

The stress of birth enhances *in vitro* spontaneous and IL-8-induced neutrophil chemotaxis in the human newborn

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The birth process induces fetal stress. Stress has profound effects on the immune system, also by acting on the trafficking of leukocytes, a process in which adhesion and chemotaxis are primordial and critical events for the development of effective antimicrobial defenses. The newborn is rapidly challenged by a microflora at the epithelia linings and therefore depending on early, innate immunity onset. The objective of the study was to investigate the immune response in cord blood from newborns in relation to different degrees of fetal stress, with focus on neutrophil chemotaxis. We analyzed *in vitro* transmigration ability of neutrophils and their CD11b expression, measured total white blood count (WBC) and the major leukocyte populations, interleukin (IL)-8, interferon (IFN)- γ , and soluble E-Selectin, as well as relevant immuno-modulating hormones in infants born at term after Cesarean section prior to the start of labor ($n = 55$), normal vaginal delivery ($n = 87$), and assisted delivery ($n = 26$). Arterial pH and lactate were used as stress markers. We found that spontaneous and IL-8-induced transmigration ability of neutrophils from newborns after normal delivery was significantly higher compared with that of neutrophils from Cesarean section or from adults. With a progressive increase in fetal stress, there were significant elevations in total WBC, in particular neutrophils and monocytes, as well as an enhanced IL-8 and soluble E-Selectin level. Assisted delivery, associated with the highest degree of fetal stress in addition had an enhanced lymphocyte and monocytes count as well as an increased IFN- γ level. There were significant direct correlations between neutrophils and monocytes, respectively, with cortisol, β -endorphin, and prolactin. Interferon- γ was directly related to dopamine, as well as to the lymphocyte and monocyte count. The setting of the HPA-axis at birth is a promoter of an alarm response and a surge of neuroendocrine immuno-modulating factors that enhances antimicrobial defenses of the newborn. We speculate that IL-8 induced by normal labor may be a priming factor for an increased neutrophil chemotaxis through the pre-activated endothelium of the fetus. Assisted delivery may trigger excessive recruitment of additional inflammatory cells and IFN- γ release.

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The birth process and postnatal adaptation are associated with leukocyte recruitment and activation (1–7), an acute phase response (8) and

inflammatory skin reactions in the human newborn (9), all together indicating an enhanced innate immunity at that time-point in life. Birth is

also accompanied by a huge surge of stress hormones (10, 11). Stress challenge has profound modulating effects on the immune system by regulating the distribution of leukocytes between different compartments of the body, a complex process, depending on the interactions between immune and endothelial cells (12, 13 reviews). Leukocyte adhesion and chemotaxis are early steps in the unspecific innate immune response. The adhesion processes involve the sequential engagement of a number of different adhesion molecules, including E-Selectin (13). E-Selectin is produced exclusively by endothelium after cytokine stimulation and is known to reduce the rolling velocity of neutrophils *in vitro* (14). The soluble form of E-Selectin originates from membrane-bound molecules due to shedding or proteolytic cleavage (15, review). High soluble E-Selectin levels are found in many chronic inflammatory conditions (15), including atopic dermatitis in children (16). Soluble E-Selectin may have proinflammatory property by chemoattraction (17) and by activating neutrophils (18). It prologs platelet activating factor (PAF)-induced neutrophil motility (19) and permits communication between PAF receptors and transient receptor potential channel (TRPC) channels in human neutrophils (20).

Neutrophils and monocytes/macrophages are crucial effector cells in innate immunity because of their ability to kill microorganism. IL-8 is a specific chemokine for the recruitment of neutrophils released by a variety of cells, including immune cells and vascular endothelial cells. Chemotactic factor-dependent neutrophil migration largely depends on CD11B/CD18 leukocyte surface molecules (13). Monocyte/macrophage, beside natural killer (NK) and T cells, can produce interferon (IFN)- γ after microbial challenge, a cytokine that regulates a variety of immunologic programs, including being the major Th1 directing cytokine (21, 22).

