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Increased maternal microchimerism after open fetal surgery

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Maternal-fetal cellular trafficking (MFCT) during pregnancy leads to the presence of maternal cells in the fetus and of fetal cells in the mother. Since this process may be altered in cases of pregnancy complications, we asked whether open fetal surgery leads to changes in microchimerism levels. We analyzed maternal and fetal microchimerism in fetuses who underwent open fetal surgery for repair of spina bifida and compared their levels to patients who had postnatal repair and to healthy controls. We found that maternal microchimerism levels were increased in patients who had open fetal surgery compared with controls. In contrast, patients who had fetal intervention at the time of delivery did not demonstrate increased microchimerism. These results suggest that open fetal surgery may alter trafficking. Given the importance of MFCT in maternal-fetal tolerance, we discuss potential implications for the field of preterm labor and transplantation tolerance.

Keywords: maternal-fetal cellular trafficking, myelomeningocele, spina bifida, microchimerism, fetal surgery

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Maternal-fetal cellular trafficking (MFCT) during pregnancy results in bidirectional passage of cells between the mother and the fetus, resulting in maternal cells in the fetus[1,2] (maternal microchimerism, MMc) and fetal cells in the mother[3-6] (fetal microchimerism, FMc). While the mechanisms of this trafficking are not known, it has been suggested that the presence of microchimeric cells in the fetus leads to the generation of fetal regulatory T cells that suppress an immune response against maternal antigens.[7] Thus, MFCT may be a component of maternal-fetal tolerance.

With advances in prenatal diagnosis and improvements in fetal surgical techniques, there are now increasing indications to perform fetal interventions for severe congenital anomalies.[8] While the field started as a way to treat severe, fatal anatomic abnormalities, with improvements in fetal surgical techniques and outcomes, it has expanded to include the repair of spina bifida (myelomeningocele), which is the first non-fatal disorder for which open fetal surgery has been performed. However, the field remains severely limited by preterm labor (PTL), as demonstrated by multiple clinical trials.[9,10] Recently, we participated in a multi-center randomized clinical trial to compare fetal surgery to post-natal repair of spina bifida.[9] This trial demonstrated that prenatal repair of the neural tube defect led to a decrease in the need for ventriculoperitoneal shunting (which carries significant morbidity) as well as an improvement in motor function and mental development. However, there was an increased risk of chorioamniotic membrane separation and of spontaneous membrane rupture, with an overall increased risk of preterm delivery in the fetal surgery group.[9] Since this trial examined a disease that did not cause fetal hemodynamic distress and included patients who underwent either pre- or post-natal repair, it was the ideal setting in which to also analyze the effects of open fetal surgical intervention on MFCT.

To determine the effects of open fetal surgery on MFCT, we collected maternal and cord blood samples at the time of birth from fetuses with spina bifida who underwent prenatal repair (n = 5), as well as those with spina bifida who were

randomized to postnatal repair (n = 6). Both of these groups were delivered by Cesarean section and healthy term patients who were delivered by Cesarean section served as controls (n = 9). We also examined trafficking in another important control group: fetuses with congenital anomalies who had an intervention at the time of birth to assist with stabilization: the ex utero intrapartum treatment (EXIT) procedure (n = 6).[11] This procedure involves a maternal hysterotomy under general anesthesia, with delivery of the fetus after securing the airway and obtaining vascular access. Four of six EXIT patients had also undergone minimally invasive fetal intervention prior to the EXIT procedure. To quantify MFCT, we used a well-established method of quantitative PCR[12] to amplify non-shared maternal alleles in fetal blood (maternal microchimerism) and non-shared fetal alleles in maternal blood (fetal microchimerism).

Although the sample size for this study was small, and there was a variation in the amount of MMc in healthy control samples, we nonetheless found a significant increase in MMc in fetuses with spina bifida who underwent a prenatal repair compared with all other groups. This observation may be because of increased recruitment of maternal cells or increased proliferation of existing maternal cells in the fetus after the surgery. Alternatively, there may be increased turnover of maternal cells in fetal blood in the control groups. In addition, we found that patients who underwent the EXIT procedure uniformly had low levels of MMc, suggesting that surgery on placental support does not immediately alter trafficking and that a subsequent period of gestation is likely necessary. These results are consistent with our finding of increased maternal cells after fetal intervention in mice[13] and we are currently exploring whether such alterations in microchimerism impact maternal-fetal tolerance during pregnancy.

