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Cellular Subsets of Maternal Microchimerism in Umbilical Cord Blood

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Abstract

Maternal microchimerism may arise in the offspring during pregnancy, and may be favorable or unfavorable. Additionally, maternal cells present in umbilical cord blood used for stem cell transplantation may affect the outcome after transplantation. The aim of this study was to evaluate the cellular subset and frequency of maternal cells in umbilical cord blood following vaginal deliveries and elective Cesarean sections where the umbilical cord clamping time was measured. A total of 44 healthy women with normal pregnancies were included in the study. Of these, 24 delivered vaginally and 20 by elective Cesarean sections. In the fresh umbilical cord blood, cellular subsets of CD3+ (T-cells), CD19+ (B-cells), CD33+ (myeloid cells), CD34+ (hematopoietic progenitor cells) and CD56+ (natural killer cells) cells were isolated and DNA extracted. A single-nucleotide polymorphism unique to the mother was identified and maternal microchimerism in the different cellular fractions was detected using quantitative real-time polymerase chain reaction with a sensitivity of 0.01%. Overall, 5 out of the 44 (11%) umbilical cord blood samples contained maternal microchimerism. The positive fractions were total DNA (whole blood, $n = 3$), CD34+ ($n = 1$), CD56+ ($n = 1$) and CD34+/CD56+ ($n = 1$). Overall, four of the five (80%) positive samples were from Cesarean sections and one was from a vaginal delivery. The conclusion from this study is that maternal microchimerism in umbilical cord blood is not a common phenomenon but includes both lymphoid and hematopoietic progenitor lineages.

Keywords

Maternal microchimerism, cell trafficking, umbilical cord blood collection, umbilical cord blood transplantation

Introduction

During pregnancy cellular trafficking over the placenta gives rise to naturally acquired microchimerism in the fetus and in the mother, fetal microchimerism (FMc) and maternal microchimerism (MMc), respectively[1]. The chimeric cells appear to persist for decades both in the healthy mother and her healthy offspring[2,3]. The significance of this exchange of a small number of semi-allogeneic cells is far from understood but has been suggested to have both adverse and beneficial effects[4–9].

In the cradle of umbilical cord blood (UCB) transplantation, concerns about 'contaminating' maternal cells were raised because of fear that the maternal cells might contribute to the development of graft versus host disease (GVHD)[10]. However, the incidence of GVHD was lower when using UCB compared with bone marrow or peripheral blood stem cells, although this has partly been attributed to the low immaturity of the UCB cells[11].

The maternal–fetal interface exhibits immunological events that include development of fetal tolerance to non-inherited maternal antigen (NIMA). The effect seems to be long-lasting since transplanted patients more often fail to react against NIMA compared with non-inherited paternal antigens and that graft survival in renal transplantations between siblings has better outcomes when the recipient's NIMA is present in the graft[12,13]. Mold et al. showed that fetal regulatory T-cells (T-regs) could suppress fetal T-cell reactivity against NIMA when maternal cells were present in low numbers. Some of the T-regs were suggested to develop into long-lived memory T-cells with the ability to suppress responses to NIMA past the fetal period[14].

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Table 1. Summary of Studies on Maternal Microchimerism in Umbilical Cord Blood.

No of samples	Frequency MMc	Method	Sensitivity	Reference
6	0/6 (0%)	PCR FISH	1/1000–10,000 1/1000	18
16	1/16 (6%)	FISH CLSM	1/10,000	19
47	1/47 (2%)	PCR	1/100	34
10	10/10 (100%)	PCR	1/100,000	35
49	7/49 (14%)	FISH	1/1000	20
38	16/38 (42%)	PCR	1/100,000	30
213	81/213 (38%)	PCR	1/100,000	15
79	1/79 (0.01%)	PCR	1/100	36
50	Plasma fract: 15/50 (30%) Cell fract: 12/50 (24%)	qRT-PCR	1/1000 1/10,000	1
152	23.4%*	Nested PRC	1/100–1000	29
51	Unsorted: 27/51 (52.9%) Cell subsets: 33.3–55.6%	qRT-PCR	1/20,000	21

CLSM: confocal laser scanning microscopy; FISH: fluorescent in-situ hybridization; MMc: maternal microchimerism; PCR: polymerase chain reaction; qRT-PCR: quantitative real-time PCR.
*Number of positive samples not presented.

