Effects of labour and medication on major lymphocyte subsets in cord blood

M. Dostál, J. Živný, P. Harting, J. Dejmek, P. A. Steerenberg, H. van Loveren and R. J. Šrám

It is envisaged that flow cytometric analysis of lymphocyte subsets in cord blood may be used as a biomarker for effects on the immune system of exposure to environmental factors. In order to investigate the possible application of this parameter, we first studied the effects of other factors that may influence the outcome of subset analysis in cord blood. FACS analysis was performed in 112 pairs of umbilical cord blood and of peripheral maternal blood sampled at labour. Whereas in maternal blood no statistically significant effects of medication during labour on T lymphocyte numbers and NK cells were found, in oxytocin- and in oxytocin and prostaglandin-treated mothers B cell numbers showed a statistically significant increase. In cord blood, the course of labour and/or medication during labour were identified as the most important factors determining distribution of major lymphocyte subsets. In cord blood after deliveries without medication or after neuroplegic analgesia (NPA), the mean percentage of cord blood T lymphocytes ($CD3^+$) was highest (59%) and that of NK lymphocytes ($CD3^-/CD16 + 56^+$) lowest (20%). The mean percentage of T lymphocytes was significantly lower (52%) and that of NK lymphocytes higher (28%) in cord blood where deliveries were done under NPA in combination with infusion of oxytocin. The combination of NPA with oxytocin and induction of labour by prostaglandin E2 led to a further reduction of T lymphocytes and an increase of NK cells (39% and 38% respectively). The changes in ratio of T and NK lymphocytes were due both to decreasing absolute counts of T lymphocytes and increasing counts of NK lymphocytes. Thus, the effects of labour and/or medication during labour must be taken into account when this parameter is applied as a potential biomarker of effects of environmental factors on the immune system.

Keywords: *cord blood, flow cytometry, lymphocyte subsets, medication, labour.*

M. Dostál *(author for correspondence) is at the Institute of Experimental Medicine, Czech Academy of Sciences, Vídeňská 1083, 142 20 Prague 4, Czech Republic;* **J. Živný, P. Harting** *are in the IInd Department of Obstetrics and Gynaecology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic;* **J. Dejmek, R. J. Šrám** *are at the Regional Institute of Hygiene of Central Bohemia, Prague, Czech Republic;* ***P. A. Steerenberg*** *and* **H. van Loveren** *are in the Laboratory of Pathology and Immunobiology, National Institute of Public Health and the Environment, Bilthoven, The Netherlands.*

Introduction

Cord blood is an easily obtained and, from an ethical point of view, an acceptable source of immunoglobulins and leucocytes. The detection of membrane marker molecules by flow cytometry (immunophenotyping) is used to study prenatal differentiation as well as functional capacity and maturity of cord blood lymphocytes. It has also been proposed to use cord blood lymphocytes phenotyping for the diagnosis of prenatally originating immunodeficiencies (Rainaut *et al.* 1987, Lucivero *et al.* 1995) and as a developmental immune marker in epidemiological studies in which the effects of environmental factors on the immune system are investigated (Vogt and Schulte 1993, Dostál *et al.* 1995).

For such purposes, the impact of factors other than those that are the subject of study on these parameters should be known. It has been shown that some factors, e.g. maternal diabetes (Giordano *et al.* 1992, Roll *et al.* 1994), maternal treatment with immunosuppressants (Takahashi *et al.* 1994), length of gestation (Wilson *et al.* 1985), infections *in utero* (Pass *et al.* 1983), influence lymphocyte subsets in cord blood or early childhood. Therefore, normal reference values are being established by examination of healthy neonates delivered at term by healthy mothers with an uncomplicated course of pregnancy. Even in such neonates the percentages of major lymphocyte subsets are influenced by sex and race (Motley *et al.* 1996). It was also found that the distribution of cord blood lymphocyte subsets differs between neonates born vaginally and by elective caesarean section (Pittard *et al.* 1989, Gasparoni *et al.* 1992, Samelson *et al.* 1992).

We have studied whether the course of vaginal delivery and/or medication during labour are associated with distribution of major lymphocyte subsets in cord blood. The present paper is based on results of phenotyping 127 pairs of maternal and cord blood sampled at deliveries in a maternity hospital during July–August 1995.

