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No direct effect of an elective caesarean section on the phenotypic and functional characteristics of maternal peripheral blood T lymphocytes

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ABSTRACT

Problem: The short term effect of the caesarean delivery on the phenotypic and functional characteristics of the peripheral blood leukocytes of the mother is unknown.
Method of study: We determined the composition and activation status of the maternal peripheral blood leukocytes isolated within 4 h before and within 24 h after elective caesarean delivery with neuraxial anaesthesia. Furthermore, we determined the proliferative and cytotoxic response of these leukocytes to several stimulators.
Results: No significant differences in the percentage of CD4+CD25bright and CD8+CD28− T cells or the expression of activation markers FoxP3, CD69 and HLA-DR were observed in peripheral blood drawn before caesarean delivery compared to after caesarean delivery. Also the alloreactive immune responses in samples taken before and after the caesarean delivery were similar.
Conclusion: Our results show that the phenotype and immune response of maternal peripheral blood T cells obtained before elective caesarean delivery are not different from those obtained after caesarean delivery. This knowledge will facilitate sample collection for future studies on the immune response in term pregnancies.

1. Introduction

Pregnancy is considered an immunological paradox [1]. The maternal immune system has to accept the semi-allogeneic fetus without abolishing the immune response towards harmful pathogens. Both maternal and fetal mechanisms of dampening the immune response to the allogeneic fetus may lead to down regulation of fetus specific effector cells resulting in successful pregnancy [2,3]. Many studies have focused on the regulation of the maternal immune system, investigating both the decidual tissues and the maternal peripheral blood. In order to compare the local immune response with the immune response in the periphery and due to logistic convenience, blood samples are mostly collected around elective caesarean section delivery. The time point of sampling around the caesarean section delivery might be of importance as several studies have shown that surgery induces suppression of the immune response.

In gynaecologic surgeries, the levels of IL-6 were shown to be elevated in women undergoing abdominal hysterectomy compared to women undergoing vaginal or laparoscopically assisted hysterectomy [4]. The effect of other types of surgery on the immune response has been extensively studied. Lymphocyte cell numbers were shown to decrease following surgery and returning to normal levels at day 7 in 35 patients with benign disease [5]. Major surgery resulted in a defective T cell response to T cell receptor and CD28 co-receptor mediated signals [6]. In addition, Cruickshank et al. [7] reported increasing levels of IL-6 within 2–4 h of incision correlating with the duration of the surgery and the extent of surgical trauma. In children undergoing elective minor surgery the neutrophil and monocyte phagocytosis and oxidative bust activity was increased at the end of surgery com-

Abbreviations: APC, Allophycocyanin; CML, cell mediated lympholysis; cpm, counts per minute; CTL, cytotoxic T lymphocyte; E:T ratio, effector:target ratio; FITC, fluorescein isothiocyanate; HLA, human leukocyte antigen; IFN, interferon; IL, interleukin; MLC, mixed lymphocyte culture; (3p) PBL, (third party) peripheral blood leukocytes; PCR, polymerase chain reaction; PE, Phycoerythrin; PerCP, Peridinin chlorophyll protein; PHA, Phytohaemagglutinin; (3p) UCB, (third party) umbilical cord blood.

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pared to before surgery [8]. Mollitt et al. [9] found increased numbers of neutrophils and decreased numbers of lymphocytes in children after elective surgery with no evidence of functional depression, suggesting an appropriate mobilization of the immune cells in response to the surgery. In contrast, a study with adult patients undergoing low to intermediate risk surgery showed reduced HLA-DR expression by monocytes at 24 h following the surgery [10]. The discrepancies between these studies can be due to technical differences, type of surgery, underlying disease and baseline immunologic status [11].

The mode of delivery may also affect the maternal immune response. Bili et al. [12] previously showed a minimal effect of delivery type on the phenotype of peripheral lymphocytes. In contrast, others showed increased cytokines after vaginal delivery compared to elective caesarean delivery [13–15]. Watanabe et al. [16] showed that the numbers of CD3+, CD4+ and CD8+ T cells were decreased during uncomplicated pregnancy compared to one month after pregnancy. Furthermore, Somerset et al. [17] found lower percentages of CD4+CD25+ Tregs in peripheral blood 6–8 weeks after delivery compared to second trimester pregnancy and Kühnert et al. [18] reported a decreased HLA-DR expression within one week after delivery compared to late pregnancy.

When investigating the maternal immune response around delivery the pre-set time point of sampling might not be met due to logistic difficulties. Even in case of elective caesarean deliveries we experienced that some samples were taken before and other after the delivery. Future studies would benefit from a certain time frame in which blood samples can be drawn without it affecting the maternal immune response. Therefore, the aim of this study was to compare the immune response before and after elective caesarean delivery. We characterized the phenotype of the maternal peripheral blood leukocytes isolated before and after caesarean delivery. Furthermore, we determined the proliferative and cytotoxic response of these leukocytes to several stimulators in order to test whether the moment of sampling affects the outcome of phenotypic and functional characterization of maternal leukocytes.

