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Maternal and Fetal Immune Response to in Utero Stem Cell Transplantation

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Abstract

Purpose of Review: In Utero Hematopoietic Cellular Transplantation (IUHCT) is a promising intervention for the non-toxic treatment of congenital disease that hinges on the assumption of fetal immunologic immaturity and an inability to reject a hematopoietic allograft. However, clinical IUCHT has failed except in cases where the fetus is severely immunocompromised. The current review examines recent studies of engraftment barriers stemming from either the fetal or maternal immune system.

Recent Findings: New reports have illuminated roles for maternal humoral and cellular immunity and fetal innate cellular immunity in the resistance to allogeneic IUHCT. These experimental findings have inspired new approaches to overcome these barriers. Despite these advances, postulates regarding a maternal immune barrier to IUHCT provide an inadequate explanation for the well-documented clinical success only in the treatment of fetal immunodeficiency with normal maternal immunity.

Summary: Characterization of the maternal and fetal immune response to allogeneic IUHCT provides new insight into the complexity of prenatal tolerance. Future work in this area should aim to provide a unifying explanation for the observed patterns of success and failure with clinical IUHCT.

Keywords

In utero transplantation; NK cells; Fetal immune system; Tolerance; Materno-fetal trafficking; IUHCT

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Compliance with Ethical Standards

Conflict of Interest : Amir Alhajjat and Aimen Shaaban declare that they have no conflict of interest.

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Introduction

In Utero Hematopoietic Cellular transplantation (IUHCT) is a promising intervention. Through the introduction of foreign antigen to the developing immature fetal immune system, induction of tolerance to donor-specific antigens and hematopoietic chimerism may be achieved providing the remarkable potential to treat congenital disease prior to the patient experiencing a single day of illness (1-5). The rationale behind IUHCT is that the prenatal engraftment of normal hematopoietic cells preempts the irreversible damage caused by congenital disorders of cellular dysfunction while avoiding the toxic myeloablation and immunosuppression typically required for successful postnatal allogeneic transplantation.

Theoretical support for IUHCT emanates from the primary assumption that the fetal immune system is immature and is therefore incapable of rejecting an allogeneic cellular transplant. Furthermore, the engraftment of allogeneic cells prior to maturation of the fetal immune system should lead to long-term donor-specific tolerance. However, clinical application of IUHCT has only been successful in the treatment of fetuses with a severely defective immune system such as in the case of severe combined immunodeficiency.(6-9) In the immunocompetent fetus, only rarely has IUHCT lead to chimerism levels adequate for the treatment of disease. These repeated failures have forced a re-examination of the central dogma for IUHCT. A better understanding of the maternal and fetal immune response is needed to overcome barriers to prenatal transplantation.

Several groups have probed barriers such as the competition for space within the hematopoietic microenvironment as well as the maternal and fetal immune responses to prenatal transplantation. As a result, the field has moved closer to the reality of IUHCT in clinical practice adding substantial new knowledge about fetal immunologic development and the interplay between the maternal and fetal immune systems in the recognition of foreign antigens.

Evidence supporting the existence of a maternal immune response to IUHCT

A number of reports in inbred strains of mice have demonstrated a difference in the engraftment rate between congenic and allogeneic IUHCT leading to postulates of immunologic rejection in the pre-thymic fetus (10, 11). However, prenatal tolerance stemming from the selective elimination of alloreactive T-cells and the expansion of allospecific regulatory cell populations should be reliably established in allogeneic transplants systems even when microchimerism exists (12-15). These observations of engraftment resistance in the pre-immune fetus despite the existence of measurable chimerism have prompted the search for a potential maternal immune response to IUHCT.