MATERIAL AND METHODS

Immunology

Maternal peripheral venous blood sampled at the time of labour and mixed arterial and venous cord blood sampled immediately after labour were collected into heparinized vacutainers (10 ml, Vacuette®, Greiner). The samples were stored in a refrigerator (at + 4 °C) in polystyrene boxes and analysed within 24 h. Lymphocytes were phenotyped in lysed whole blood using a FACSort flow cytometer, Simulset software, and Simultest IMK lymphocyte kit of monoclonal antibodies (all from Becton Dickinson Immunocytometry Systems). The following lymphocyte subsets were determined: $CD3^+$ T lymphocytes, $CD3^+/CD4^+$ T-helper lymphocytes (CD4), $CD3^+/CD8^+$ T-cytotoxic/suppressor lymphocytes (CD8), $CD3^-/CD19^+$ B lymphocytes, $CD3^-/CD16 + 56^+$ natural killer (NK) lymphocytes. Problems with contamination of cord blood lymphocytes with nucleated red blood cells (Harris *et al.* 1994) are most easily solved by a lysed whole blood method. The Simulset software (Becton Dickinson Immunocytometry Systems) provides the three part differential of leucocytes in the gate, based on the CD45/CD14 staining. Correspondence of the percentage of identified lymphocytes (T + B + NK) to the percentage of lymphocytes in the gate was used to control the quality of staining. The values were converted to percentages of the total counts of lymphocytes, i.e. of the sum of T, B and NK lym

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Material

The lymphocytes of maternal and cord blood of 112 Caucasian women and their neonates born vaginally without anaesthesia at the same maternity hospital in July–August 1995 were phenotyped. There were 100 neonates delivered at gestational age 38–41 weeks, three neonates at week 37 and nine neonates at week 42. In 92 samples of cord blood, total counts of leucocytes, erythrocytes and platelets, and haemoglobin content were determined immediately after delivery at the Laboratory of Hematology, IInd Department of Obstetrics and Gynaecology. Total lymphocyte counts were deducted from the white blood cell counts and the differential determined by SIMULSET software.

The following data were available from the medical records:

Medication during labour: NPA – neuroplegic analgesia (the mixture of dolsin (100 mg), protazin (50 mg) plegomazin (25 mg) and dihydroergotoxin (0.6 mg) administered i.m. in a dose adjusted to the body weight), OG – i.v. infusion of 2 IU oxytocin in 5% glucose, PGE – induction of labour by prostaglandin E2 (Prostin E2, Upjohn).

Newborn: gestational age in weeks determined by menstrual data, time of birth, interval between the rupture of membranes and delivery, presence of stained amniotic fluid, gender, birth weight, diagnosed infection, major congenital defects.

Mother: age, body weight, body height, number of previous births and of spontaneous or induced abortions, gestational diabetes, hypertension in pregnancy, incompetence of the cervix, diagnosed or reported bleeding during pregnancy, infectious diseases, administration of antibiotics during pregnancy, diseases before pregnancy, previous usage of contraceptives, smoking, history of allergy.

To analyse the effects of labour on lymphocyte subsets in cord blood, neonates delivered vaginally without anaesthesia were divided into five groups differing in medication used during labour:

1. vaginal delivery without any medication (group NONE, 22 pairs of blood samples)
2. vaginal delivery in neuroplegic analgesia (group NPA, 47 pairs)
3. vaginal delivery in NPA, infusion of oxytocin (group NPA/OG, 29 pairs)
4. vaginal delivery with induction of labour with prostaglandin E2 and NPA (group PGE/NPA, 8 pairs)
5. vaginal delivery in NPA, induction of labour with prostaglandin E2, oxytocin (group PGE/NPA/OG, 6 pairs)

Statistics

The test of goodness of fit, regression analysis and multifactorial analysis of variance (Statgraphics 5.0) were used to identify association of maternal and labour-associated variables with percentages of the lymphocyte subsets. The significance of differences between the mean values of lymphocyte subsets in groups differing by medication at labour was tested using the non-parametric analysis of variance Kruskal–Wallis (Theodorsson-Norheim 1986). Differences at the level 0.05 and less are considered statistically significant.

Results

Cord blood

The numbers of T lymphocytes showed a normal distribution, whereas distributions of NK and B lymphocytes were not normal ($p < 0.05$). The varying proportions of T and NK lymphocytes was the most conspicuous feature of our data. Pharmacological induction/stimulation of labour in vaginal deliveries were identified as the most important factor influencing relative counts of T lymphocytes in cord blood.

