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ALTERATIONS IN MATERNAL-FETAL CELLULAR TRAFFICKING AFTER FETAL SURGERY

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Abstract

Background/Purpose—Bi-directional trafficking of cells between the mother and the fetus is routine in pregnancy and a component of maternal-fetal tolerance. Changes in fetal-to-maternal cellular trafficking have been reported in prenatal complications, but maternal-to-fetal trafficking has never been studied in the context of fetal intervention. We hypothesized that patients undergoing open fetal surgery would have altered maternal-fetal cellular trafficking.

Methods—Cellular trafficking was analyzed in patients with myelomeningocele (MMC) who underwent open fetal surgical repair (n=5), MMC patients who had routine postnatal repair (n=6), and normal term patients (n=9). As a control for the fetal operation, trafficking was also analyzed in patients who were delivered by an ex utero intrapartum treatment (EXIT) procedure (n=6). Microchimerism in maternal and cord blood was determined using quantitative real-time PCR for non-shared alleles.

Results—Maternal-to-fetal trafficking was significantly increased in patients who underwent open fetal surgery for MMC compared to normal controls, postnatal MMC repair, and EXIT patients. There were no differences in fetal-to-maternal cell trafficking between groups.

Conclusion—Patients undergoing open fetal surgery for MMC have elevated levels of maternal microchimerism. These results suggest altered trafficking and/or increased proliferation of maternal cells in fetal blood and may have important implications for preterm labor.

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Keywords

Fetal surgery; myelomeningocele; spina bifida; maternal-fetal cellular trafficking; microchimerism; preterm labor; EXIT

Maternal-fetal cellular trafficking (MFCT) is the bidirectional passage of cells across the placenta that results in the presence of fetal cells in mothers [1–4] and of maternal cells in offspring [5, 6]. Increased amounts of fetal cells and cell-free DNA have been seen in maternal serum after fetal intervention [7] and may be a marker for preterm labor (PTL) [8, 9]. However, trafficking in the other direction (maternal into fetal) is less well understood and has not been investigated in the context of pregnancy complications.

Normal cellular trafficking in pregnancy may be a component of maternal-fetal tolerance. It has recently been reported that the presence of maternal cells in fetuses ("maternal microchimerism") may lead to the formation of fetal regulatory T cells which protect against an immune response against the mother [10]. Thus, alterations in trafficking may be related to the breakdown of tolerance between the mother and the fetus. Since preterm labor, a possible consequence of such a breakdown in tolerance, remains the Achilles' heel of fetal intervention [11], it is important to study whether MFCT is altered in the context of fetal surgery.

We have previously described increases in maternal-to-fetal cellular trafficking after fetal intervention in mice, with particular increases in maternal T cells found in fetal blood after allogeneic hematopoietic stem cell transplantation [12]. However, maternal microchimerism patterns after fetal intervention in humans have not been studied. We examined patients undergoing fetal surgery for repair of myelomeningocele (MMC) to test the hypothesis that cellular trafficking would be altered after open fetal surgery. We chose to study MMC patients since they are free of other underlying hemodynamic or hematologic abnormalities that may affect trafficking. In addition, since it is a nonlethal disease, there is a control group of patients with the same disease who do not undergo fetal intervention. We report that maternal microchimerism is significantly increased in patients undergoing fetal MMC repair compared to normal controls.

1. Materials and Methods

This study was approved by the University of California, San Francisco institutional review board (#10-00350). Informed consent was obtained from all participants.

1.1. Cohorts and controls

Women carrying fetuses with MMC and normal term controls were prospectively recruited to participate in this study between 2009–2011. MMC patients were concurrently enrolled in the recently published Management of Myelomeningocele Study (MOMS) randomized controlled trial [13] and thus underwent either open hysterotomy fetal surgery with subsequent Cesarean delivery ("prenatal MMC" group) or planned Cesarean delivery with postnatal repair ("postnatal MMC" group) at University of California, San Francisco. Given this delivery method, normal term pregnancies that underwent planned Cesarean delivery without the onset of labor were included as controls. Normal controls were also included from the Perinatology Research Branch in Detroit, MI as part of an ongoing collaboration. As a control for trafficking during an operation on placental support, 6 patients who underwent ex utero intrapartum treatment (EXIT) procedure [14] for various fetal anomalies were also examined. Charts were reviewed for maternal history, operative reports, and perinatal course.

1.2. Fetal interventions

Open fetal surgery for repair of MMC was performed as detailed in Adzick et al [13]. EXIT procedures were performed as previously described [15] with maternal general anesthesia to maximize uteroplacental circulation.