Mechanistic support for a maternal immune response to IUHCT comes from earlier work by Merianos et al. (16). The authors initially demonstrated that about 70% of prenatal allogenic chimeras lost engraftment between 2-4 weeks of age, whereas all congenic recipients remained engrafted. Serological examination of the recipient dams revealed the existence of circulating alloantibodies. This humoral response was temporally related to the loss of

chimerism in injected pups as it peaked at 2-5 weeks after IUHCT. It was postulated that the dams were sensitized at the time of the IUHCT leading to the emergence of alloantibodies that could be subsequently transferred through maternal milk to the fetus leading to the rejection. In support of this hypothesis, the authors found that when the pups were fostered by naive mothers, all of the recipients remained chimeric. The mechanism through which the transferred alloantibodies led to allograft rejection was not clarified and the possibility of maternal cellular rejection in the recipient offspring was not excluded.

In addition to alloantibodies, maternal cell microchimerism resulting from maternal→fetal cellular trafficking may also be a source of graft rejection in prenatal transplants. Bi-directional cellular trafficking has been recognized to occur in normal pregnancy(17, 18) and the clinical implications of maternal microchimerism in tolerance to solid organ transplantation(19, 20), autoimmunity and bone marrow transplantation have been described (21, 22). More recently, maternal microchimerism was demonstrated to play a pivotal role in promoting cross-generational reproductive fitness thereby providing a rationale for the natural persistence of this phenomenon (23). The significance of maternal microchimerism in IUHCT is an intriguing question that was initially examined by Nijagal et al(24). The authors were able to demonstrate that murine fetuses contain a significant number of trafficked maternal cells at baseline which increase significantly after prenatal injections. Importantly, the composition of the trafficked cells differed between the maternal blood and the fetuses suggesting that trafficking is an active and selective process rather than mechanical “leakage” due to the fetal injection. Furthermore, in this body of work, the authors found that significant numbers of T cells traffic from the mother into the fetus and proposed that a maternal T cell response was responsible for the lower early engraftment rates in allogeneic vs. congenic IUHCT. In support of this, higher engraftment rates were seen with the use of T cell deficient mothers or by matching the donor cells with the maternal MHC antigens thereby avoiding the potential for a maternal T cell response in either setting. The authors concluded that maternal T cells persisted in the chimeric offspring for months after birth and led to chronic rejection. However, no maternal cells could be found within the recipient at any point beyond the fetal period making it difficult to reconcile engraftment loss by this mechanism that occurred months later. Despite these unresolved issues, the finding of maternal cells in the fetal immune system illustrates the potential complexities involved in prenatal transplantation and clearly warrants further study.

Evidence supporting a fetal immune response to IUHCT

Although significant support for a maternal immune barrier to IUHCT exists in some murine models of IUHCT, the clinical significance of this barrier in human IUHCT remains questionable. Early clinical observations with IUHCT support the existence of a fetal rather than maternal barrier to IUHCT as the greatest clinical success has been seen in the treatment of SCID and x-SCID using paternally derived transplants (6-8, 25) with predictable failure in the treatment of sickle cell disease and thalassemia (26, 27). Given that the maternal immune response has been intact in these cases regardless of their outcome, an independent role for the maternal immune response to clinical IUHCT does not seem apparent.

Similarly, rejection by maternal immune cells does not provide a standalone explanation for the patterns of graft rejection seen in murine models of IUHCT. This is most evident with the observation that engraftment or rejection may occur in alternating littermates exposed to the same maternal influence (10, 11). The rejection may occur within the first few weeks after birth or may follow months of decay in the chimerism level whereas parallel transplantation in a naturally tolerant congenic strain combination revealed remarkably stable engraftment in nearly all recipients even at the lowest chimerism levels Furthermore, rejection in the allogenic recipients was complete with no detectable chimerism and normalization of the alterations in host lymphocyte populations that are typically seen even in the setting of microchimerism In our laboratory, this pattern was found to be independent of a potential maternal immune response as the rate of rejection was unaffected by the use of naïve foster dams and no maternal cells have been detected anywhere within the recipient mice after birth(11, 28, 29). A detailed assessment of the transplanted offspring revealed that engraftment or rejection in littermates correlated with a level of initial chimerism greater than or less than 1.8% of circulating cells (chimerism threshold). With increasing numbers of cells in the transplant inoculum, higher levels of early chimerism were achieved eventually resulting in all of the littermates attaining levels above the chimerism threshold and none of them rejecting their graft (11).