The mean percentage of T lymphocytes (Table 1) was highest (61.4%) in newborns delivered without any medication. The mean percentages of T lymphocytes in newborns of mothers delivering under neuroplegic analgesia in combination with oxytocin (52%) or administered combination PGE/NPA/OG (38.5%) were significantly lower than in group NONE. A lower percentage of T lymphocytes was associated with a higher percentage of NK lymphocytes and in the group PGE/NPA/OG also by a non-significant increase in the percentage of B lymphocytes. Changes in percentages of CD4 and CD8 lymphocytes reflect the changes of total T lymphocytes. With the treatment PGE/NPA/OG, the percentage of CD8 lymphocytes decreased to a lesser extent than that of CD4, thus the CD4 : CD8 index was lower than in the other groups (Figure 1).

The mean absolute counts of cord blood leucocytes and lymphocytes in the group PGE/NPA were significantly lower than the mean counts in the other groups (Table 2) and were reflected by low mean counts of all lymphocyte subsets. The mean counts of lymphocyte subsets in groups with similar total counts of lymphocytes (i.e. after exclusion of PGE/NPA group) show association with medication during labour. The mean count of T lymphocytes is highest and the count of NK lymphocytes lowest in deliveries without medication and they decrease and increase, respectively, with increasing medication during labour. Also, the mean count of B lymphocytes is highest in neonates delivered without medication. The differences between the mean counts of CD3 and CD4 lymphocytes are statistically significant.

There were no differences between haemoglobin content and counts of erythrocytes and platelets (data not shown).

Maternal blood

The mean percentages of T lymphocytes in groups of mothers differing in medication during labour are in the range 68.9 (no medication) to 53.7 (group PGE/NPA/OG). The mean percentages of NK lymphocytes are in the range from 24.4 (no medication) to 33.0 (group PGE/NPA/OG). These differences are statistically insignificant (Table 3). Thus, only the percentage of B lymphocytes changes with medication during labour, being significantly higher in mothers treated with oxytocin (groups NPA/OG and PE/NPA/OG).

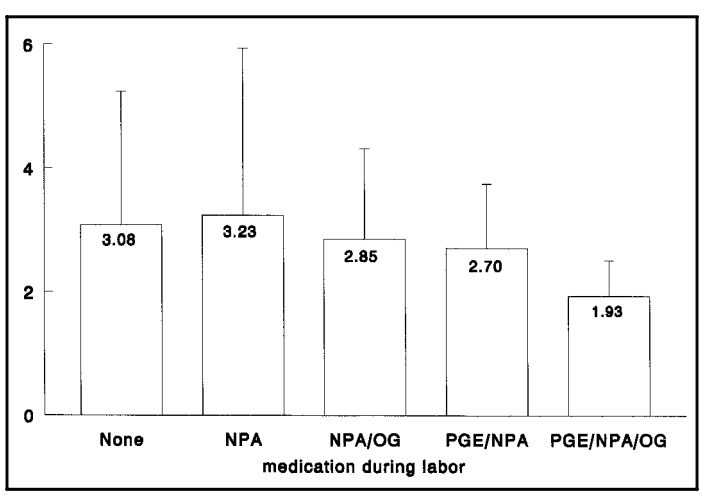

Figure 1. The CD4/CD8 index (mean, SD) of co

Group: Medication:		1 None N = 22	2 NPA N = 47	3 NPA/OG N = 29	4 PGE/NPA N = 8	5 PGE/NPA/OG N = 6	LS
T lymphocytes	Mean	61.4	56.8	52.0[1]	57.1	38.5[1–4]	$p < 0.01$
$CD3^+$	SD	9.6	13.3	12.1			
B lymphocytes	Mean	21.1	20.6	19.6	18.5	24.0	NS
$CD3^-/CD19^+$	SD	10.7	9.5	7.1			
NK lymphocytes	Mean	17.4	22.7[1]	28.2[1,2]	24.1	37.5[1,2]	$p < 0.01$
$CD3^-/CD16 + 56^+$	SD	6.8	9.7	11.8			
CD4 lymphocytes	Mean	44.8	41.9	39.4[1]	43.6	25.2[1–4]	$p < 0.01$
$CD3^+/CD4^+$	SD	9.6	9.8	10.0			
CD8 lymphocytes	Mean	18.0	17.0	15.1	16.5	13.3	NS
$CD3^+/CD8^+$	SD	6.2	8.2	5.1			

Table 1. Relative values of major lymphocyte subsets in cord blood.
Key: LS, Level of significance tested by non-parametric analysis of variance Kruskal–Wallis; NS, non-significant difference.
[1–4] Statistically significant difference against all groups.
[1] Statistically significant difference against group NONE.
[2] Statistically significant difference against group NPA.