Our hypothesis in this study was that the process of birth, associated with stress and major physiologic changes, including the establishment of a commensal microflora, could have an impact on the innate immune response of the newborn in terms of leukocyte recruitment and chemotaxis. We particularly focused on neutrophil function as these cells act as primary responders to challenges. Therefore, we investigated the inflammatory immune response in newborns after different modes of delivery and different degrees of fetal stress, in relation to circulating levels of relevant immuno-regulatory hormones such as cortisol, prolactin (PRL), β -endorphin, and catecholamines. We also analyzed spontaneous and

IL-8-induced chemotaxis as well as CD11b expression of neutrophils obtained after different delivery modes, measured circulating IL-8, IFN- γ , and soluble E-Selectin levels as well as the major leukocyte populations.

Methods

Study populations

The ethics committee of Karolinska University Hospital approved the study and parents gave their informed consent to participate. All pregnancies were uncomplicated and all deliveries were at term gestation. Mothers were non-smoking. A total number of 168 infants were studied after three different types of deliveries: Cesarean section (C-section) due to previous section, cephalo-pelvic disproportion, or humanitarian reasons in spinal anesthesia and prior to the start of labor ($n = 55$), normal delivery ($n = 87$), and surgical/assisted delivery ($n = 26$). The last group included infants delivered with vacuum extraction ($n = 17$) and acute C-section ($n = 9$), due to imminent risk for fetal asphyxia. All infants, 72 boys and 96 girls, had an adequate birth weight for gestational age and a further uncomplicated neonatal period. Peripheral blood obtained from seven healthy adults was also included. Data of the study populations are shown in Table 1.

Sample collection and preparation. Blood samples were collected at delivery by double clamping the umbilical cord at the placental end and the infant end within 5 s after birth. Umbilical arterial blood samples were collected for the determination of: pH and lactate with a standard analyzer (AVL-OMN19, Gratz, Austria), a β -endorphin, and catecholamines. Venous blood was collected in tubes containing 10 IE heparin (Kabi AB, Stockholm, Sweden) and 500 E aprotinin (Bayer AB, Stockholm, Sweden)/ml whole blood. Plasma was obtained by centrifugation (2400 *g*) at 4°C for 10 min. For serum preparation, blood was collected in sterile tubes

Table 1. Anthropometrical and clinical data of the study populations

	Cesarean section ($n = 55$)	Normal delivery ($n = 87$)	Instrumental delivery ($n = 26$)
Maternal age (yr)	32 $\pm$ 5	30 $\pm$ 5	31 $\pm$ 5
Primi/multipara	21/34	42/45	18/8
Gestational time (wk)	38 $\pm$ 1	39 $\pm$ 1	40 $\pm$ 1
Infant's sex boy/girl	23/32	36/51	16/10
Infant's birth weight (g)	3459 $\pm$ 425	3587 $\pm$ 419	3440 $\pm$ 377

Values are expressed as mean $\pm$ s.d.

without additives, allowed to clot for 30 min and centrifuged (10 min, 2400 *g*).

Analysis of biomarkers. Levels of IL-8 were measured in plasma by IL-8 US Kit (BioSource International, Camarillo, CA, USA), sensitivity 0.7 pg/ml, and E-Selectin by ELISA Test Kit (R&D Systems, Minneapolis, MN, USA), sensitivity 0.1 ng/ml, according to the instructions of the manufacturers. Levels of IFN- γ were measured by ELISA (R&D Systems), sensitivity 8.0 pg/ml, and values below the lowest kit calibrator were interpolated by including a zero calibrator using a spline function, thus obtaining a calibration curve between 0 and 500 pg/ml. Cortisol and PRL were measured in serum by time-resolved immunofluorometric assays using AutoDelfia (Perkin Elmer, Turku, Finland).