Drawing on observations from the clinical and laboratory experience with IUHCT that suggested improved success when the fetus was NK cell deficient(30), we examined whether fetal NK cells mediated delayed allograft rejection in murine IUHCT. In support of this hypothesis, all recipients above the chimerism threshold demonstrated allospecific NK cell tolerance. Furthermore, when NK cells were depleted from sub-threshold chimeras, rejection did not occur as expected. Rather, the recipients maintained stable engraftment. As a final evaluation of this mechanism, when NK cells were allowed to recover, the engraftment was lost (11).

These findings were the first confirmation of the involvement of the innate immune system in the rejection of a prenatal allograft which had been suggested by the clinical experience. The identification of the chimerism threshold as a reliable predictor of engraftment suggested that NK cell education was essential to durable NK cell tolerance. Subsequent experiments led to the characterization of a sophisticated process for prenatal NK cell allospecific education akin to thymic T-cell selection that resulted in the elimination of alloreactive (hostile) phenotypes from the mature pool of NK cells in stable chimeras (28, 29). In this process, NK cells that expressed allospecific activating Ly49 receptors without co-expression any of the allospecific Ly49 inhibitory receptors were deleted or rendered functionally anergic. Here, NK cell education hinged on allorecognition by the activating receptor and subsequent upregulation of the inhibitory receptors in a developmentally-limited window (29). These changes were proven to be remarkably stable in mature chimeras even in the face of a potent viral infection (31) and were supported by adjunct mechanisms such as MHC transfer (trogocytosis) that provided sustained cisrecognition of donor ligand in the absence of trans- interaction (32). The essence of this quantitative model for NK cell education is that a minimal amount of donor chimerism is required for induction and maintenance of NK cell tolerance thereby establishing a theoretical explanation for many of the observations of clinical IUHCT.

The delineation of an immune barrier to *human* IUHCT that is rooted in the innate immune system awaits further study. However, a seminal report from our laboratory revealed that the potential for fetal NK cell allorecognition may begin in the first trimester of human development through the expression of killer immunoglobulin-like receptors (KIR) which are homologous to murine Ly49 receptors. Although the exact timing is unknown, we were able to demonstrate that only a handful of first trimester NK cells (10 weeks of gestation) expressed surface KIR whereas a significantly higher frequency of second trimester NK cells (14 weeks of gestation) expressed surface KIR (33). Similar to mice, interactions with MHC class Ia ligands appears to influence the KIR repertoire of mature human NK cells(34). Coupled with the observation that cytotoxic function is linked to the expression of KIR receptors(35), an early NK cell mediated immune barrier to allo-IUHCT seems likely. A subsequent report confirms these findings but failed to demonstrate allospecific cytotoxicity. (36) Further investigation is needed in this area.

A unifying explanation for the maternal and fetal Immune response to IUHCT.

The finding that prenatal allospecific tolerance requires chimerism levels beyond microchimerism suggests that subtle variations in numbers of transferred cells may be important in the engraftment outcome of IUHCT littermates. Experimental protocols that involve injection of small amounts of fetal hematopoietic cells directly into the recipient's fetal liver likely deliver a lesser load to the placenta than corresponding protocols calling for the injection of large amounts of fetal or adult hematopoietic cells into the fetal bloodstream. These latter techniques may contribute to maternal sensitization through a breach in the fetal-maternal blood barrier or perhaps selective transport of maternal T-cells into the fetal circulation.

