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Immune Response Parameters During Labor and Early Neonatal Life

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Abstract. *Aim: Selected cytokines, associated with Th1 and Th2 immune response and inflammation, were studied in order to evaluate the relation between their release into maternal and neonatal circulation, during labour, and after birth, in comparison with those in adults. Materials and Methods: Cytokine concentrations were determined by very sensitive immunoassays, in maternal serum (MS), umbilical cord (UC), neonatal serum, the 1st (1N) and 5th (5N) day postpartum and in adult controls. Results: Both IL-2 and IL-4 cytokine concentrations in UC were markedly elevated, compared to adult and MS ones. IL-2 decreased significantly in 5N, while IL-4 remained unchanged. IFN-γ UC values were significantly lower than those in adults and MS, increasing significantly in 5N. Neonatal serum sIL-2R and sIL-4R were markedly higher than those in adults and MS. IL-1β, IL-6, sIL-6R, sTNFRI and sTNFRII concentrations in MS and all with TNF-α in neonatal serum were significantly higher than in adults. IFN-γ, IL-1β, IL-6, TNF-α, IL-2R, IL-4R concentrations in MS, 1N and 5N were dependent on the mode of delivery. Conclusion: The results of this comparative study are indicative for a meaningful role for the studied cytokines and their receptors in: i) the development of neonatal immune system, ii) the regulation of immune response during labour and early life, and iii) the initiation of the processes of labour.*

The foetus, during parturition, is suddenly transferred from an overprotective intrauterine environment into a hostile world, exposed to foreign antigens. The effective protection of the foetus from these antigens depends on the promptness of its immunological mechanisms, which constitute a complicated network with stimulative and suppressive interactive systems (1).

The high frequency and severity of infections in newborns indicate the immaturity of the neonatal immune system, especially that of the premature ones (2). Despite this relative inexperience, its defence mechanisms are noteworthy. The cells and functions which constitute its immune response appear very early in embryonic life. At birth, several parameters of immune activity are incomplete, due, mainly, to the lack of antigenic stimulations during intrauterine life. However, during parturition and the first days of life, the maturation of immune mechanisms proceeds rapidly (3).

For a better understanding of the neonatal immune system, it seems better to be studied in terms of the maternal one, since they temporarily co-exist during pregnancy. Moreover, during the perinatal and early neonatal life, the immune behaviour in the neonate reflects the interactions and adaptation mechanisms of embryonal and maternal immune system throughout its intrauterine life (4-7).

Embryonal and placental trophoblast tissues express several cytokines that during embryonic life regulate haematopoiesis, modulate the defence against inflammation, protect the embryo from maternal rejection and function as non-haematopoietic growth factors (8).

The action of cytokines may be (i) autocrine, when the cytokine acts on the cell that secretes it, (ii) paracrine, when the target is restricted to the immediate vicinity of cytokine secretion, and (iii) endocrine, when the cytokine diffuses to distant regions of the body (carried by blood or plasma).

Cytokines are critical in the development and functioning of both the innate and adaptive immune response, although not limited just to the immune system. They are often secreted by immune cells that have encountered a pathogen, thereby activating and recruiting further immune cells to increase the system's immune response. Cytokines are also involved in several developmental processes during embryogenesis.

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Placental cytokines modify the maternal immunity in order to block rejection of the embryos. For example, interleukin (IL)-4, IL-10 and interferon (IFN)-α enhance maternal humoral immunity and suppress the cellular one (9). In addition, proinflammatory cytokines, such as IL-1β and IL-6, being active components of the acute phase reaction, promote prostaglandin biosynthesis, induce modification in the myometrium (10), stimulate the dilatation of the cervix and, finally, induce labour. Normal labour is probably controlled by relative processes as in inflammation.

Our research is a comparative study, focused on the relation between cytokine release into the maternal circulation, during labour, as well as into the neonatal circulation during and after birth, in comparison with that of controls.