β -Endorphin determination

Cord blood samples were collected in pre-chilled Vacutainer tubes (Becton Dickinson, Meylan, France), containing 0.47 M potassium EDTA, supplemented with an enzyme 'inhibitory cocktail' consisting of aprotinin (500 KIU) and *N*-ethyl maleimide (30 μ g) per ml of whole blood. Blood samples were mixed carefully and placed on ice. After centrifugation (3000 *g*, 10 min at 4°C) the plasma was acidified by adding 5 M HCl (40 μ l/ml plasma). Samples were stored in -70°C until extraction. The peptide was extracted on SEP-Pak Vac RC C-18 cartridges (Millipore, Milford, MA, USA). About 4 ml of the acidified plasma were applied on the cartridge. After washing with 10 ml 0.1% trifluoroacetic acid and then eluted with 4 ml 70% methanol/0.1% trifluoroacetic acid. The eluates were dried in a Speedvac concentrator (Labrecycler, Inc., Gaithersburg, MD, USA). Evaporated plasma samples were reconstituted in ice-cold methanol/0.1 M HCl (1:1) for the peptide assay. The β -endorphin peptide was labeled with ^{125} I using chloramine-T and purified with reverse-phase HPLC using a gradient of 15–40% acetonitrile in 0.04% trifluoroacetic acid. Plasma samples were diluted 1:3 and incubated with antiserum for 24 h at 4°C before adding the labeled peptide solution. A double antibody technique was applied for the separation of bound and free antigen as previously described (23). The detection limit was 1.5 fmol.

Catecholamines' determinations

Catecholamines were analyzed in EDTA-plasma after acidification with perchloric acid to a final

concentration of 0.2 M and the addition of dihydroxy-benzylamine as internal standard in each sample. The samples were injected onto the Reprosil Pur C ^{18} reversed phase column (150 $\times$ 4 mm; 3 μ m) for separation. The mobile phase consisted of 0.055 M sodium acetate, 0.5 mM octansulfonic acid, 0.01 mM Na $_2$ EDTA, and 10% methanol (pH was adjusted to 4.1 with acetic acid) at a constant flow rate of 0.7 ml/min with a HPLC pump (580, ESA). First, the samples were passed through a guard cell (5021, ESA) with an applied oxidizing potential of 150 mV. The electrochemical detection was thereafter accomplished using a coulometric detector (Colochem II 5200A, ESA) connected with an analytical cell (5011, ESA) that analyzed the samples (coulometric electrode: 400 mV). The sample concentrations were determined by comparison with the standard peaks from injection of known concentration of noradrenaline/norepinephrine, adrenalin, and dopamine, and thereafter corrected for the recovery, comparison between the internal standard in the sample and the 100% internal standard injected. Total white blood count (WBC) and differential count was determined by routine automated laboratory analysis.

Adhesion molecule (CD11b) and transmigration kinetics

Preparation of blood leukocytes. About 10 ml blood was collected from the umbilical cord and from healthy, non-allergic blood donors (*n* = 4), aged between 26 and 40 yr, in citrate tubes (Vacutainer, 5 ml, with 500 μ l of 0.129 M Citrate-Na (Becton-Dickinson, Plymouth, UK).

Erythrocytes were specifically hemolyzed by adding 3 ml of NH $_4$ Cl-EDTA [154 mM NH $_4$ Cl, 10 mM KHCO $_3$, 0.1 mM EDTA (pH 7.2)] to 150 μ l of blood. The suspensions were incubated for 5 min at 15°C and centrifuged at 300 *g* for 5 min at 4°C. The leukocyte preparations were washed in 2 ml of 4°C 0.15 M PBS (pH 7.4). The leukocyte pellets were kept on ice until further treatments.

In vitro leukocyte activation. Leukocyte preparations from blood samples after normal delivery (*n* = 8), after C-section (*n* = 5) and adults (*n* = 4) were resuspended in HEPES-buffered RPMI 1640 medium (GIBCO, Paisley, UK), in the presence of the following stimuli: recombinant human IL-8 (rh; Becton-Dickinson), three different IL-8 concentrations (10, 100, and 1000 ng/ml) and two time-points (15, and 30 min) were used for *in vitro* stimulation to observe their effect on CD11b upregulation as previously described (24), in buffer alone at 4 and 37°C

were run in parallel. The leukocytes were subsequently washed with 2 ml of PBS (300 g, 5 min) and kept on ice until immunostained.