1.3. Sample collection

Cord blood samples for all infants were obtained at the time of delivery, after cleaning the umbilical cord with alcohol to avoid contamination with maternal blood. Maternal blood was collected within 24 hours of delivery. All blood samples were initially collected in EDTA containing tubes and an aliquot of whole blood was frozen for PCR analysis. Blood collected from Detroit was shipped on the day of delivery by overnight mail on ice and processed upon arrival. All blood processing was performed within 36 hours of delivery and samples were stored at −80°C.

1.4. Quantitative real-time PCR

Researchers blinded to patient groups quantified maternal and fetal microchimerism using a qRT-PCR assay that has been previously validated [16] and used in the quantification of maternal blood in fetal samples [10]. Briefly, paired maternal and cord blood samples were first genotyped for 12 HLA-DR and 12 In-Del alleles to determine non-shared (“informative”) alleles between the mother and the fetus after extracting genomic DNA. The presence of microchimeric cells was then determined by amplifying for the non-shared maternal alleles in cord blood or the non-shared fetal alleles in maternal blood. Samples were also amplified with primers specific for HLA DQ-alpha to determine the concentration of total genomic DNA in each specimen. The concentration of total DNA and minor-type DNA (resulting from microchimerism) was calculated by comparing the cycle threshold of the sample to those from parallel amplifications of 10-fold serial dilutions of standards with a known cell count. True negative (samples from unrelated individuals) and true positive (samples with a known concentration of spiked cells) were consistently evaluated correctly (data not shown). The lower limit of detection of this assay has been established to be between 0.001–0.0001% depending on the DNA input and primer pair [16].

1.5. Statistical Analysis

Pairwise comparisons were performed using the Mann-Whitney U test for non-parametric, or t test for parametric data. Groupwise comparisons were performed using the Kruskal-Wallis oneway analysis of variance by ranks with a Dunn's post hoc test. A p value of less than 0.05 was considered statistically significant. Statistics were performed using Prism 5.0 (GraphPad Software, Inc., La Jolla, CA).

2. Results

2.1. Demographic and operative characteristics

Relevant demographic and operative characteristics for the groups are summarized in Table 1. Cell trafficking data were analyzed for a total of 26 pregnancies. 9 were normal, uncomplicated patients who had cesarean deliveries at term without labor. 11 patients had fetal MMC, of which 5 underwent open fetal surgery at 24±1.3 weeks and 6 underwent postnatal surgery after planned delivery at 37 weeks. 6 patients underwent EXIT procedures for the following indications: cervical teratoma (n=1), congenital high airway obstructive syndrome (CHAOS, n=1), sacrococcygeal teratoma (SCT, n=2), and tracheal occlusion (TO) for congenital diaphragmatic hernia (CDH, n=2). Of note, 4 of the EXIT patients underwent single port minimally invasive fetal interventions during pregnancy: one patient with SCT underwent radiofrequency ablation of the tumor at gestational age 25 5/7 weeks (EXIT at 26

6/7 weeks), one patient with CHAOS underwent fetal bronchoscopy with tracheoplasty at 27 1/7 weeks (EXIT at 35 5/7 weeks), and the 2 patients with CDH underwent tracheal balloon insertion at 27 weeks (EXIT at 29 and 36 weeks, respectively).

There were no differences in maternal age or infant gender between groups. As expected, the prenatal MMC surgery group delivered significantly earlier than both the postnatal MMC ($p=0.02$) and normal groups ($p<0.01$) in spite of routine post-operative tocolysis. The onset of PTL after open fetal surgery was also reported in the results of the larger trial comparing surgical outcomes in these patients [13]. Operative time for prenatal MMC repair was significantly longer than for EXIT procedure, where EXIT operative time was defined as surgical start until delivery of the fetus ($p<0.01$).

2.2. Cellular trafficking

Maternal-to-fetal trafficking (maternal microchimerism)—Informative (non-shared) alleles were identified in 25 of 26 samples (Figure 1). Maternal microchimerism was detectable in the 6 of 9 patients undergoing normal Cesarean delivery and undetectable in 2 patients (median ± interquartile range: 0.0015 ± 0.015%); microchimerism could not be evaluated in one normal patient due to the lack of an informative allele. These values were consistent with the largest published series of cord blood microchimerism [5]. Maternal cells were detected in all 6 MMC patients undergoing postnatal repair (0.0145 ± 0.029%). Maternal microchimerism was significantly increased in the 5 MMC patients who had fetal surgery (0.2377 ± 0.099%, $p<0.05$ vs. postnatal MMC by Mann-Whitney test and $p< 0.05$ compared to normal controls and to EXIT by Kruskal Wallis with Dunn's post test). Interestingly, the highest level of MFCT (3.45%) was detected in an open fetal surgery patient whose postsurgical course was complicated by prolonged oligohydramnios and recurrent contractions with threatened PTL that continued for one month after her surgery. Maternal microchimerism was low in 5 EXIT patients and undetectable in 1 (0.0080 ± 0.013%).