According to the protocol of this study, serum concentrations of IL-2, IFN-γ, IL-4, IL-1β, IL-6, tumour necrosis factor-α (TNF-α) and their soluble receptors: sIL-2R, sIL-4R, sIL-6R, sTNFRI (p55) and sTNFRII (p75) were measured during parturition and early life, in order to evaluate their role in the initiation of normal labour and the development of immune response after birth.

Materials and Methods

The study was approved by the Ethics Committee of our University Hospital and informed written consent was acquired from the participating mothers and controls on admission to the Clinic. Fifty-five healthy, non-smoking pregnant women (mean age: 24.6±4.6 years; range: 21-40 years) with a singleton uncomplicated pregnancy and their healthy full-term neonates, appropriated for gestational age, were included in this study. The demographic data of the neonates are presented in Table I. Placentas were normal in appearance and weight (mean±SD, 444±28 g). Fifty-five consenting adult controls also participated in the study (25 healthy non-pregnant women and 30 healthy male blood donors, mean age±SD, 25.8±4.8 years). Clinical examinations of neonates, their mothers and controls, as well as serum reactive protein (CRP) determinations were performed and all participants were judged infection-free.

Blood was obtained from i) mothers (MS) during the first stage of labour; in cases of Caesarean section, MS was collected before anaesthesia; ii) umbilical cord (UC) after double clamping of the cord, in the second stage of labour; iii) neonates on the 1st (1N) and 5th (5N) day after birth and iv) adult controls. The blood was collected in pyrogen-free tubes and was immediately centrifuged after clotting; the supernatant serum was kept at –30°C until assay.

Cytokine concentrations were determined using high sensitive immuno-enzymatic techniques (high sensitivity micro-ELISA). With an extension of the standard curve at very low concentrations and the application of a logistic program for the best fit, very high sensitivity was achieved. Performance characteristics of the reagents used and the new analytical criteria are given in Table II.

Statistical analysis included non-parametric tests [Wilcoxon test, Spearman correlation coefficient and Kruskal-Wallis analysis of variance (ANOVA)] and parametric tests (*t*-test, Pearson's correlation coefficient, one-way ANOVA), since data from IL-4, sIL-4R, IFN-γ, IL-1β presented abnormal distribution, while those from the remaining cytokines showed normal distribution, respectively (Kolmogorov-Smirnov test). Cytokine concentrations in MS were compared with those of 25 healthy non-pregnant women, while neonatal cytokine concentrations were compared with those of all 45 adult controls.

Table I. *Clinical characteristics of participating neonates.*

Gender	
Female	n=28
Male	n=27
Birth weight (g), mean±SD (range)	3322 g±390 (2520-4120)
Weeks of pregnancy, mean±SD (range)	39.2±0.9 (38-41)
Mode of delivery	
Elective Caesarean section	n=20
Normal vaginal delivery	n=35
Apgar score	8-10

Results

Summarized data are given in the Tables III, IV and V.

Serum IL-2 concentrations in UC were markedly higher, compared with those in MS and controls ($p<0.01$), decreasing significantly afterwards up to 5N ($p<0.001$).

Serum sIL-2R in MS, UC, 1N and 5N were significantly higher than those in controls ($p<0.0001$), and increased greatly from UC to 5N ($p<0.0001$). Both 1N and 5N sIL-2R concentrations were considerably higher in neonates born vaginally (840±204.5 U/ml and 1662±518 U/ml, respectively), than in those delivered by elective Caesarean section (592±224 U/ml and 1163±496 U/ml, respectively, $p<0.01$).

IL-4 concentrations did not differ significantly between UC, 1N and 5N. However, levels in these samples were markedly elevated compared to those in MS ($p<0.001$, $p<0.0005$ and $p<0.0001$, respectively) and controls ($p<0.05$, $p<0.01$, and $p<0.005$, respectively).