Although the initial apprehension about what effect the semi-allogeneic maternal cells could have when transplanted together with the UCB, ideas about beneficial consequences arose, namely the graft versus leukemia (GVL) effect[15]. Recently Van Rood et al. reported that patients with acute myeloid leukemia had more rapid engraftment, less GVHD and lower relapse rates if the patient shared the same NIMA as the UCB donor[16]. Later, the same group showed indirect evidence that immunity against the inherited paternal antigens (IPAs) by co-transmitted maternal T-cells in UCB transplantations may contribute to a GVL effect[17].

Several studies on MMc in UCB have yielded different results depending on the method (Table 1). The frequency of MMc in UCB at term varies between 0–100% in different studies with a median value of 23%. Cellular subsets of MMc have been sparsely investigated[18–21].

With the recent proposal that MMc may be a part of the GVL effect in UCB transplantation, a better characterization of the maternal cellular traffic across the placenta is needed. Based on our previous findings of maternal CD3+, CD19+, CD45+ and CD34+ cells widely spread in second trimester tissues[22], our aim with the present study was to investigate distinctive cellular subsets of MMc in UCB. In addition, we wanted to investigate whether labor affects the frequency of maternal cells. In all cases the umbilical cord was clamped at a well-defined time that is in line with the clinical praxis in Sweden when UCB is collected for clinical banking.

Materials and Methods

UCB Samples

In Sweden parents giving birth at two hospitals can altruistically donate UCB to Sweden's national UCB bank. All collection procedures are standardized and are performed in as sterile a way as possible by dedicated midwives.

Collection at vaginal birth. After birth the umbilical cord is clamped after approximately 1 minute. The umbilical cord is washed at least three times with chlorhexidine, the umbilical cord vein is punctured and the UCB collected into a UCB collection bag with sodium citrate (MSC1206DU, Macopharma, Mouvax, France) by gravitation before the placenta is delivered.

Collection at elective Cesarean sections. The baby is delivered and the umbilical cord is clamped after approximately 30 seconds. Thereafter the process is as described for vaginal deliveries. The clamping time was recorded with a timer. Peripheral blood (in ethylenediaminetetraacetic acid) was collected from the women in conjunction to the birth.

Ethics. The study was approved by the Regional Ethics Committee in Stockholm, Sweden (approval number 2012/480-31/3), and performed in accordance with the Helsinki Declaration. Informed oral and written consent was obtained from all participants.

Table 2. Patient and Umbilical Cord Blood Characteristics.

	Whole group	Vaginal	CS
Age mothers (years)	30 (±4)	28 (±4)	31 (±4)
Gestational age (weeks+days)	39+1 (±1+1)	39+3 (±1+2)	38+6 (±1+0)
Weight baby (grams)	3394 (±302)	3427 (±324)	3355 (±276)
Clamping time (seconds)	52 (±25)	71 (±20)	30 (±4)
WCB (10^8/l)	78 (±19)	81 (±22)	75 (±16)
Total amount WCB (10^8)	6.7 (±1.7)	6.9 (±1.5)	6.7 (±1.8)

CS: Cesarean section (elective); WCB: white cell blood count.

Patient Characteristics

A total of 44 healthy women with normal full-term pregnancies (38+0 to 41+6 weeks+days) were recruited where the white cell blood count ($<10\times10^8$) was not sufficient for clinical banking of UCB. The inclusion and exclusion criteria of Sweden's national UCB bank were followed. All women gave birth on their back. The patient characteristics and laboratory data are summarized in Table 2.

Cell Separation

To evaluate lineage-specific chimerism in the UCB, separations of CD3+ (T-cells), CD19+ (B-cells), CD33+ (myeloid cells), CD34+ (hematopoietic progenitor cells) and CD56+ (natural killer (NK) cells) cells were made by means of immunomagnetic beads according to the manufacturer's instructions (Dynal Biotech, Oslo, Norway). The UCB was divided into six equal parts and immunomagnetic bead selection was performed for one marker per UCB volume. The purity of the positively-selected cell populations was tested by flow cytometry (not after each cell separation), and was found to be between 90–95% pure.