Fetal-to-maternal trafficking (fetal microchimerism)—Informative alleles were identified in 24 of 26 samples (Figure 2). There was no significant difference in the amount of fetal-to-maternal cell trafficking (FMCT or fetal microchimerism) detected in any group.

Discussion

This is the first study to examine maternal-to-fetal cell trafficking in the context of fetal intervention. Although the sample size is small, our results suggest that maternal microchimerism is increased in patients undergoing open fetal surgery followed by subsequent Cesarean delivery but not in patients who undergo fetal surgery during birth while still on placental support (EXIT procedure).

The finding of increased in maternal cells in fetuses that have undergone open fetal surgery suggests that cellular trafficking is altered after fetal surgery. An alternative explanation, however, is that fetal surgery prompts proliferation of maternal cells that have already crossed--perhaps as a result of the inflammatory signals that are triggered after surgery. We examined MFCT in EXIT patients to determine whether undergoing surgery while on placental support leads to immediate leakage of maternal cells into the fetus; our results suggest that changes in MFCT are more delayed. The continued gestational period that follows prenatal MMC surgery, but not EXIT procedure, may allow time for the activation of cellular mechanisms that regulate trafficking and/or the proliferation of trafficked maternal cells. Since several of the EXIT patients had minimally invasive procedures prior to the EXIT, our data also suggest that open hysterotomy fetal surgery leads to more alterations in maternal microchimerism than fetoscopy or percutaneous interventions.

In this study, we have not defined whether any particular maternal cell types are preferentially recruited or proliferated following fetal surgery. Our animal data suggest that granulocytes are the predominant cell type in normal trafficking, with an increase in T cell trafficking after cellular transplantation [12]. While this human study was performed with whole blood, it would be possible to perform qRT-PCR on sorted cells to determine if microchimerism is increased in particular cell populations and whether particular chemokines recruit distinct cell types across the placenta.

Fetal-to-maternal cellular trafficking was not increased in our study. This finding may due to the difficulty in detecting small changes in levels of fetal cells in the larger maternal blood volume or because trafficked fetal cells may have diminished survival in maternal blood. Another explanation may be that signals leading to trafficking across the placenta are unidirectional and governed by particular chemokines, rather than a general “leakiness” of the placenta. An increase in cell-free fetal DNA has been previously reported after less invasive fetal interventions, such as with laser coagulation for twin-twin transfusion syndrome [7]. It is possible that a surgical intervention focused on the placenta leads to more perturbations in cell trafficking, compared to the non-placental operations in our study.

MFCT is a component of maternal-fetal tolerance, and it is possible that alterations in trafficking perturb this balance, resulting in preterm labor. In this study, the prenatal MMC group with higher trafficking was born at an earlier gestational age, but our study does not establish a causal relationship between trafficking and PTL. In addition, higher trafficking was not seen in several fetuses that delivered preterm via EXIT, indicating that there is not an absolute correlation between prematurity and increased microchimerism. We are currently studying cellular trafficking in PTL patients secondary to non-surgical causes as well as whether trafficking alters immune reactivity between the mother and fetus to further characterize the relationship between trafficking and PTL. A careful analysis of the specific types of cells which traffic and the signals that promote their migration may help determine if there is a causal relationship between trafficking and PTL and may uncover strategic targets for the prevention of preterm birth.

The long-term clinical significance of increased maternal microchimerism in patients is unknown. Increased maternal microchimerism has been described in patients with a variety of diseases such as type 1 diabetes mellitus [17], neonatal lupus syndrome-congenital heart block [18], and biliary atresia [19–22]. However, it is unknown whether these increased maternal cells in the offspring directly contribute to the etiology of neonatal disease or are simply proliferating in response to injury. Microchimerism may be tolerizing or sensitizing to non-inherited maternal antigens [23–26] and may therefore have implications for graft tolerance in some transplantation settings such as living-related bone marrow [27] or liver transplantation [28]. Thus, the study of the effects of fetal intervention on maternal microchimerism may have vital clinical significance for prenatally treated diseases that may require postnatal organ transplantation, such as in patients with posterior urethral valves.

Prenatal surgery for myelomeningocele (MMC) is the most common open fetal intervention performed worldwide [29] and is the only open fetal surgery that has demonstrated efficacy in a randomized controlled trial [13]. Given the anticipated expansion of open fetal surgery following the promising results of the MOMS trial, our finding of increased maternal microchimerism with this approach may have clinical implications for the future health of these patients.