Immunostaining and flow cytometry. Leukocytes were incubated with phycoerythrin-conjugated mAb directed against CD11b (DAKO A/S, Glostrup, Denmark) at a final concentration of 50 $\mu\text{g}/\text{ml}$ for 30 min on ice and thereafter were washed once in 3 ml of 4°C PBS-EDTA (300 g, 6 min). The final preparations of mixed leukocytes were analyzed and counted in an EPICS XL (Beckman Coulter, Inc., Hialeah, FL, USA) flow cytometer (Fig. 1).

Transmigration assay. Blood samples after normal delivery ($n = 6$), C-section ($n = 3$) and from adults ($n = 3$) were analyzed. The transmigration assay was performed using 24-well microchemotaxis chamber with a polyethylene terephthalate-treated membrane (BD Falcon™, San Jose, CA, USA) which has a high pore density membrane (2.0×10^6 pores/ cm^2 , 3.0 μm in diameter) for maximum permeability. Insert filters were coated with collagen IV. Briefly, for IL-8-induced neutrophil transmigration, Ficoll-separated (Pharmacia & Upjohn, Uppsala, Sweden) granulocytes were resuspended in RPMI alone and added to the upper chamber. Buffer supplemented with IL-8 (100 ng/ml; BD) was added to the lower chamber. Cells that transmigrated into the lower chamber ('transmigrated cells') were, together with the original cell suspension ('total cells'), subjected to the further analysis. A ratio between

transmigrated cells and total cells was calculated for each experimental setting and expressed as percentage of transmigrated cells ('transmigration index'), as previously reported (25). The transmigration assay was run for 180 min, and the transmigration index was calculated at stated time-points. Cell viability, assessed by annexin/pi staining, was $>95\%$ and cell recovery was $>95\%$.

Statistics

Because the values of most of the studied metabolic endocrine variables followed a non-Gaussian distribution, these data are given as median (range) and we used the Kruskal–Wallis rank test, followed by the Mann–Whitney *U*-test to evaluate possible differences between groups. Simple regression analysis was performed to evaluate the relationship between variables by group. Data on cell migration kinetics and CD11b expression are given as median and interquartile range. We used analysis of variance followed by Mann–Whitney *U*-test to establish differences between groups. Interactions within groups were calculated with the Tukey test.

Results

Biomarkers and WBC

A progressive increase in fetal stress occurred in normal and assisted delivery, respectively, compared with C-section, as reflected by significant changes in umbilical arterial pH and lactate. Total WBC increased significantly after normal and furthermore after assisted delivery. Labor-induced leukocytosis was due to an increase in neutrophils concomitant with an elevation of total monocytes, but also of lymphocyte after assisted delivery. IL-8 and E-Selectin increased stepwise and significantly with progressive fetal stress. Data are summarized in Table 2.

Relationships between WB cells and biomarkers

There was a positive relationship of total WBC with (i) cortisol: $r = 0.55$, $n = 133$, $p < 0.0001$, (ii) PRL: $r = 0.43$, $n = 52$, $p < 0.001$, and (iii) endorphin: $r = 0.37$, $n = 133$, $p < 0.001$.

There was a positive relationship between neutrophil count and (i) cortisol: $r = 0.57$, $n = 130$, $p < 0.001$, (ii) log β -endorphin: $r = 0.41$, $p < 0.01$, $n = 52$, and (iii) lactate: $r = 0.53$, $p < 0.0001$, $n = 88$, and a negative correlation