Serum sIL-4R concentrations were notably higher in MS compared with those in controls ($p<0.0001$), UC ($p<0.0005$), 1N ($p<0.03$) and 5N ($p<0.0001$). MS sIL-4R values were dependent considerably on the mode of delivery, being higher in cases of vaginal delivery (median 148 pg/ml, range 95-398 pg/ml) compared with those of elective Caesarean section (median 119 pg/ml, range 59-235 pg/ml; $p<0.05$). Serum sIL-4R values were significantly higher in all three neonatal samples compared with those in controls ($p<0.0001$), being higher in 1N than in UC ($p<0.01$), and declining in 5N ($p<0.01$). A strong negative correlation was found between sIL-4R and IL-4 concentrations both in 1N ($r=–0.48$, $p<0.002$) and 5N ($r=–0.45$, $p<0.006$) samples.

Serum INF-γ levels in UC were significantly lower than those in adults ($p<0.04$), showing, however, a significant increase in 5N ($p<0.03$). INF-γ concentrations in MS and UC were dependent on the mode of delivery, being

Table II. *Performance characteristics and trade names of reagent used.*

Cytokines	Type of analysis	Sensitivity	Intra-assay CV%	Inter-assay CV%	Trade names
IL-2	EASIA	0.1 IU/ml	5.7	7.5	MEDGENIX, EASIA, Fleurus, Belgium
sIL-2R	ELISA	50 U/ml	5.2	6.5	Cellfree® IL-2R, T Cell Sciences, USA
IFN-γ	MicroELISA	3.0 pg/ml	2.6	6.4	Quantikine® IFN-γ, R&D Systems, USA
IL-4	MicroELISA	0.13 pg/ml	4.6	5.8	Quantikine® hIL-4 HS, R&D Systems, USA
sIL-4R	MicroELISA	5 pg/ml	2.6	5.2	Quantikine® hIL-4sR, R&D Systems
IL-1β	ELISA	4.3 pg/ml	8.2	10.4	Biokine® IL-1β T cell Diagnostics
IL-6	MicroELISA	0.094 pg/ml	5.9	16.5	Quantikine® hIL-6 HS, R&D Systems
sIL-6R	MicroELISA	6.5 pg/ml	2.6	4.2	Quantikine® hIL-6sR, R&D Systems
TNF-α	MicroELISA	0.12 pg/ml	5.9	12.6	Quantikine® TNF-α HS, R&D Systems
sTNFRI	MicroELISA	30 pg/ml	2.9	3.7	Quantikine® sTNFRI, R&D Systems
sTNFRII	MicroELISA	10 pg/ml	2.5	3.5	Quantikine® sTNFRII, R&D Systems Inc. MN 55413, USA

Table III. *Concentrations of IL-2, sIL-2R, IL-4, sIL-4R in maternal serum (MS), umbilical cord (UC) blood and neonatal serum in the 1st (1N) and 5th (5N) day postpartum, compared to those in adult controls.*

Serum samples	IL-2 (IU/ml) Mean±SE	sIL-2R (U/ml) Mean±SD	IL-4 (pg/ml) Median (range)	sIL-4R (pg/ml) Median (range)
MS	0.125±0.02	682±318	0.110 (0.019-2.6)	132.5 (59-398)
UC	0.48±0.22	695±205	0.195 (0.0-1.926)	75.5 (39.5-212)
1N	0.32±0.16	820±39.0	0.190 (0.0-1.965)	100 (48-312)
5N	0.23±0.07	1632±84.6	0.204 (0.066-0.699)	74.0 (30- 218)
Adult controls	0.137±0.06	490±32	0.133 (0.0-0.410)	38.5 (20-88)

Table IV. *Concentrations of IFN-γ, IL-1β, IL-6 and sIL-6R in maternal serum (MS), umbilical cord (UC) blood and neonatal serum in the 1st (1N) and 5th (5N) day postpartum compared to those in adult controls.*