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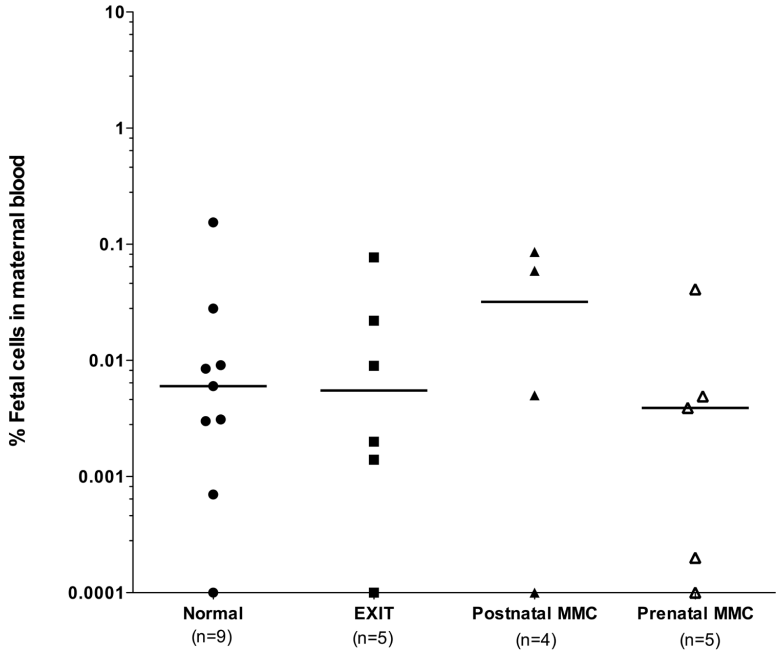

Figure 1.

Maternal microchimerism. The percentage of maternal cells in cord blood was significantly different among groups (Kruskal Wallis for all groups, p=0.01. *= p<0.05 by pairwise comparison using Mann-Whitney; **= p<0.05 by Kruskal-Wallis with Dunn's post-hoc comparison.) MMC = myelomeningocele, EXIT = ex utero intrapartum treatment. Maternal to fetal cellular trafficking could not be analyzed in one normal patient due to an absence of non-shared alleles on PCR.

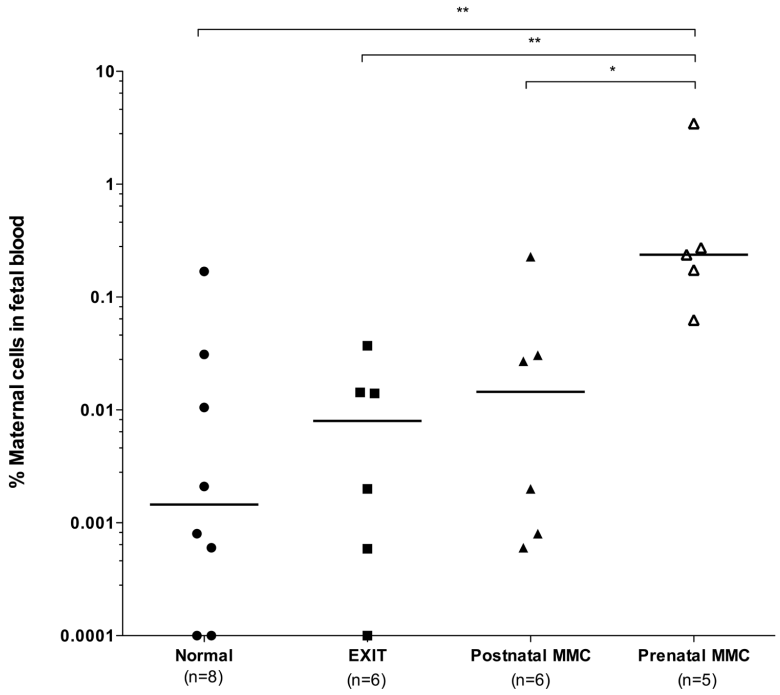

Figure 2.

Fetal microchimerism. The percentage of fetal cells in maternal blood was not significantly different among groups. MMC = myelomeningocele, EXIT = ex utero intrapartum treatment. Fetal to maternal cellular trafficking could not be analyzed in two postnatal MMC patients due to an absence of non-shared alleles on PCR.

Table 1

Patient demographics.

	N	Maternal age (years)	GA at minimally invasive procedure (weeks)	GA at open fetal surgery (weeks)	Operative time (minutes)	GA at delivery (weeks)	Infant gender (%male)
Normal	9	28 ± 6.5	n/a	n/a	n/a	39 ± 0.9	67%
EXIT	6	29 ± 5.5	27 ± 0.7	n/a	36.5 ± 7.8 *	32 ± 3.9	67%
Postnatal MMC	6	25 ± 1.5	n/a	n/a	n/a	37 ± 0.9	83%
Prenatal MMC	5	26 ± 2.8	n/a	24 ± 1.3	131 ± 29	34 ± 2.5 **	20%

GA = gestational age, n/a = not applicable

* p<0.01 compared to operative time for open fetal surgery by t test.

** p=0.02 vs postnatal MMC, p<0.01