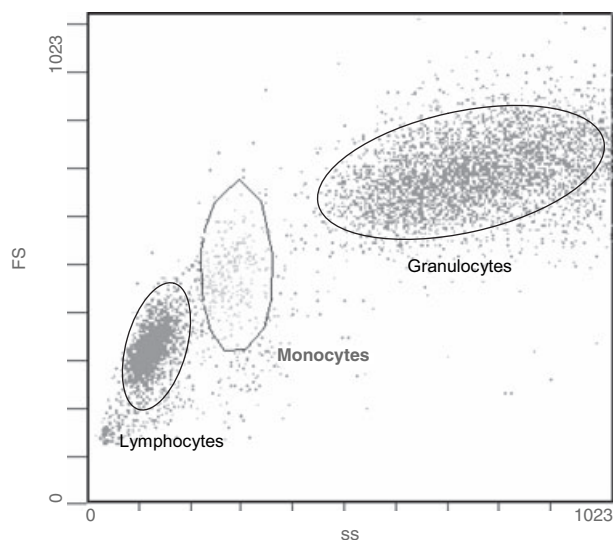


Fig. 1. A representative scatter histogram illustrating peripheral leukocyte subpopulations isolated from cord blood after normal delivery by flow cytometer.

Table 2. Biomarkers, total WBC and major leukocyte populations in cord blood from newborns born at term gestation after C-section, normal and assisted delivery

	C-section (n = 47)	Normal delivery (n = 73)	Surgical/assisted delivery (n = 26)
pH	7.31 (7.23–7.40)	7.29 (7.17–7.39)*	7.19 (7.02–7.34)**
Lactate (mm)	1.7 (1.3–3.9)	4.4 (1.8–7.0)**	8.1 (4.6–11.1)**
IL-8 (ng/l)	2.2 (0.7–4.1)	2.8 (0.7–20.7)***	3.8 (1.7–34)*
IFN- γ (pg/ml)	0	0 (0–17.7)	0.6 (0–16.7)****
E-Selectin (μ g/l)	43.1 (25–85.3)	52.6 (29.2–92.2)***	70.7 (27.8–127.7)*
Cortisol (nm)	155 (95–312)	477 (241–964)****	538 (340–901)
Prolactin (μ g/l)	177 (96–413)	187 (89–648)	247 (106–848)***
β -Endorphin (pm)	87 (5–152)	125 (33–314)	124 (41–566)
Norepinephrine (pmol/ml)	nd	26.5 (4.2–449.8)	48.5 (29.2–166.7)***
Epinephrine (pmol/ml)	nd	3.2 (0.5–38.7)	7.6 (0.8–29.9)***
Dopamine (pmol/ml)	nd	1.4 (1–1.1)	1.8 (3.3–2.5)***
Total WBC (10^9 /l)	11.5 (5.8–17.9)	14.7 (8.2–32.2)****	20.4 (14.1–32.3)****
Neutrophils	5.7 (1.9–11.3)	8.3 (3.1–19.1)****	11.7 (3.7–21.7)**
Basophils	0.1 (0–0.6)	0.1 (0–1.3)	0.1 (0–0.8)
Eosinophils	0.4 (0–0.1)	0.4 (0–1.3)	0.5 (0.3–2.5)
Lymphocytes	4.5 (4.0–7.4)	4.4 (2.2–11.3)	7.2 (4.7–11.8)****
Monocytes	0.8 (0.4–2)	1.1 (0.1–3.9)**	2.6 (0.4–2.6)***

C-section, Cesarean-section; nd, not determined.

We compared C-section with normal delivery, and normal delivery with assisted delivery (Mann–Whitney *U*-test). Values are median (range). The number of infants for each variable analyzed was 17–47 for C-Section, 18–73 for normal delivery, and 9–26 for assisted delivery.

* $p < 0.01$, ** $p < 0.001$, *** $p < 0.05$, **** $p < 0.0001$.

between neutrophil count and arterial pH: $r = -0.31$, $p < 0.001$, $n = 98$. There was a positive correlation between monocyte count and cortisol: $r = 0.42$, $p < 0.0001$, $n = 130$.

E-Selectin was directly related to lactate: $r = 0.27$, $p < 0.007$, $n = 27$ and indirect to arterial pH: $r = -0.37$, $p < 0.0001$, $n = 93$. There were positive correlations between IFN- γ and (i) dopamine: $r = 0.50$, $p < 0.001$, $n = 37$, (ii) monocytes: $r = 0.29$, $p < 0.001$, $n = 133$, and (iii) lymphocytes: $r = 0.28$, $p < 0.001$, $n = 128$.

