. Statistics

Statistical analysis included parametric tests for IL-2 and sIL-2R (t-test, one-way ANOVA) and non-parametric tests for IL-4, sIL-4R and IFN-γ (Wilcoxon test, Kruskal–Wallis ANOVA, Spearman rank correlation coefficient), since data presented normal and abnormal distribution, respectively. The level of \( p < 0.05 \) was considered statistically significant.

3. Results

Summarized data on cytokine concentrations in umbilical cord, neonatal serum day 1 and day 5 and in healthy adults are given in Table 2.

IL-2 concentrations showed a significant decrease from 0.48 ± 0.22 IU/ml in UC to 0.23 ± 0.12 IU/ml in 5N \( (p < 0.001, \text{one-way ANOVA}) \), still remaining markedly elevated compared to those in healthy adults \( (p < 0.0001) \).

In contrast, sIL-2R concentrations were found to increase significantly from 695 ± 205 U/ml in UC to 1560 ± 638 U/ml in 5N \( (p < 0.0001, \text{one-way ANOVA}) \). sIL-2R concentrations in UC were significantly higher than those in adults \( (p < 0.0001) \).

IL-4 concentrations did not differ significantly among UC, 1N and 5N while sIL-4R showed the highest values in 1N. However, both IL-4 and sIL-4R concentrations in UC were significantly elevated, compared to those in adults \( (p < 0.05; p < 0.0001, \text{respectively}) \). A negative correlation was noticed between IL-4 and sIL-4R concentrations in 1N \( (r = −0.48; p < 0.002) \) and 5N \( (r = −0.45; p < 0.002) \) neonatal samples.

IFN-γ concentrations in UC were significantly lower compared to those in healthy adults \( (p < 0.04) \) showing however, a significant increase in the 5th day of life \( (p < 0.03) \).

We calculated median IFN-γ to mean IL-2 (IFN-γ/IL-2) and median IFN-γ to median IL-4 ratios (IFN-γ/IL-4) in neonatal samples and in healthy adults (Table 3). There is an

<table>
<thead>
<tr>
<th>Sample</th>
<th>IL-2 (IU/ml)</th>
<th>sIL-2R (U/ml)</th>
<th>IL-4 (pg/ml)</th>
<th>sIL-4R (pg/ml)</th>
<th>IFN-γ (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Umbilical cord</td>
<td>0.48 ± 0.22a</td>
<td>695 ± 205bc</td>
<td>0.20 (0–1.93)d</td>
<td>76 (39–212)e</td>
<td>3.7 (0–10.5)f</td>
</tr>
<tr>
<td>Neonate day 1</td>
<td>0.32 ± 0.16e</td>
<td>785 ± 318h</td>
<td>0.19 (0–1.97)</td>
<td>100 (48–312)</td>
<td>5.5 (0–17.7)</td>
</tr>
<tr>
<td>Neonate day 5</td>
<td>0.23 ± 0.12h</td>
<td>1560 ± 638b</td>
<td>0.20 (0–0.70)</td>
<td>74 (30–218)</td>
<td>7.2 (0–23.2)</td>
</tr>
<tr>
<td>Healthy adults</td>
<td>0.14 ± 0.08</td>
<td>487 ± 163</td>
<td>0.13 (0–0.41)</td>
<td>38 (20–88)</td>
<td>4.7 (0–21.4)</td>
</tr>
</tbody>
</table>

a \( p < 0.0001 \) one-way ANOVA.

b \( p < 0.001 \) one-way ANOVA.

c \( p < 0.001 \) compared to healthy adults.

d \( p < 0.05 \) compared to healthy adults.

e \( p < 0.001 \) compared to healthy adults.

f \( p < 0.04 \) compared to healthy adults.

g \( p < 0.03 \) compared to 5N.

h \( p < 0.001 \) compared to healthy adults.
Table 3
Cytokine ratios during early neonatal period

<table>
<thead>
<tr>
<th>Sample</th>
<th>IFN-γ/IL-4</th>
<th>IFN-γ/IL-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Umbilical cord</td>
<td>18.5</td>
<td>7.7</td>
</tr>
<tr>
<td>Neonate day 1</td>
<td>28.9</td>
<td>17.2</td>
</tr>
<tr>
<td>Neonate day 5</td>
<td>36.0</td>
<td>31.3</td>
</tr>
<tr>
<td>Healthy adults</td>
<td>36.2</td>
<td>33.6</td>
</tr>
</tbody>
</table>

increase trend in both ratios from UC to 5N, where they reach levels comparable to adults.

