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# The maternal immune response inhibits the success of in utero hematopoietic cell transplantation

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**In utero hematopoietic cell transplantation (IUHCTx) is a promising strategy for the treatment of congenital stem cell disorders. Despite the purported immaturity of the fetal immune system, the clinical success of this strategy has been limited by poor engraftment of transplanted cells. The fetal host immune system is thought to be the major barrier to achieving successful IUHCTx. Since the fetal immune system is immature, however, we hypothesized that the maternal immune response may instead pose the true barrier to IUHCTx. We have demonstrated that maternal T cells traffic into the fetus after allogeneic in utero transplantation and that these lymphocytes play a critical role in limiting engraftment. Furthermore, we have shown that MHC matching the donor cells to the mother improves engraftment in the unmatched fetus. These results help renew interest in using the fetal environment to treat patients with congenital stem cell disorders.**

**Key words:** maternal microchimerism, in utero transplantation, stem cell transplantation, maternal immune response, maternal-fetal cellular trafficking

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In utero hematopoietic cell transplantation (IUHCTx) is a promising technique to treat congenital hematological defects and induce immune tolerance to alloantigens in the fetus. Theoretically, introducing allogeneic cells during the period of thymic education to self antigens should lead to deletion of donor-specific T cells by negative section.[1] The resulting antigen-specific tolerance can therefore minimize the need for myeloablation during postnatal stem cell or organ transplantation.[2-4] Despite the theoretical advantages of allogeneic cell transplantation in the fetus, the clinical success of IUHCTx in humans has been limited. With the exception of severe combined immunodeficiency,[5,6] the use of IUHCTx to treat other prenatally diagnosed diseases such as hemoglobinopathies has been unsuccessful (reviewed in ref. 7).

There are numerous barriers to the success of IUHCTx such as limited space in hematopoietic niches and the lack of competitive advantage of transplanted cells (reviewed in ref. 8). In addition, it is possible the fetal immune system is mature enough to reject the transplanted cells. The idea that the fetal immune system may be a barrier to IUHCTx was first demonstrated in the mouse model by Peranteau et al. who observed a clear difference in the engraftment of congenic vs. allogeneic cells.[9] We have obtained similar results in our mouse model of IUHCTx in that there is a significantly lower frequency of chimerism when allogeneic hematopoietic cells (derived from fetal liver, FL) are transplanted compared to congenic FL transplantation.[10] These observations have created an important conundrum in the field: why can the fetus be tolerized to some foreign antigens it encounters during development, such as non-inherited maternal antigens (NIMAs),[11-13] and not to those which are transplanted in utero?

One potential explanation is that it is the maternal, not fetal, immune response that limits engraftment after IUHCTx. Indeed, a study by Merianos et al. demonstrated that maternal alloantibodies, transmitted through breast milk after birth, can lead to rejection of transplanted cells.[14] In addition to antibodies, it is known that maternal cells also traffic into the fetus. Such trafficking is a normal

part of pregnancy, and it has been suggested that this phenomenon contributes to maternal-fetal tolerance by inducing the formation of Tregs that prevent an anti-maternal immune response.[11] Since maternal cells are likely present in fetuses at the time of in utero transplantation, we hypothesized that they could influence donor cell engraftment. We first documented that significant numbers of maternal cells are present in the circulation of unmanipulated mouse fetuses at midgestation (E12.5–E15.5) although these cells are not detectable by flow cytometry after birth. When we performed IUHCTx on E13.5 and analyzed the fetuses 5 days later, we noted a striking increase in trafficking compared to uninjected pups, with a significant population of maternal T cells found in these fetuses.[10] Thus, although the mouse fetus does not have many mature T cells at the time of transplantation, cellular trafficking brings maternal T cells in contact with the transplanted allogeneic cells.

We then asked whether engraftment after IUHCTx would increase if the mother, but not the fetus, lacked T or B cells. Using knockout female mice bred to wildtype male mice (such that the fetuses were immunocompetent), we found that 91% of fetuses whose mothers were T cell deficient were chimeric after IUHCTx, whereas B cell deficiency did not rescue engraftment.[10] These results confirmed that maternal T cells play a critical role in inhibiting engraftment. The obvious implication of these results is that donor cells that are MHC matched to the mother should improve engraftment after IUHCTx. To show this definitively, we used the F1 backcross model described by Zhang and Miller[15] and generated crosses in which the transplanted B6 cells were matched to the mother and allogeneic to half of the fetuses. Transplanting donor FL that was matched to the mother resulted in equivalent rates of engraftment in both the matched and mismatched fetuses, demonstrating that it is the maternal, not fetal, immune response which is the main barrier.[10]

A key question raised by these experiments is the mechanisms by which maternal T cells limit engraftment. The two main possibilities are that maternal cells directly respond to the transplanted allogeneic cells or that they indirectly influence the fetal immune system to reject the allogeneic transplant. Even after IUHCTx, we have found that the proportion of maternal cells in fetal lymphoid tissues and peripheral blood decreases to almost undetectable levels in neonatal mice 2 and 3 weeks after in utero transplantation (Nijagal A and Wegorzewska M, unpublished data). The lack of persistent maternal lymphocytes in recipient mice suggests that trafficked maternal cells indirectly influence engraftment by priming the fetal immune system. For example, maternal T cells could induce fetal antigen presenting cells (APC) to undergo precocious maturation involving the expression of key APC costimulatory markers, including B7-1, B7-2 and CD40. Alternatively, maternal T cells could stimulate fetal T cells to become activated.

If this model of maternal cells influencing the fetal immune system is correct, it may have implications for the role of maternal microchimerism in other diseases. Maternal cells have been found at a higher frequency in human autoimmune diseases such as Type I diabetes[16] and inflammatory diseases such as biliary atresia,[17] suggesting that these cells could play a role in the pathogenesis of these conditions. An alternative theory is that maternal microchimerism may be important for fetal tissue repair during inflammation.[16] While our results do not distinguish between these possibilities, they do indicate the importance of defining which cells are trafficking and the molecular pathways that allow them to cross the placenta. We speculate that trafficking may be regulated by specific cues released by the fetus and/or placenta in response to danger signals. For example, fetal intervention (which, in our mouse model, involves significant trauma to the fetal liver) may induce particular changes in the chemokine/chemokine receptor profiles at the maternal-fetal interface that encourage lymphocyte trafficking. Interestingly, maternal T cells were increased only when allogeneic cells were transplanted, suggesting further specificity in this system. Clinically, it is possible fetal distress (due to congenital anomalies or after fetal surgery) may also lead to alterations in maternal-fetal cellular trafficking. If maternal cell trafficking is a critical component of maternal-fetal tolerance, alterations in trafficking may then correlate with the breakdown of tolerance and the onset of preterm labor. Since preterm labor remains the Achilles' heel of fetal intervention, we are actively investigating this question in our fetal surgery patients.

IUHCTx holds great promise for the treatment of congenital hematopoietic diseases. Although barriers such as space and host cell competition may still limit the clinical success of IUHCTx, our findings provide important insights into the immune barrier for IUHCTx. The immune barrier may result from the conversion of normally tolerogenic maternal-fetal immune crosstalk to pathogenic immune responses as result of pro-inflammatory signals released during fetal intervention. Our results suggest that changes in maternal microchimerism play a critical role in influencing the balance between fetal tolerogenic and pathogenic immune responses. Experiments in both small and large animal models of IUHCTx will be crucial to help understand the function of maternal microchimerism and its relation to fetal stem cell engraftment and maternal-fetal tolerance.

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