2. Materials and methods

2.1. Study subjects

This study included 22 women who delivered by elective caesarean section with neuraxial anaesthesia and with uneventful postoperative hospital course. Clinical data were summarized from the medical records and are shown in Table 1. Peripheral blood samples were collected within 4 h before caesarean delivery and within 24 h after caesarean delivery. The mean time of sampling after caesarean delivery was six hours. Umbilical cord blood (UCB) samples were collected directly after caesarean delivery. Leukocytes were isolated from these heparinised blood samples by Ficoll-hypaque (LUMC pharmacy, Leiden, The Netherlands) density gradient centrifugation. The cells were washed twice with 0,9% NaCl solution and frozen in RPMI 1640 (Gibco, Paisley, Schotland) with 25% Fetal Calf Serum (Greiner) and 10% DMSO (dimethylsulfoxide, Fluka Chemica, Buchs, Switzerland). The cells were frozen at −80 °C and stored in liquid nitrogen until phenotypical and functional analysis. HLA typing of the mother and the new born child was performed at the typing laboratory of the Leiden University Medical Center using sequence specific oligonucleotides (SSO) PCR. The presence of HLA specific antibodies was determined in plasma by Luminex. Plasma samples with a Luminex score $\geqslant 2$ were considered to contain HLA specific antibodies.

The study protocol was approved by the ethics committee of the Leiden University Medical Center (LUMC), and informed consent of every patient was obtained.

Table 1
Pregnancy characteristics.

Pregnancy		
	Gestational age (weeks)	38 (36–40)
	Indication caesarean section	
	- Breach presentation	14 (64%)
	- Caesarean previous pregnancy	3 (14%)
	- Other	5 (22%)
	Anaesthesia	
	- Combined spinal-epidural	2 (9%)
	- Epidural	8 (36%)
	- Spinal	12 (55%)
Mother		
	Age (years)	33 (26–39)
	Highest diastolic pressure (mmHg)	77 (60–100)
	Gravidity	3 (1–5)
	Parity	1 (0–3)
	Spontaneous abortion	1 (0–4)
Child		
	Birth weight (g)	3222 (2485–3900)
	Gender (male)	8 (40%)
HLA		
	HLA matches	
	- Class I	3 (3–4)
	- Class II	2 (2–4)
	HLA mismatches	
	- Class I	2 (1–3)
	- Class II	1 (0–2)

2.2. Phenotypic characterization of maternal lymphocytes

The maternal lymphocytes were phenotyped using flow cytometric analysis. The analysis was performed with a standardized protocol and gating strategies previously described [19]. Shortly, the percentage of different lymphocyte populations within peripheral blood samples was calculated. A life-gate was set around viable leukocytes and CD45+CD14− cells were gated to analyse the various lymphocyte populations. The antibodies used include: CD4-FITC, CD14-FITC, CD69-FITC, HLA-DR-FITC, CD8-PE, CD25-PE, CD3-PerCP, CD4-APC, CD28-APC and CD45-APC. The expression of FoxP3 was determined in four samples using an APC anti-human FoxP3 staining Set (eBioscience, San Diego, CA). Analysis was performed on a Calibur flow cytometer using Cellquest Pro software (Becton Dickinson).

2.3. Mixed lymphocyte culture

Mixed lymphocyte cultures (MLC) were used to analyse the proliferative response of maternal peripheral leukocytes (responders) towards several stimulators. The stimulators used in this study were leukocytes isolated from UCB of the new born child, a third party UCB (3p UCB) and third party peripheral blood leukocytes (3p PBL). The third party stimulators were selected to have one or two HLA-DR mismatches with respect to the maternal HLA typing. The HLA-DR typing of the responders and stimulators are shown in Table 2. Responders (50,000 cells per well) were cultured together with 50,000 irradiated (3000 rad) stimulator cells in triplicate wells in round-bottom 96-well plates (Greiner Bio-one). The cells were cultured in RPMI 1640 with 2 mM L-glutamine (Gibco, Paisley, Scotland) and 10% pooled human serum in a total volume of 200 µl. The cultures were incubated for 6 days at 37 °C in a humidified atmosphere containing 5% CO_2. On day 5, 50 µl supernatant was taken from each well for cytokine analysis and ^{3}H-thymidine was added. ^{3}H-thymidine incorporation was measured on day 6 by liquid scintillation spectroscopy using a beta-plate counter (PerkinElmer, Waltham, MA). The stimulation index was calculated by dividing the median counts per minute