Our results are consistent with published studies on MMc. For example, it has been reported that there is a variable range of MMc in cord blood samples from healthy controls.[1] HLA compatibility may be an important factor in determining MMc: a study of 120 maternal-fetal pairs found MMc to be associated with the HLA-DQB1 allele, suggesting that specific fetal and maternal compatibility may trigger the trafficking of maternal cells into the fetus,[14] a variable that we did not study in this small series. Another factor that may contribute to increased microchimerism may be fetal distress or inflammation since the production of cytokines and chemokines in this context may alter trafficking. We are currently examining trafficking and other parameters in cohorts of fetuses with congenital anomalies that cause hemodynamic compromise to address this point.

Changes in trafficking after fetal intervention have also been examined by several investigators, with an emphasis on detection of fetal DNA in maternal blood. Wataganara and colleagues reported increased levels of circulating fetal DNA in maternal circulation after laser ablation of inter-twin vessels for twin-twin transfusion syndrome (TTTS).[15] In particular, longer operation time, the total number of chorionic vessels ablated, and in utero fetal demise of at least one twin were associated with elevated levels of fetal DNA at 24 h, suggesting that detection of circulating fetal DNA is a possible marker for trophoblast injury.[15] However, a subsequent study of circulating free mRNA levels after fetoscopic procedures (including laser ablation for TTTS and tracheal occlusion for congenital diaphragmatic hernia) were not elevated.[16] It is possible that differences in the kinetics of free DNA and mRNA may account for the discrepancy between these results. In our study, we did not detect increased levels of fetal microchimerism in mothers of any group. This may because the signals leading to increased trafficking (or increased proliferation of trafficked cells) may be uni-directional, or that it is difficult to detect small changes in fetal cells among the larger maternal blood volume.

While the description of altered microchimerism levels is interesting, we are most interested in deciphering the possible functional significance of these findings. One potential area of impact is in living-related transplantations in which the mother serves as the donor for her child. For example, patients with posterior urethral valves undergo prenatal interventions for diagnosis and therapy and some of these patients require renal transplantation postnatally,[17] sometimes from a parental donor. Although a national study of renal transplantation did not show a survival benefit with maternal transplantation,[18] there is improvement in graft survival with sibling transplantation if the donor expresses non-inherited maternal antigens.[19] A potential benefit of maternal organ transplantation may be discerned in other transplantation settings with milder rejection. For example, we recently reported that patients with biliary atresia (who have increased MMc at baseline[20]) have improved survival with maternal liver transplantation compared with paternal.[21] For hematopoietic stem cell (HSC) transplantation, it has been reported that transplantation of maternal HSC improves survival[22] and decreases the risk of graft vs. host disease.[23] Thus, it is possible that elevated levels of MMc may improve tolerance to maternal tissues in some settings. However, microchimerism may be tolerizing or sensitizing to non-inherited maternal antigens[24-26] and it is possible that increased MMc may instead lead to heightened rejection of maternal organs, especially if increased maternal trafficking occurs in the setting of surgical inflammation. 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 solid organ or hematopoietic stem cell transplantation.

Another unanswered question is whether alterations in MMc can contribute to preterm labor. In our study, the gestational age at delivery of the fetal surgery group was significantly lower than that of the postnatal control group (34 ± 2.5 weeks vs. 37 ± 0.9 weeks, p = 0.02). However, several patients in the EXIT group also had PTL but did not exhibit high MMc, indicating that even if there is some association of trafficking with PTL, it is not absolute. It is possible that certain situations (such as open surgery, membrane separation, prenatal infection and HLA disparity between the mother and fetus) may predispose to increased MMc.

Understanding the implications of trafficking will ultimately depend on

determining the mechanisms that contribute to trafficking, the cell types that cross and their interactions with components of the maternal-fetal interface that usually limit trafficking.[27] Such studies are possible in the murine model, using tools such as GFP transgenic mice[28,29] or congenic alleles of CD45[13] to identify the lineage of trafficked maternal cells, in combination with transgenic and knockout models to identify molecular mechanisms. However, given the differences in placental anatomy between mouse and humans, murine observations will need to be confirmed in patient specimens. Ultimately, a more refined understanding the potential link between fetal surgery, cellular trafficking, and preterm labor may lead to new therapies for preterm labor.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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