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Inflammatory cytokines in newborn infants

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SERUM levels of IL-1β, IL-6 and TNF-α were measured in 48 healthy, termed neonates on the 1st (N1), 5th (N5) and 40th (N40) day after birth, compared with those in maternal serum (MS), umbilical cord (UC) and adult controls. Cytokine values in N1 and N5 were significantly elevated, than those in UC and in controls ($P<0.0001$). IL-1β and IL-6 declined significantly from N1 to N40 ($P<0.0001$), while TNF-α increased significantly from N1 to N5 and declined thereafter. MS∞IL-1β and IL-6, but not MS∞TNF-α, were significantly higher than those of controls ($P<0.0001$). IL-1β values depended on the mode of delivery. In conclusion, the increased concentrations of IL-1β, IL-6 and TNF-α during the perinatal period might suggest their involvement in an inflammation-like process during normal parturition, and reflect also a newborn immune response to the stress of delivery and environmental changes.

Key words: IL-1β, IL-6, TNF-α, Neonatal serum, Maternal serum, Umbilical cord, Labour, Neonatal immune response, Perinatal period, Early neonatal life, Normal values of serum inflammatory cytokines in neonates

Introduction

The inflammatory cytokines, interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) are important mediators of host response to stress and infection. All three cytokines have already been found in cells, tissues and fluids associated with pregnancy.[1–5] It has been hypothesized that IL-1β may play a significant role in the embryogenesis, development and differentiation of the fetus by regulating the growth and differentiation of tissues and/or organs.[2] Moreover, IL- 1β, stimulating prostaglandin biosynthesis by intrauterine tissues, may signal the onset of labour and also strongly upregulate IL-6 production. In term pregnancy IL-6 may orchestrate biochemical, immunological and physiological changes that contribute to maternal and fetal survival.[3] In addition, TNF-α, detected in the placenta, amniotic fluid and decidua, may contribute to the regulation of cell proliferation at the uteroplacental unit.[6,7]

Elevated values of IL-1β, IL-6 and TNF-α during both term and preterm labour have already been reported in amniotic fluid samples,[2,3,8–11] in maternal serum[7,12–14] and in cervicovaginal secretions;[15–16] but, little is known about the presence of these inflammatory cytokines in early neonatal life. For this reason, our aim was to study the concentrations of IL-1β, IL-6 and TNF-α in newborn infant serum on the first days after birth, compared with those in maternal and umbilical cord serum during parturition, in order to investigate the changes of all three inflammatory cytokines during the perinatal period.

Patients and methods

This study was approved by the Ethics Committee of the Areteion Teaching Hospital. Following informed maternal consent, peripheral blood samples were collected from 48 healthy, termed neonates (clinical characteristics in the Table 1), during the 1st (N1), the 5th (N5) and the 40th (N40) days after birth. In 25 of these cases, maternal serum (MS) during the first stage of labour and umbilical cord (UC) serum in the second stage of labour were also obtained. Serum samples from 25 healthy adults were also analysed simultaneously, as controls.

Clinical examination of neonates, their mothers and controls, as well as serum reactive protein (CRP) determinations were performed and all subjects were judged infection-free.

Blood samples, collected in pyrogen-free tubes, were centrifuged at once after clotting and stored aliquotted in –30°C until assay. All three cytokines

Table 1. Clinical characteristics of the studied neonates

Sex: Females	$n = 28$
Males	$n = 20$
Birthweight (mean, range)	3322 g (2300–4380 g)
Weeks of pregnancy (mean, range)	40 (37–42)
Mode of delivery	
elective caesarean section	$n = 13$
urgent caesarean section	$n = 6$
vaginal delivery	$n = 29$
Apgar scores	9–10

were determined using solid phase sandwich enzyme immunoassays. Sensitivity, interassay CV% and trade names were, for IL-1β: 4.3 pg/ml, 8.2, Biokine® IL-1β; for IL- 6: 0.7 pg/ml, 4.4, Biotrak IL-6 Amersham Life Science; and for TNF-α: 3.0 pg/ml, 8.2, TNF-α EASIA™, Medgenix Diagnostics.

Data were analysed using non parametric methods, as they did not show a normal distribution (Kolmogorov-Smyrnov test). A Wilcoxon paired or non paired test, as appropriate, was used in comparing the distribution of cytokines in the different serum samples, while Kruskal-Wallis ANOVA was used to compare the cytokine values among the three groups according to the mode of delivery. Correlations were evaluated with Spearman rank correlation coefficients.