Expression of cell surface adhesion molecule CD11b on neutrophils in neonates and adults

The expression of CD11b on neutrophils obtained from cord blood after normal delivery and C-section and from adults incubated with IL-8 at 10 ng, was higher than on neutrophils incubated with RPMI alone, both at 15 and 30 min ($p < 0.001$). The expression of CD11b, after incubation with IL-8 (10 ng for 15 min and 100 ng for 30 min), on neutrophils from adults was higher, compared with that on corresponding cells from both normal delivery and C-section (both $p < 0.001$), see Fig. 2 for details.

In vitro transmigration kinetics of neutrophils from neonates and adults with and without IL-8

Spontaneous transmigration of neutrophils obtained after normal delivery, without IL-8, was significantly higher at 120 min, compared

with that in neutrophils from C-section and from adults (for both, $p < 0.05$). A gradual increase in neutrophil transmigration with IL-8 stimuli occurred reaching a plateau at 120 min, both in neutrophils from normal delivery ($p < 0.0001$) and in neutrophils from adults ($p < 0.001$). The IL-8-induced transmigration of neutrophils from normal delivery was significantly higher at 120 min compared with C-section ($p < 0.001$) and adults ($p < 0.05$). For details, see Fig. 3.

Discussion

The major findings in this study are that neutrophils obtained after normal delivery had a higher transmigration ability compared with neutrophils obtained after C-section or from adults, both spontaneously and after IL-8 induction, which reflects cell activation during labor. Soluble E-Selectin increased after normal delivery, indicating that also fetal vascular endothelial activation occurs during labor. Furthermore, the normal birth process induced an increased circulating IL-8 level as well as an increased number of neutrophils and monocytes. Lymphocytes and IFN- γ increased only after the more stressful, assisted delivery. The CD11b expression of neutrophils from newborns was not affected by the delivery stress and was lower compared with adults, as previously reported (26). The increased transmigration ability was not associated with a concomitant increase in CD11b expression, a finding in line with results from human adults (27).