Serum samples	IFN-γ (pg/ml) Median (range)	IL-1β (pg/ml) Median (range)	IL-6 (pg/ml) mean±SD	sIL-6R (pg/ml) mean±SD
MS	4.14 (0.0-11.17)	191 (15-464)	8.3±4.6	26096±8489
UC	3.72 (0.18-10.46)	18.5 (0.0-148)	5.5±3.6	32345±9043
1N	5.50 (0.0-17.66)	57.3 (14.5-240)	12.9±2.75	36635±10379
5N	7.19 (0.0-23.2)	33.0 (0.0-160)	6.9±3.2	43650±10119
Adult controls	4.65 (0.0-21.36)	3.0 (0.0-76)	1.9±0.6	24926±9682

significantly higher in cases of vaginal delivery [4.8 pg/ml (1.06-11.17) and 3.9 pg/ml (0.18-10.45), respectively], compared with those in Caesarean section [3.9 pg/ml (0.0-8.7), $p<0.005$ and 3.4 pg/ml (0.0-6.8), $p<0.007$, respectively]. Both INF-γ/IL-2 and IFN-γ/IL-4 ratios in UC were significantly lower compared with those in MS and controls ($p<0.001$ and $p<0.03$, respectively), showing, however, a critical increase in 5N ($p<0.001$ and $p<0.03$, respectively).

Serum IL-1β concentrations in MS were extremely elevated compared to those in all three neonatal samples and in controls ($p<0.0001$). IL-1β values in 1N were

Table V. *Concentrations (mean±SD) of TNF-α, sTNFRI and sTNFRII in maternal serum (MS), umbilical cord (UC) blood and neonatal serum in the 1st (1N) and 5th (5N) day postpartum compared with those in adult controls.*

Serum sample	TNF-α (pg/ml)	sTNFRI (pg/ml)	sTNFRII (pg/ml)
MS	3.66±0.82	1320±418	1939±605
UC	5.80±1.93	2050±708	3124±792
1N	6.20±1.87	3366±950	4241±957
5N	8.40±3.37	2155±613	3567±784
Adult controls	3.20±0.95	933±315	1344±290

significantly higher than those in UC ($p<0.0001$), in 5N ($p<0.006$) and in controls ($p<0.0001$). IL-1β values in MS, UC and IN depended strongly on the mode of delivery being higher in vaginal delivery than in Caesarean section ($p<0.002$), ($p<0.03$) and ($p<0.001$ respectively).

Serum IL-6 levels in 1N were strongly elevated compared to those in MS ($p<0.002$), UC and 5N ($p<0.0001$), as well as in the controls ($p<0.0001$). All three neonatal values were significantly higher, than control ones ($p<0.001$-0.0001). Cytokine concentrations in UC and MS were dependent on the mode of delivery, showing higher values in cases of vaginal delivery (7.7±3.8 pg/ml), (11.4±1.9 pg/ml) respectively) than in Caesarean section (3.5±1.8 pg/ml) ($p<0.04$), (2.95±1.23 pg/ml; $p<0.0005$ respectively). A strong correlation was found between IL-6 values in UC and 1N (r=0.8, $p<0.005$).

Serum sIL-6R concentrations in all three neonatal samples showed a significant continuous increase from UC to 5N ($p<0.002$). 1N and 5N sIL-6R values were significantly higher than those in MS ($p<0.01$ and $p<0.0001$, respectively) and controls ($p<0.01$ and $p<0.0002$, respectively), while no significant difference was found among UC, MS and controls. A strong correlation was observed between sIL-6R concentrations in UC and 1N (r=0.85, $p<0.002$) and 1N and 5N (r=0.8, $p<0.0001$).

Serum TNF-α, sTNFRI and sTNFRII concentrations in all three neonatal samples were significantly elevated compared with those in MS and controls ($p<0.0001$), showing critical alterations during the fifth postnatal day. Analytically, TNF-α values increased continuously and considerably from UC up to 5N ($p<0.0001$), while its two receptor values increased sharply, from UC to 1N ($p<0.0001$ and $p<0.001$, respectively), declining significantly thereafter in 5N ($p<0.0001$ and $p<0.01$, respectively). TNF-α values in UC, 1N, 5N and sTNFRI in UC were significantly dependent on the mode of delivery ($p<0.05$, $p<0.03$, $p<0.04$ and $p<0.01$, respectively), with higher values in vaginal delivery than in Caesarean section.