Results

Cytokine concentrations, as median values and ranges, in the different serum samples are given in Table 2.

- All three cytokines were significantly elevated in the neonatal samples N1 and N5, compared with those found in UC and in adult controls ($P<0.0001$).
- Neonatal serum values of IL-1β and IL-6 declined significantly from N1 to N40 ($P<0.0001$), while those of TNF-α increased significantly from N1 to N5 ($P<0.0001$) and declined thereafter.
- Umbilical cord values of TNF-α were significantly higher than those in controls ($P<0.0001$), but lower than in N40. The UC values of IL-1β and IL-6 were higher, not significantly though, than those in adult controls and in neonatal samples N40.
- Maternal serum IL-1β and IL-6, but not TNF-α values were significantly higher than those in controls ($P<0.0001$).
- Maternal serum IL-1β values were also significantly higher than those in N1 ($P<0.0001$), while those of IL-6 and TNF-α were significantly lower ($P<0.0001$).
- Serum IL-1β values were strongly dependent on the mode of delivery in UC ($P<0.03$), in N1 ($P<0.001$), in MS ($P<0.02$), and marginally in N5 samples ($P<0.055$), the lowest being found in the cases with elective caesarean section and the highest in those with an urgent one.
- A significant correlation was observed between IL-1β values in MS and UC (r=0.77; $P<0.001$), in MS and N1 (r=0.6; $P<0.009$), in MS and N5 (r=0.5; $P<0.03$) and especially in N1 and N5 (r=0.9; $P<0.0001$). No correlation was found between either IL-6 or TNF-α values in the same samples.
- Serum values of IL-1β and TNF-α in N1 were strongly interrelated (r=0.7; $P<0.01$).

Discussion

The patterns of inflammatory cytokines IL-1β, IL-6 and TNF-α in newborn infants during the perinatal period demonstrated significant changes in cytokine values, related to the time after birth. TNF-α presented significantly elevated umbilical cord values, compared with those in controls and in maternal serum, with a significant increase in neonatal serum on the 1st and further on the 5th day after birth, declining slowly thereafter. The concentrations of IL-1β and IL-6 in umbilical cord were also higher, marginally though, than those in controls, increasing markedly further on the 1st day after birth and declining thereafter, until normalization 40 days later.

These data, concerning elevated values of all three inflammatory cytokines in healthy newborn infants, are the new findings of this study and may possibly reflect an immune response of neonates to the stress of delivery and environmental influences during the early neonatal life. This hypothesis is also supported by the dependence of IL-1β values in UC and N1 on the mode of delivery and the elevation of cytokine values in UC, mainly of TNF-α, compared with those in controls.

To the best of our knowledge, serum levels of these inflammatory cytokines in healthy, non-infected neonates during the early neonatal period are reported here for the first time. Since all these pregnancies were uneventful and led to the birth of healthy, termed newborns, these levels may be considered to be a good reflection of the normal range.

Table 2. Cytokine values (median, range) in maternal serum (MS), umbilical cord (UC) and newborn infant serum, in the 1st (N1), 5th (N5) and 40th (N40) days after birth and in adult controls

Samples	*n*	IL-1β pg/ml	Significance *P**	IL-6 pg/ml	Significance *P**	TNF-α pg/ml	Significance *P**
MS	25	188 (15–464)	0.0001	5.7 (0.9–68)	0.0001	5.8 (2.5–10)	NS
UC	25	20.6 (0.0–148)	0.055	2.1 (0.7–36.0)	0.06	14.5 (8–19)	0.0001
N1	48	58.0 (14.5–240)	0.0001	21.0 (6.0–680)	0.0001	29.2 (17–54)	0.0001
N5	48	30.0 (0.0–160)	0.0001	6.7 (2.4–10.5)	0.0001	42.0 (18–79)	0.0001
N40	13	0.8 (0.0–80)	NS	1.07 (0.7–11)	NS	30.0 (27–44)	0.0001
Controls	25	14.0 (0.0–76)		1.8 (0.5–2.6)		4.8 (0.0–1.3)	

*Compared with controls.

It has been hypothesized that the presence of increased TNF-α values in pyar and breast milk may induce the developmental and maturational processes of the immune system in neonates.[17,18] However, our TNF-α values in umbilical cord showed a significant elevation already during birth, before breast feeding, strengthening our suggestion, that normal term labour itself, might constitute an inflammation-like process, that promotes the activation of the newborn immune system.