In the studies by Merianos et al, which demonstrated that a maternal allospecific humoral response prevented long-term engraftment, a large number of adult donor bone marrow cells were used (16). This may have resulted in the transfer of a large number of mature T-cells to the fetus with a resulting graft-vs-host reaction diminishing the maternal fetal barrier. With exposure of the mature maternal immune system to the donor cells, a significant maternal immune response may have led to precipitous fetal loss prenatally and a massive allospecific humoral response postnatally. The capability of the maternal immune system to respond to fetal alloantigen after IUHCT and cause precipitous fetal loss was explored Wegorzewska et al (37). In their study, the fetal injection of PBS alone led to an increased fetal loss in allogenic matings. The authors reasoned that a remote breach of the feto-maternal barrier within the placenta resulted from the prenatal injection leading to maternal exposure to fetal antigen with subsequent fetal resorption.

Furthermore, it has also been shown that fetal surgery increases the amount of maternal-to-fetal cellular trafficking in humans that varies according to the technical intervention. Saadai et al. measured the cord blood microchimerism in human fetuses that underwent open fetal repair for myelomeningocele (MMC), ex utero intrapartum treatment (EXIT) or postnatal MMC and compared those levels to that found in the cord blood of normal term neonates

(38). The authors found that almost all newborns that had undergone open fetal surgery exhibited increased levels of maternal microchimerism. Interestingly, patients who underwent EXIT, had low levels of maternal microchimerism comparable to the levels seen in normal deliveries. Similar findings of maternal microchimerism have also been reported in monochorionic-diamniotic twins undergoing fetoscopic laser photocoagulation of shared placental vessels (39, 40). The mechanism in which fetal surgery affects the fetal-maternal blood barrier remains to be elucidated. Notwithstanding, additional studies will be needed to evaluate how percutaneous procedures such as IUHCT affect cellular trafficking during pregnancy.

Closing knowledge gaps in larger animal models and moving forward with clinical IUHCT

Within the complexity of the fetal and maternal alloimmune response, it is likely that numerous parameters influence the success of IUHCT. The contributions of these components in the murine and canine studies of IUHCT have influenced the design of newer research protocols for IUHCT. However, the inherent differences between these models and humans and their potential impact on the maternal-fetal immunity of IUHCT should be reconciled with large animal studies in which the immunologic disparity, placentation and cellular kinetics of human development can be effectively modeled. Human protocols for IUHCT are likely to involve smaller doses of donor cells that are delivered more precisely. Strategies that permit precise percutaneous delivery of the transplant while limiting the disruption of the maternal-fetal immune barrier are favored. The use of a maternal donor cell source or a source that is immunologically matched to the mother is a logical approach to escape the maternal immune response.

As IUHCT again moves toward clinical study, an opportunity arises to enhance the existing understanding of the immunologic response to IUHCT and confirm the translational relevance of recent findings from animal studies (2). To this end, future clinical protocols should include detailed studies of cell homing, early engraftment levels, maternal-fetal cell trafficking, maternal immune responses and allospecific tolerance in innate and adaptive fetal immune cells. Accomplishing this task will require innovative techniques in non-invasive cellular tracking as well as invasive approaches to fetal blood and tissue sampling. These assessments will almost certainly add risk and costs beyond previous efforts but are essential to ensure advancement in understanding with each of these very rare clinical cases. Focused preclinical studies in large animals such as the macaque may ease the translation of more provocative approaches to IUHCT that include targeted conditioning of the fetal immunologic or hematopoietic systems. Finally, a standardized protocol based upon the principles set forth by collaborative academic societies such as the International Fetal Transplantation and Immunology Society (www.fetaltherapies.org) will greatly facilitate the establishment of international registries and cooperative study (2).

As we continue to explore prenatal transplantation, a better characterization of the feto-maternal barrier will be fashioned. Ultimately, this new knowledge will guide future aspects

of IUHCT and influence investigations into NIMA tolerance, preterm labor, and other maternal, obstetric and childhood immunologic conditions.

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