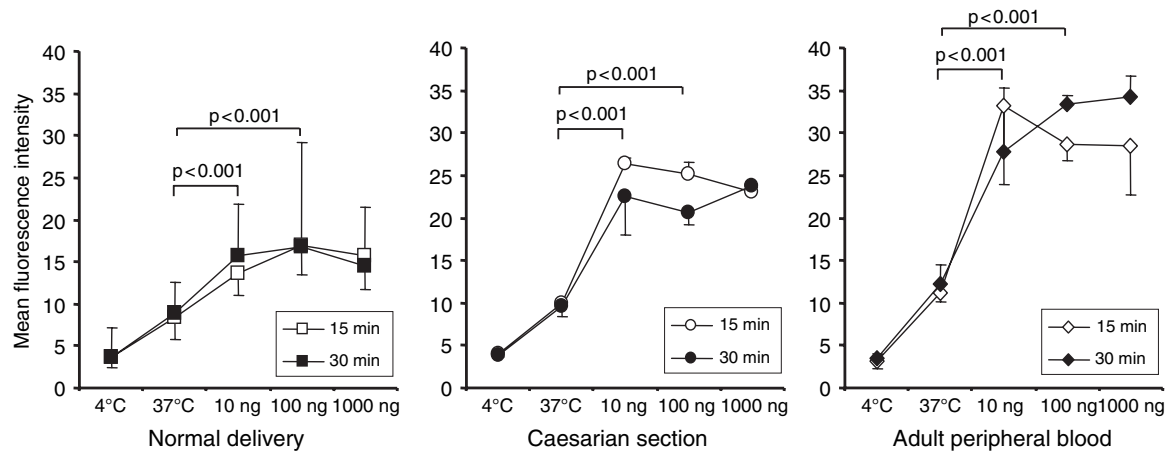


Fig. 2. Expression of CD11b on neutrophils obtained from cord blood after normal delivery ($n = 8$) and Cesarean-section ($n = 5$) and from adults ($n = 4$). The neutrophils are treated at $+4^{\circ}\text{C}$ with RPMI alone, and at $+37^{\circ}\text{C}$ with interleukin (IL)-8 at three different concentrations: 10, 100, and 1000 ng/ml for 15 and 30 min. Results, expressed as MFI units, are presented as median and interquartile range. The expression of CD11b on neutrophils from normal delivery and caesarean section and from adults incubated with IL-8 was higher than on corresponding cells incubated with RPMI alone, at both 15 and 30 min (for all, $p < 0.001$). The CD11b expression on neutrophils from adults after 15 min incubation with 10 ng IL-8 (*) was higher compared to that of neutrophils from normal delivery and C-section (for both $p < 0.001$). The CD11b expression on neutrophils from adults after 30 min incubation with 100 ng IL-8 at 37°C (‡) was higher than that on neutrophils from normal delivery and C-section (for both $p < 0.001$).

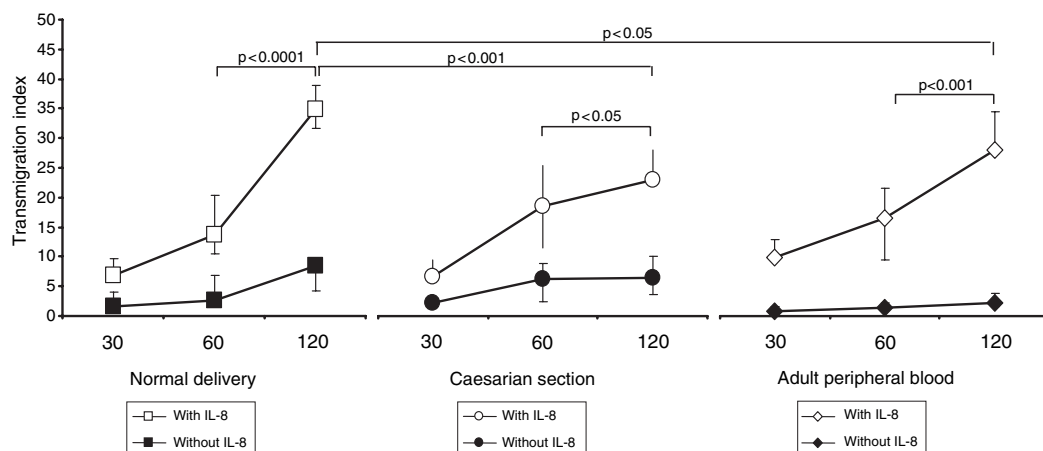


Fig. 3. The effect of interleukin (IL)-8 on *in vitro* transmigration of neutrophils obtained from cord blood after normal delivery ($n = 6$) and Cesarean-section (C-section; $n = 3$) and from adults ($n = 3$). Results expressed as transmigration index are presented as median and interquartile range. The spontaneous transmigration of neutrophils from normal delivery without IL-8 (*) was higher at 120 min compared to that of neutrophils from C-section and from adults at corresponding times ($p < 0.05$, for both). There was a significant increase at 120 min of IL-8-induced transmigration of neutrophils from normal delivery ($p < 0.0001$) which was higher compared to that of neutrophils from C-section (‡, $p < 0.001$) and neutrophils from adults (‡, $p < 0.05$).

These data show the early capacity of the newborn to mount a powerful, inflammatory immune response during the process of normal labor. Why should newborns be prone to inflammatory reactions? The redistribution and activation of immune cell under the stress of labor may be an adaptive response designed to enhance the vigilance and capacity of the immune system respond to challenges at the major defense

barriers of the body, skin, and mucosal linings. In this way, the immune system is alerted of the oncoming commensal microflora and potential pathogens. The interaction between microbes and host at the epithelial linings is an active process where the early, innate immune response plays a pivotal role for survival but also constitutes a factor for the maturation of the immune system of the newborn (28). It is also possible

that neutrophils at birth are primed to other regulatory immunologic effects, besides being antimicrobial, as indicated by a change in their transcriptional program upon transmigration into the skin (29). In this context, the labor-induced fetal vascular endothelial activation combined with enhanced transmigration ability of neutrophils, critical effector cells in mucosal-epithelial defense, makes sense.