Similarly, we have already reported elevated serum values of soluble interleukin-2 receptors (sIL-2R) in umbilical cord and in serum samples from healthy term neonates on the 1st and 5th < day after birth, increasing progressively from the 1st to the 3rd sample and strongly dependent on the mode of delivery.[19] The elevation of this cytokine, a characteristic marker of immune activation, was attributed to the dynamic expansion of the neonatal immune system, in response to environmental changes, during and after birth in the early neonatal period. This phenomenon was also reflected in the elevation of other growth factors, as we have previously reported for angiogenin levels in the perinatal period.[20]

We have recently also found, however, soluble intercellular adhesion molecule-1 (sICAM-1) levels in neonatal serum samples on the 1st day of life to be lower than those in controls, though increasing progressively thereafter and exceeding those of controls 1 month after birth.[21] These differences in expression of cytokines during parturition may be due to their differentiated roles in the cytokine cascade during inflammation and immune process.

Moreover, in agreement with previous reports,[7,18,22–24] this study demonstrated very increased values of IL-1β and IL-6, but not of TNF-α, in maternal circulation during the first stage of labour. The values of IL-1β were strongly related to the mode of delivery, a finding consistent with the dominant role of this cytokine in the initiation of parturition.[1–3] The elevation of maternal serum IL-1β and IL-6 values seems to reflect a systemic reaction in the mother to her fetus and might be analogous to the cytokine increase observed in the serum of renal transplant recipients, before acute rejection of the graft.[25] The final increase at the onset of labour could be a similar 'sign of rejection'.[7] Another possible explanation might be the physical effort of giving birth, because prolonged exercise is associated with increased production of IL-6 from stimulated blood mononuclear cells.[26]

In contrast, the low values of TNF-α in maternal serum suggest a paracrine action for this cytokine in maternal and fetal tissues, as they regulate the growth of trophoblast during pregnancy and contribute to the immune processes, associated with labour.[24]

Generally, IL-1β, IL-6 and TNF-α released by chorion, amnion and decidua and acting on these membranes, maternal serum cytokine values reflect functions that take place in the gestational tissues and depend on the permeability of these tissues by these proteins. Thus, our elevated maternal serum IL-6 and low TNF-α are in agreement with a previous report,[27] regarding the permeability of fetal membranes by these cytokines. In contrast, our results on increased maternal serum IL-1β values do not agree with the latter study.

In conclusion, the presence of increased inflammatory cytokines IL-1β, IL-6 and TNF-α during the early neonatal period (a) suggests their contribution to the immune system alterations that may signal or propagate the onset of parturition and (b) reflects also a neonatal immune response to the stress of delivery and environmental changes in the first days after birth.