Group: Medication:		1 None N = 19	2 NPA N = 38	3 NPA/OG N = 24	4 PGE/NPA N = 6	5 PGE/NPA/OG N =5	LS
Leucocytes	Mean	17.3	15.8	17.3	12.3[1,3]	17.30	$p < 0.01$
	SD	4.39	4.80	4.66			
Lymphocytes	Mean	5.41	4.36	4.77	3.24[1,3]	4.25	$p < 0.01$
	SD	1.77	1.40	1.73			
T lymphocytes	Mean	3.30	2.51[1]	2.44[1]	1.62[1,2]	1.55[1,2]	$p < 0.01$
$CD3^+$	SD	1.18	9.02	1.05			
B lymphocytes	Mean	1.13	0.80	0.91	0.62[1,3]	0.82	NS
$CD3^-/CD19^+$	SD	0.56	0.26	0.31			
NK lymphocytes	Mean	0.94	1.05	1.42	0.99	1.87	NS
$CD3^-/CD16 + 56^+$	SD	0.53	0.68	0.88			
CD4 lymphocytes	Mean	2.46	1.81[1]	1.82[1]	1.21[1]	0.97[1,2,3]	$p < 0.01$
$CD3^+/CD4^+$	SD	1.07	0.67	0.83			
CD8 lymphocytes	Mean	0.94	0.75	0.72	0.47	0.53	NS
$CD3^+/CD8^+$	SD	0.38	0.37	0.34			

Table 2. The absolute counts of lymphocytes in cord blood ($\times 10^9 \times l^{-1}$).
Key: LS, Level of significance tested by non-parametric analysis of variance Kruskal–Wallis; NS, non-significant difference.
[1] Statistically significant difference against group NONE.
[2] Statistically significant difference against group NPA.
[3] Statistically significant difference against group NPA/OG.

Group: Medication:		1 None N = 22	2 NPA N = 47	3 NPA/OG N = 29	4 PGE/NPA N = 8	5 PGE/NPA/OG N = 6	LS
T lymphocytes	Mean	68.9	63.9	65.1	58.0	53.7	NS
$CD3^+$	SD	9.4	8.5	10.9	14.9	9.8	
B lymphocytes	Mean	6.5	8.5	10.1[1]	9.8	13.0[1]	$p < 0.05$
$CD3^-/CD19^+$	SD	3.2	4.9	4.2	5.2	7.5	
NK lymphocytes	Mean	24.4	27.5	24.6	32.5	33.0	NS
$CD3^-/CD16 + 56^+$	SD	9.1	9.7	11.6	15.0	12.7	
CD4 lymphocytes	Mean	35.3	31.9	36.8	34.0	33.0	NS
$CD3^+/CD4^+$	SD	10.3	8.5	8.2	11.2	5.9	
CD8 lymphocytes	Mean	33.1	31.6	29.7	23.0	23.2	NS
$CD3^+/CD8^+$	SD	8.4	10.4	7.6	7.0	7.5	

Table 3. Percentages of lymphocyte subsets in peripheral maternal blood.
Key: LS, Level of significance tested by non-parametric analysis of variance Kruskal–Wallis; NS, non-significant difference.
[1] Statistically significant difference against group NONE.

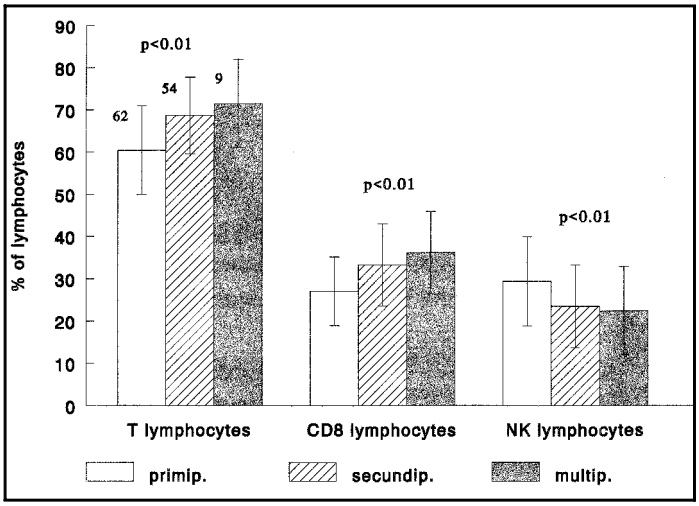

Figure 2. Major lymphocyte subsets (mean, SD, *N*) in maternal peripheral blood at labour of primigravida, secundigravida and multigravida mothers. The statistical significance was tested by non-parametric analysis of variance Kruskal–Wallis.