DNA was extracted from cell-separated UCB, whole UCB and maternal blood using an automatic MagNa pure machine (Roche, Basel, Switzerland). Genomic DNA concentrations were measured using NanoDrop (Thermo Scientific, Waltham, MA, USA).

Quantitative Real-Time Polymerase Chain Reaction

The methodology of chimerism analysis with quantitative real-time (qRT) polymerase chain reaction (PCR) has been described previously.[23] Initially, screening of the mother and the child was performed by using a small amount of DNA (10 ng) in a PCR assay. The allelic markers used were: S01a, S02, S03, S04a, S05b, S07a, S07b, S08b, ID1, ID2, ID4 and ID7. If no differences were found, the pair was screened for additional 10 markers (S01b, S04b, S06, S09b, S010a, S011b, ID9, ID10, ID11 and ID12). For each biallelic system, one of the primers was from the polymorphic region to specifically amplify each allele, whereas the second primer and the probe were common to both alleles. An allele was considered informative when it was positive for maternal DNA and negative for the child's DNA. Detection and quantification with two markers were performed with the 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA), using TaqMan technology. The mean value of both markers was used for final quantification. The amount of amplifiable DNA in each sample was assessed by parallel amplification of the reference gene glyceraldehyde phosphate dehydrogenase (GAPDH). All the samples were run in duplicate, and both maternal, UCB and control DNA were included in each run. Relative quantification of recipient DNA was calculated according to the $\Delta\Delta$Ct method (Applied Biosystems, user bulletin 2), using GAPDH as a reference gene and the maternal DNA sample as a calibrator. The amount of DNA used in the studies varied between 150–500 ng. The specificity and sensitivity of the qRT-PCR method was determined for all markers included in this study using artificial DNA mixtures and varying DNA amounts. A sensitivity of 1/10,000 (i.e. 0.01%), was reached and no false positive results were found using 40 cycles of PCR amplification, which was confirmed with spike-in control assays.

Statistics

Data were analyzed using the nonparametric Mann–Whitney U test (comparing medians between groups) or Chi-square test (comparing proportions between groups). We also used multivariate logistic regression for the modeling of the factors involved in the positive MMc findings. Statistical significance was set at $p < 0.05$. Statistical analysis was performed using statistical packed Statistica 12 (StatSoft, Tulsa, OK, USA).

Results

UCB Contains Maternal Cells from Different Lineages

A total of 5 out of 44 (11%) UCB samples were positive for maternal microchimerism (Table 3). Two samples were positive only in the total DNA fraction (cases 5 and 17), one in the CD34+ fraction (case 8), one in the CD56+ fraction (case 37), and one in the three fractions of total DNA, CD34+ and CD56+ (case 6). The estimated concentration of maternal DNA ranged from 0.01% to 1.5% (Table 3 and Figure 1). All five positive samples were from babies with healthy mothers of whom four were delivered by elective Cesarean section (80%) and one vaginally (20%). In the group of negative samples, 16 of 39 (41%) mothers were delivered by Cesarean section and 59% vaginally. The median (quartile range) clamping time for vaginal deliveries was 62 (60–84) seconds and for Cesarean sections 30 (30–30) seconds, which were significantly different ($p < 0.001$). The median (quartile range) clamping time in the positive group ($n = 5$) was 30 (30–35) seconds compared with 60 (30–69) seconds in the negative group ($n = 39$). This difference was

Table 3. Summary of Maternal Microchimerism-Positive Umbilical Cord Blood Samples.

Patient no	Mode of delivery	Gestational age (weeks+days)	Parity	Previous fetal loss	Clamping time (seconds)	Gender child	WCB (10^8/L)	% MMc in CD34	% MMc in CD56	% MMc in total DNA
5	CS	38+1	0	0	30	B	84	0	0	0.01
6	CS	38+6	1	1	30	B	100	1.5	0.3	0.2
8	CS	39+0	0	1	30	B	61	0.5	0	0
17	CS	38+4	2	1	35	B	45	0	0	0.1
37	vaginal	38+5	1	0	60	G	95	0	0.07	0

B: boy; CS: Cesarean section (elective); G: girl; MMc: maternal microchimerism; WBC: white cell blood count.