References

1. Miller LC, Isa S, LoPreste G, Schaller JG, Dinarello CA. Neonatal interleukin-1 beta, interleukin-6, and tumor necrosis factor: cord blood levels and cellular productio. *J Pediatr* 1990; **117**: 961–965.
2. Tsunoda H, Tamatani T, Oomoto Y, *et al.* Changes in interleukin –1 levels in human amniotic fluid with gestational ages and delivery. *Microbiol Immunol* 1990; **34**: 377–385.
3. Romero R, Avila C, Santhanam U, Sehgal PB. Amniotic fluid interleukin 6 in preterm labor. Association with infection. *J Clin Invest* 1990; **85**: 1392–1400.
4. Gunn L, Hardiman P, Tharmaratnam S, Lowe D, Chard T. Measurement of interleukin-1 alpha and interleukin-6 in pregnancy-associated tissues. *Reprod Fertil Develop* 1996; **8**: 1069–1073.
5. Olah KS, Neilson JP, Johnson PM. Interleukin-6 in amniotic fluid at forewater amniotomy compared with hindwater samples in women in spontaneous labour. *Eur J Obstet Gynecol Reprod Biol* 1995; **60**: 65–67.
6. Jaattela M, Kuusela P, Saksela E. Demonstration of tumor necrosis factor in human amniotic fluids and supernatans of placental and decidual tissues. *Lab Invest* 1988; **58**: 48–52.
7. Opsjon SL, Wathen NC, Tingulstad S, *et al.* Tumor necrosis factor, interleukin-1 and interleukin-6 in normal human pregnancy. *Am J Obstet Gynecol* 1993; **169**: 397–404.
8. Rivero-Marcotegui A, Larranaga-Azcarate C, Ceres-Ruiz R, Carcia-Merlo S. Polymorphonuclear elastase and interleukin-6 in amniotic fluid in preterm labor (letter). *Clin Chem* 1997; **43**: 857–859.
9. Coultrip IL, Lien JM, Gomez R, Kapernick P, Khoury A, Grossman JH. The value of amniotic fluid interleukin-6 determination in patients with preterm labor and intact membranes in the detection of microbial invasion of the amniotic cavity. *Am J Obstet Gynecol* 1994; **171**: 901–911.
10. Ghidini A, Jenkins CB, Spong CY, Pezzulo JC, Salafia CM, Eglinton GC. Elevated amniotic fluid interleukin-6 levels during the early second trimester are associated with greater risk of subsequent preterm delivery. *Am J Reprod Immunol* 1997; **379**: 227–231
11. Romero R, Mazor M, Sepulveda W, Avila C, Copeland D, William J. Tumor necrosis factor: in term and preterm labor. *Am J Obstet Gynecol* 1992; **166**: 1576–1587.
12. Lahan N, Rice GE, Bishop GJ, Hansen MB, Bendtgen K, Brennecke SP. Elevated plasma interleukin-6: a biochemical marker of human preterm labour. *Gynecol Obstet Invest* 1993; **36**: 145–147.
13. Greig PC, Murtha AP, Jimmerson CJ, Herbert WNP, Roitman-Johnson B, Allen J. Maternal serum interleukin-6 during pregnancy and during term and preterm labor. *Obstet Gynecol* 1997; **90**: 465–469.
14. Arntzen KJ, Lien E, Austgulen R. Maternal serum levels of interleukin-6 and clinical characteristics of normal delivery at term. *Acta Obstet Gynecol Scand* 1997; **76**: 55–60.
15. Steinborn A, Kuhnert M, Halberstadt E. Immunomodulating cytokines in term and preterm parturition. *J Perinat Med* 1996; **24**: 381–390.
16. Rizzo G, Capponni A, Rinaldo D, Tedeschi D, Arduini D, Romanini C. Interleukin-6 concentrations in cervical secretions identify microbial invasion of the amniotic cavity in patients with preterm labor and intact membranes. *Am J Obstet Gynecol* 1996; **175**: 812–817.
17. Rudlof AE 1992. Tumor necrosis factor-a in human milk. *Pediatr Res* 1992; **31**: 29–33.
18. Ellis LA, Mastro AM, Picciano MF. Do milk-born cytokines and hormones influence neonatal immune cell function? *J Nutr* 1997; **127**: 985S–988S.

19. Sarandakou A, Phocas I, Giannaki G, Rizos D, Davri Y, Zourlas PA. Immunomarkers in newborn infants. In: Cosmi EV, Di Renzo GC, eds. *Proceedings of 2nd World Congress of Perinatal Medicine*. Rome: Monduzzi Editore, 1993; 307–310.
20. Malamitsi-Puchner A, Sarandakou A, Giannaki G, Rizos D, Phocas I. Changes of angiogenin serum concentration in the perinatal period. *Pediatr Res* 1997; **41**: 909–911.
21. Phocas I, Sarandakou A, Giannaki G, Malamitsi-Puchner A, Rizos D, Zourlas PA. Soluble intercellular adhesion molecule-1 in newborn infants. *Eur J Pediatr* 1997; **157**: 153–156.
22. Austgulen R, Lien E, Liabakk NB, Jacobsen G, Arntzen KJ. Increased levels of cytokines and cytokine activity modifiers in normal pregnancy. *Eur J Obstet Gynecol Reprod Biol* 1994; **57**: 149–155.
23. Buonocore G, De Filippo M, Gioia D, Picciolini E, *et al.* Maternal and neonatal plasma cytokine levels in relation to mode of delivery. *Biology of the Neonate* 1995; **68**: 104–110.
24. Laham N, Brennecke SP, Bendtzen K, Rice GE. Tumor necrosis factor-alpha during human pregnancy and labor: maternal plasma and amniotic fluid concentrations and release of intrauterine tissues. *Eur J Endocrinol* 1994; **131**: 607–614.
25. van Oers MHJ, van der Heyden AAPAM, Aarden LA. Interleukin 6 (IL-6) in serum and urine of renal transplant recipients. *Clin Exp Immunol* 1988; **71**: 314–319.
26. Haar PM, Petersen BK, Fomsgaard A. Effect of physical exercise on in vitro production of interleukin-1, interleukin-6, tumour necrosis factor-α, interleukin2 and interferon-γ. *Int Sports Med* 1991; **12**: 223–227.
27. Kent AS, Sullivan MH, Elder MG. Transfer of cytokines through human fetal membranes. *J Reprod Fertil* 1994; **100**: 81–84.

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