The percentage of B lymphocytes is also correlated (data not shown) with a number of maternal factors: age, body weight, body height, length of pregnancy, time interval between rupture of membranes and delivery. The highest correlation coefficient is that for maternal age, its value reaches –0.243 only.

Differences in lymphocyte subsets between primigravida and multigravida mothers are shown in Figure 2. Mothers delivering for the first time have significantly lower proportions of CD8 lymphocytes and higher proportions of NK lymphocytes than mothers delivering for the second or more time (Figure 2).

Discussion

The samples of cord and maternal blood from 112 vaginal deliveries without anaesthesia were divided into five groups according to medication during labour (cases with atypical medication were not included). The percentage of T lymphocytes in our samples was significantly correlated with medication during labour. Induction/stimulation of labour with oxytocin and/or prostaglandin E2 is associated with a decreased percentage of T lymphocytes and increased percentage of NK lymphocytes (when compared with neonates born without medication). The contribution of factors such as length of gestation, neonatal sepsis, congenital defects, or maternal disease to the total variance of proportion of T lymphocytes was less than that of medication during labour. It must however be mentioned that this may in part be due to a low incidence of such newborns in our cohort.

Our data show that changes in proportions of T and NK lymphocytes in groups differing in medication at labour are due to both decreasing counts of T lymphocytes and increasing counts of NK lymphocytes. The mean counts of B lymphocytes are also somewhat higher in the group NONE than in pharmacologically-assisted deliveries.

Interlaboratory comparisons of data on cord blood lymphocyte subsets are confounded by differences in staining procedures (full blood ×isolated lymphocytes), selection of antibodies to surface markers (CD markers versus sIg for B lymphocytes, $CD3^-/CD56^+$ versus $CD3^-/CD16 + 56^+$ staining for NK lymphocytes) and correction for the purity of lymphocyte gate when converting values to a percentage of total lymphocytes (see discussion by Motley *et al.* (1996)). Also, the criteria for selection of the normal newborns may differ. For instance Kontny *et al.* (1994) excluded 168 newborns which did not fulfil their criteria for healthy newborns from a population of 221 newborns delivered at term with normal weight for age. Nevertheless, the published percentages of T lymphocytes are similar to our values for newborns delivered without medication or in NPA (Pittard *et al.* 1989, Erkeller-Yuksel *et al.* 1992, Raes *et al.* 1993, Kontny *et al.* 1994, Motley *et al.* 1996).

At present, we have no data to explain why the neonates born after induction/stimulation of labour by PGE and oxytocin have low numbers of T lymphocytes and high numbers of NK lymphocytes. Theoretically, this may be explained by the following reasons: 1. the differences existed before labour started; 2. they reflect redistribution of lymphocytes during labour due to transplacental effects of drugs; 3. they reflect redistribution of lymphocytes due to maternal stress and/or to foetal hypoxia. If so, medication during labour is in fact a surrogate for the course of labour.

Pittard *et al.* (1989) explained the labour-associated decrease in counts of CD3 lymphocytes (compared with neonates born by elective Caesarean section) by the effects of increased neonatal levels of circulating catecholamines and cortisone, or by physiological alterations due to mild ischaemia or hypoxia of delivery.

With maternal lymphocytes, only the percentages of B lymphocytes differ significantly in relation to medication. They are increased in groups NPA/PGE/OG and NPA/OG. In fact, also the mean values of T and NK lymphocytes have a trend similar to changes seen in cord blood – the percentages of NK lymphocytes increase and these of T lymphocytes decrease when induction/stimulation of labour is used.

In total, the distribution of the main lymphocyte subsets in cord blood of neonates born vaginally is influenced by a number of factors. We have found that in addition to the factors reported in the literature (gestational age, maternal physiology and pathology, neonatal pathology, race and sex), the course of labour and/or medication during labour are related to changes in both percentages and absolute counts of T and NK lymphocytes in cord blood. Induction/stimulation of labour with prostaglandin E2 and/or oxytocin is associated with decreasing proportions of T lymphocytes and increasing proportions of NK lymphocytes.

It is envisaged that distribution of major lymphocyte subsets in cord blood may be used as a biomarker for effects on the immune system of exposure to environmental factors. For instance, the results of phenotyping cord blood lymphocytes of 65 Gypsy newborns suggest an association of changes in the ratio of T and NK lymphocytes with maternal smoking before or during pregnancy (Dostál *et al.* 1997). Our present results clearly show that the effects of labour and/or medication during vaginal delivery must also be

this parameter is applied as a potential biomarker of effects in epidemiological studies on developmental toxicity.

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