Our data provide evidence that IL-8 released at low doses during labor may be a physiologic mechanism of neutrophil priming leading to enhanced chemotaxis. The increased IL-8 level observed after birth may reflect a spillover from activated vascular endothelial cells and/or circulating immune cells and implicates neutrophil activation and inflammation. IL-8 is produced both by cells of the immune system (monocytes, T cells, neutrophils, NK cells) and non-immune cells (endothelial cells, fibroblast, adipocytes, epithelial, and adrenal cells). IL-8- expression is regulated at the transcriptional level by a coordinated stimulation of NF- κ B (30, 31). The main biologic activity of IL-8 is as a neutrophil chemotactic factor, but it also affects neutrophil degranulation, respiratory burst and promotion of adherence to endothelial cells (32).

It is known that E-Selectin expression is restricted to endothelial cells. Thus, increased soluble E-Selectin at birth reflects immunoactivation of the endothelium, directly related to the degree of fetal stress as indicated by the significant correlation with the stress markers, arterial pH, and lactate. Increased expression of E-Selectin has been demonstrated in vessels of the skin in healthy, 1-day-old infants (6).

The neuro-endocrine and the immune system interact reciprocally in controlling homeostasis of the organism (12). Redistribution of immunocytes, particularly neutrophils, between different compartments of the immune system is of great importance in maintaining effective cell-mediated immune responses during stress (33, 34). Elevation in circulating cortisol is the clearest marker of HPA-axis activation in humans, a phenomenon also occurring at birth (11). Adrenal steroids increase de-margination of neutrophils, prolong neutrophil half-life in circulation (35–37) and either reduce or has no effect on CD11b expression (38–40).

Prolactin is a peptide/cytokine secreted by the anterior pituitary gland with multiple functions, including effect on the immune system. It has been suggested that effects of PRL on immune function *in vivo* may be more apparent when the organism is subjected to stress challenge (41). PRL attenuates hydrocortisone-induced CD11b expression

on human CD8+ leukocyte (42), an effect that may be relevant in connection with birth and fetal stress by reducing the risk of pathological tissue inflammation. In fact, PRL release was only observed in babies after assisted delivery.

The endogenous opioid, β -endorphin released from the anterior hypophysis, increases neutrophil oxidative burst activity and CD11b expression *in vitro* (43). We found that the mode of delivery did not significantly affect the fetal β -endorphin level. However, total WBC and particularly the neutrophil count, was directed related to the peptide level, arguing for a role of endomorphins in mediating HPA-axis induced immune activity in newborns.

Dopamine, the major adrenomedullary hormone, affects trafficking of lymphocytes by inducing migration and homing of naive CD8+ T cells both in humans and mice (44). Dopamine was significantly related to the level of IFN- γ , indicating its potential role in the activation of lymphocytes at birth.

Interferon- γ increased only in babies delivered by assisted methods and was related to the dopamine level. It may derive from activated monocytes and/or NK and T cells as reflected by an elevation of both total monocyte and lymphocyte count in these infants and by the positive correlations between these cell types and the cytokine. The pathophysiologic meaning of this finding is still to be determined. But, it is possible that higher levels of stress present after some assisted deliveries may be harmful as increased IFN- γ has been reported in newborns with brain damage (45).

In conclusion, this study assembles a picture of the newborn infant's capability to mount a powerful inflammatory immune response with increased trafficking of neutrophils and monocytes, including vascular endothelial activation. We speculate that, IL-8 induced during the birth process, combined with a complex neuro-endocrine immunomodulatory network, primes neutrophil's functional responsiveness, resulting in an enhanced chemotaxis.

Acknowledgments

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