Discussion

The development of immunity in neonates may be visualized as a series of adaptive cellular responses to an ever-changing and potentially, hostile, environment. The foetal immune system tends to develop a Th2-type immune response, due to the production of Th2-promoting factors, such as IL-4, IL-10 and prostaglandin E from the placenta (11). Consequently, umbilical cord cells are incapable of developing efficient Th1-mediated responses, being massively inclined towards Th2 reactions. Hence, a high release of IL-4 would be expected, accompanied by a decrease in the production of IFN-γ, resulting in an initial dysregulation of IFN-γ and IL-4 in neonates (12).

In accordance with these immunological demands, our findings clearly demonstrated elevated IL-4 concentrations in umbilical cord and 1st and 5th day neonatal samples compared to those in maternal and adult control ones, accompanied by very low IFN-γ umbilical cord concentrations.

On the other hand, 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 differentiation. However, cord blood T-cells lack the capability for inducing the production of IFN-γ, irrespective of IL-2 and IL-2R up-regulation (13). Indeed, IL-2 is a potent IFN-γ-inducing cytokine and is produced by both memory and naïve neonatal and adult T-cells in nearly equal amounts. Therefore, the striking discrepancy between neonatal and adult IFN-γ production is not related to IL-2 deficient induction of IFN-γ (14).

Consistent with the above remarks, our results demonstrated clearly elevated concentrations of IL-2 in umbilical cord and in the 1st day neonatal samples, while IFN-γ values were significantly lower than those in controls and mothers. This observation indicates a differentiation in the functional maturation of the immune response, and may possibly constitute a developmental characteristic in the ontogeny the immune system (11, 15).

In contrast to IL-2, soluble receptor sIL-2R values increased significantly during the neonatal period, which may be attributed to the newborn's immune system response to a changing internal and external environment (4). The dependence of sIL-2R on the mode of delivery, with higher values in neonates delivered vaginally compared to those delivered by elective Caesarean section is strongly related to a stimulation of the cytokine network (16, 17). The exact function of sIL-2R has not been clearly clarified. It is suggested that it should be an immunoregulatory molecule, acting competitively with the membrane receptor for binding with IL-2 and therefore suppress systemic or local immune reactions (18).

Concerning IL-4 and sIL-4R, our data demonstrated elevated concentrations in all three neonatal samples. Both these cytokines have been immunohistochemically localized in the term placenta and the immediate tissues at the maternal-fetal interface (19), indicating the crucial role of IL-4 in the immunobiology of pregnancy. A central biological function of IL-4 is the induction of differentiation of $CD4^+$ Th precursor T lymphocytes into Th2 T-cells, in both humans and mice (20). An important finding of this above study is that not only memory cells, but also naïve human $CD4^+$ T-cells can be an initial source of IL-4 during primary immune responses.

The rise of sIL-4R levels in neonatal serum in the first day after birth reflects an endogenous regulation of IL-4 activity, which is supported by the strong negative correlation calculated between IL-4 and its receptor in first and fifth day

postpartum neonatal samples. Because of its dependence on the mode of delivery, it amplifies the beneficial influence of vaginal delivery towards the activation of the immune system.

Additional findings of interest in the present study include the dependence of maternal and cord blood serum IFN-γ on the mode of delivery. IFN-γ values were higher in mothers giving birth vaginally than in those delivered by elective Caesarean section. Similarly, babies born vaginally had higher concentrations of IFN-γ than those delivered by elective Caesarean section. Therefore, labour appears to be responsible for an increase in the release of maternal IFN-γ and other cytokines. A possible explanation could be the enhanced absorption of lipopolysaccharides in the maternal gut during labour, leading to an increase in synthesis of some cytokines (21). Thus, labour possibly induces the production of such important modulators of immune responses, both in mothers and their newborn infants, strengthening maternal and neonatal defence against perinatal infections (15, 21).