4. Discussion

The fetal immune system strongly favors the development of Th2-mediated immunity, due to the production of the Th2-promoting factors, as IL-4, IL-10 and prostaglandin E2 from the placenta [1]. Postnatally, newborn infants, like murine neonates, are selectively impaired in the development of Th1 memory effector function and are biased to Th2 function at all phases of an immune response [3]. Consequently, umbilical cord cells would be expected to produce 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 [4]. However, it is also likely that at birth the main source of these cytokines in the neonatal plasma is from the placenta [5].

The proliferative response of T lymphocytes on their exposure to antigens is the results of two major events: the generation of the T cell growth factor interleukin-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) [6]. Cord blood and neonatal serum T cells synthesize sufficient amounts of IL-2, express IL-2R and are able to form a functionally intact high affinity receptor complex to support T cell growth and proliferation [7]. Moreover, IL-2, a potent IFN-γ inducing cytokine is produced in nearly equal amounts by both memory and naïve T cells from neonatal and adult blood. Therefore, the striking discrepancy between neonatal and adult IFN-γ production does not seem to be related to deficient IL-2 induction of IFN-γ [8]. Consistent to these immunological demands, our findings demonstrate clearly elevated concentrations of IL-2 in umbilical cord and in the 1st day samples, as well as highly increased IL-4 levels in all three neonatal samples, compared to those in adults.

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, able to secrete IL-4, IL-5 and IL-13, but without the ability to produce IFN-γ [9].

The lack of sufficient IFN-γ production after birth may contribute to impaired neonatal antiviral responses and facilitate allergic sensitization [10]. Thus the IL-4 levels in serum, but not 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 [11] and the severity of atopic state correlates with the degree of imbalance in IL-4 and IFN-γ production [12]. IFN-γ deficiency in human neonates may represent a developmental phenomenon, possibly associated with cellular responsiveness of neonatal T cells to cytokines, critical to Th1 differentiation and in translational and post-translational defect [13,14].

In agreement with previous studies [4,13], our findings indicate very low IFN-γ concentrations in umbilical cord, increasing, though, significantly thereafter in the 5th day after birth. The most likely cause for increased IFN-γ levels during the 1st week of life is from mononuclear response to microbial stimulation – possibly resulting in immune maturation – as the neonatal gut becomes colonized with various microflora [15].

Concerning sIL-4R concentrations, they were significantly elevated, compared to adult values in all three neonatal samples, the highest being found in the 1st day after birth. Our findings are consistent with a previous study of sIL-4R in infants, where the highest values were found in healthy children in comparison with other who suffered from asthma or acute respiratory infection [16]. The rise of sIL-4R concentrations in neonatal serum in the 1st day after birth reflects possibly an endogenous regulation of IL-4 activity. This possibility is also supported by the strong negative correlation that was found between IL-4 and its receptor in neonatal samples of the 1st and 5th day postpartum. Recently, there has been a great interest in using sIL-4R for the safe and effective therapy of asthma, without the use of corticosteroids [17].

Lastly, an important finding of this study is the increase trend of both IFN-γ/IL-2 and IFN-γ/IL-4 ratios from cord serum up to the 5th day of life in the studied healthy, term neonates. It is evident that newborn infants’ cells can rapidly differentiate into cells with adult-like functional capacities. Thus, the relative immunodeficiency, immaturity, or lack of experience of the neonate is most likely 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, the findings of this study indicate a dysregulation among IL-2 and IL-4 and IFN-γ concentrations during the 1st day after birth, favoring a more precocious expression of IL-2 and IL-4 against IFN-γ that seems to be ameliorated in the end of the 1st week of life.

References