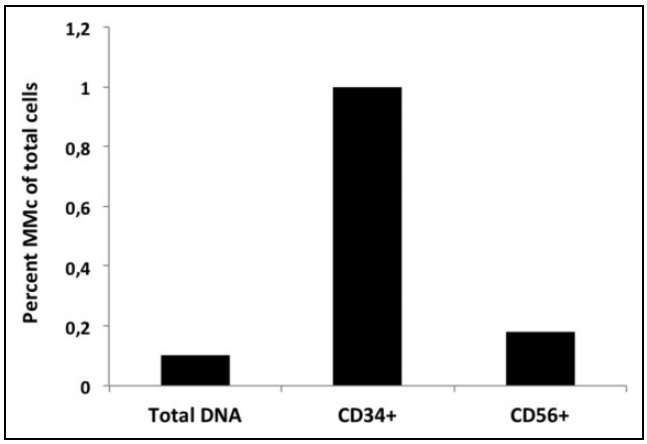

Fig 1. Percent MMc of total cells in the fractions of total, CD34+ and CD56+ cellular fractions in positive umbilical cord blood samples.
MMc: maternal microchimerism.

not significantly different. Overall, four of five positive samples were found in the Cesarean section group (4/20) in contrast with only one in the vaginal delivering group (1/24). However, this difference was not significant. Even if it is clear that most of the positive cases were found in the Cesarean section group, which also had significantly shorter clamping times, there was no statistically significant explanation for this finding. Thus, we used multivariate logistic regression on MMc in UCB as an outcome variable and the type of delivery and clamping time as explanatory variables. None of these variables could explain the positive finding of MMc.

Overall, four of the five (80%) mothers of the positive babies had been pregnant before (54% in the negative group). Of these, two of them (40%) had had a previous delivery (38% in the negative group), two (20%) had experienced at least one fetal loss (miscarriage or elective abortion) but no previous delivery (negative group 38%), and one (20%) had experienced at least one fetal loss and had given birth before (20% in the negative group). The mean white blood cell count per liter in the UCB samples of the positive group was 77×10^8 (SD$\pm$23) and in the negative group, 80×10^8 (SD$\pm$21), which was not significantly different.

Discussion

It is well known that maternal cells persist in the blood of healthy individuals through childhood and into middle age[24]. The present study shows an MMc frequency of 11% in UCB, which corresponds with the incidence in previous studies, although the range is very large (Table 1). However, the contributing cell lineages in MMc have not been thoroughly examined in UCB. So far, only four previous studies have investigated this, yielding varying results: MMc was identified in CD3+, CD8+, memory and naïve T-cells and in B-cells, NK cells, monocytes and hematopoietic progenitor cells with a frequency between 0 to 55.6%[18–21]. The most recent study used flow cytometry sorting of the cell lineages and a more sensitive PCR method than the previous studies, and they reported the highest number of positive cell lineages (memory and naïve T-cells, B-cells, NK cells, monocytes), and the highest level of MMc in the investigated cell lineages[21]. In the present study, MMc was detected in cellular subsets of CD34+ and CD56+ and in total DNA, but not in CD3+, CD19+ or CD33+ cell fractions. Only one sample exhibited MMc in all three fractions, whereas the other four showed MMc merely in one fraction. There were two samples that demonstrated positive MMc in either the CD34+ or CD56+ fraction, but not in the total DNA fraction. These may be false negative results and explained by the dilution effect; total DNA was isolated from a separate portion of whole blood whereas the cells were purified and concentrated when selecting them with magnetic beads. Comparably, the two samples displaying MMc only in the total DNA may not be true MMc, but merely maternal cell-free DNA present in the serum or plasma.

The GVL effect in hematopoietic stem cell transplantation is assumed to be exerted by the transplanted T-cells or NK cells[25,26]. The suggestion by Van Rood et al. that maternal T-cells with immunity against the fetal IPA may increase the GVL effect in recipients where the IPA was shared between the donor and recipient made us hypothesize that MMc in CD3+ cellular subsets would be common in UCB[17]. However, in the present study maternal CD3+ cells were not demonstrable in any sample. This is in contrast with three other studies that reported T-cells of maternal origin[19–21]. However, in two samples in the present study, CD56+ cells were detected that might exhibit similar GVL effect as T-cells after transplantation[26].