On the other hand, elevated values of IL-1β, IL-6 and TNF-α demonstrated in maternal serum, cord blood and neonatal samples; these results are in accordance with previously published reports (22-24). It has been suggested that these three inflammatory cytokines are important mediators of host response to stress and infection (25) and have been already found in cells, tissues and fluids associated with reproduction and pregnancy (26-28). It has been hypothesized that IL-1β may play a significant role in the embryogenesis, development and differentiation of the foetus by regulating the growth and differentiation of tissues and/or organs (29). Moreover, IL-1β, by stimulating prostaglandin biosynthesis from intrauterine tissues may signal the onset of labour (28). In term pregnancy, IL-6 may orchestrate biochemical, immunological and physiological changes that contribute to maternal and embryonal survival (30). In addition, TNF-α, detected in the placenta, amniotic fluid and decidua, may contribute to the regulation of cell proliferation at the uteroplacental unit, promoting apoptosis by its soluble receptor sTNFRI (p55) (26, 31, 32).

The new finding in our study is the elevation, gradual or rapid, of all three cytokines and their soluble receptors in all neonatal samples. Moreover, an interesting finding may be the dependence of all these inflammatory cytokines on the mode of delivery. These observations strengthen the hypothesis that normal-term labour constitutes an inflammatory process, promoting the activation of the neonatal immune system during parturition and early life.

The elevation of maternal IL-1β and IL-6 values seems to reflect a systemic reaction from the mother to her embryo and might be analogous to cytokine increase, observed in the serum of renal transplant recipients before acute graft rejection (33). Another possible explanation might be the physical effort of giving birth, because prolonged exercise is associated with increased production of IL-6 (34). On the contrary, low maternal TNF-α values have been described in previous studies (35, 36), suggesting a local or paracrine action of this cytokine in the placenta, decidua and chorionic membranes. TNF-α seems to be the initial mediator in the cytokine network, inducing the release of other cytokines such as IL-6 (35).

An impressing finding of our study is the concurrent elevation of all three soluble receptors sIL-6R, sTNFRI and sTNFRII in maternal serum and neonatal samples. A possible explanation for this significant elevation might be their regulatory effect against the inflammatory cytokine activity. It is well known that inflammatory cytokines, produced during inflammatory and other immune processes, may cause systemic or local toxic reactions. For this reason, their biosynthesis and release are continuously controlled and their activities are narrowed with negative feedback mechanisms (36).

Soluble receptors belong to the type II inhibitors which compete with the membranic ones for cytokine binding. They act as protective mechanisms against the systemic and potentially harmful activities of cytokines, allowing their local expression and amplifying their paracrine (beneficial) actions (36, 37). Soluble sIL-6R, like interleukin-6, has been histochemically detected in placental trophoblast (38), in the developing embryo, and especially in its adrenal glands, possibly contributing to development of the embryo (39).

As reported previously, the concentrations of sTNFRI and sTNFRII in maternal serum, increased significantly during labour, reflecting a respective increased production of TNF-α and its implication in the process of labour. Because the half-life of TNF-α soluble receptors is longer than that of TNF-α, it has been suggested that both soluble receptors are a 'sign' of cytokine activation, as well as markers of the activation of immune response, for a long period after the normalization of TNF-α (40).

In conclusion, the results of this comparative study do not reflect the totality of functions for each immune parameter. However, they give a measure of each cytokine release that seems to be proportional to its whole functions. According to this precondition our findings are as follow: i) Serum concentrations of selected cytokines associated with Th1 and Th2 immune response and inflammation are elevated during early neonatal life; ii) An alteration between Th1 and Th2 cytokine release occurs after birth favouring an earlier expression of IL-2 and IL-4, compared to IFN-γ that seems to be ameliorated up to fifth postpartum day; iii) The release of most cytokines is dependent on the mode of delivery, with significantly higher values in vaginal delivery than those in Caesarean section; iv) Serum concentrations of inflammatory cytokines are elevated during labour, indicating their involvement in the initiation of labour and activation of

neonatal immune system; v) Serum concentrations of soluble receptors of all three inflammatory cytokines are elevated, suggesting their important role in the regulation of immune response during labour and early life.

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