If maternal CD34+ cells in a given UCB graft have the same capacity of self-renewal, differentiation and engraftment in a recipient as the donor's CD34+ cells are not known. Hypothesizing that the maternal CD34+ in UCB share the same properties as other CD34+ cells, they would be capable of differentiation into all hematological cellular subsets. Indeed, hematopoietic progenitor cells, T-cells, B-cells, NK cells, monocytes/macrophages and granulocytes of maternal origin have been detected in the circulation of adults[27,28]. However, we cannot be certain that the origin of the maternal CD34+ cells is hematopoietic since CD34+ is also expressed on endothelial and mesenchymal lineages, although it would be unlikely.

There are two studies that have investigated MMc in larger populations (152 and 213 samples)[15,29]. The prevalence of MMc in the two studies were 23.5 and 38%, and no differences were seen between vaginal deliveries and Cesarean sections deliveries in contrast with the present study where the data indicate that the mode of delivery has an impact on MMc (positive samples = 80% elective Cesarean sections, negative samples = 41% elective Cesarean sections). However, since the number of positive samples was limited, it is difficult to be conclusive about any difference. In the study of Scaradavou et al., the duration of labor did not affect the incidence of MMc leading to the hypothesis that contractions during labor are not mechanistically involved in transfer of maternal cells into the fetus[15]. They also hypothesized that the passage of MMc occurs before term, which is in line with our present study with the highest MMc in elective Cesarean sections with no labor and with our previous findings of MMc in the cellular subsets of CD3+, CD19+, CD45+ and CD34+ in multiple tissues of second trimester fetuses[22].

In the present study four out of five UCB samples that were MMc positive were derived from boys. Since several previous studies on MMc in UCB used fluorescent in situ hybridization as a detection method (analyzing XX in male UCB), possible sex differences in harboring MMc have not been feasible to study. The three previous studies that enclosed samples from both sexes did not reveal any sex differences, suggesting that the male dominance of positive samples in the present study is a random finding[15,29,30].

Studies have shown that late umbilical cord clamping (after 3 minutes) may be beneficial for the child[31,32]. However, late clamping is not compatible with clinical collection of UCB due to limited retrieval of cells. Therefore, in vaginal deliveries the umbilical cord is clamped after 1 minute in clinical collection of UCB in Sweden. In the present study, four of the five positive samples of UCB were from elective Cesarean sections with a median clamping time of 30 seconds compared with 57 seconds in the entire group. A total of 57 seconds is probably much longer than the average clamping time in previous studies of MMc in UCB due to the praxis of 'active management of labor' that has been prevailing for the past decades. The lower frequency of MMc in the present study may be due to a less sensitive method compared with previous studies, but might also be due to an overall longer clamping time. Why more maternal cells would transfer into the baby with a shorter clamping time is an open question.

To summarize, the present study confirms previous studies that MMc is not a very common phenomenon in UCB, and the mode of delivery or clamping time might influence the transfer of cells. The cells involved in MMc include hematopoietic progenitors and mature lineages with recognized advantages in hematopoietic stem cell transplantation. Larger studies on specific cell populations with well-defined high sensitivity methods including functional tests are required to determine the true frequency of this phenomenon. Finally, to address the hypothesis that MMc in UCB mediates a GVL effect, it would be desirable to demonstrate microchimeric cells in the recipient exhibiting this effect. As suggested by van Besien et al., this would be 'the proof in the pudding', but to prove a direct effect is a formidable methodological challenge[33].

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Declaration of Conflicting Interests

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Ethical Approval

Ethical approval to report this study was obtained from the Regional Review Board in Stockholm, Sweden (approval number 2012/480-31/3).

Statement of Human Rights

All procedures in this study were conducted in accordance with the Regional Review Board in Stockholm approved protocols (approval number 2012/480-31/3).

Statement of Informed Consent

Written and oral informed consent was obtained from the patients for their anonymized information to be published in this article.

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