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CONCISE REVIEW

The Theoretical Basis of In Utero Hematopoietic Stem Cell Transplantation and Its Use in the Treatment of Blood Disorders

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Since its conception, prenatal therapy has been successful in correction of mainly anatomical defects, although the range of application has been limited. Research into minimally invasive fetal surgery techniques and prenatal molecular diagnostics has facilitated the development of in utero stem cell transplantation (IUT)—a method of delivering healthy stem cells to the early gestation fetus with the hope of engraftment, proliferation, and migration to the appropriate hematopoietic compartment. An area of application that shows promise is the treatment of hematopoietic disorders like hemoglobinopathies. The therapeutic rationale of IUT with hematopoietic stem cells (HSCs) is based on the proposed advantages the fetal environment offers based on its unique physiology. These advantages include the immature immune system facilitating the development of donor-specific tolerance, the natural migration of endogenous hematopoietic cells providing space for homing and engraftment of donor cells, and the fetal environment providing HSCs with the same opportunity to survive and proliferate regardless of their origin (donor or host). Maternal immune tolerance to the fetus and placenta also implies that the maternal environment could be accepting of donor cells. In theory, the fetus is a perfect recipient for stem cell transplant. Clinically, however, IUT is yet to see widespread success calling into question these assumptions of fetal physiology. This review aims to discuss and evaluate research surrounding these key assumptions and the clinical success of IUT in the treatment of thalassemia.

Keywords: in utero hematopoietic stem cell transplantation, β-thalassemia, fetal surgery

Introduction

Major questions in the field of pregnancy and fetal development have been answered in the last 50 years through advancements in obstetric research and technology. Our understanding of maternal and fetal immunology and hematopoiesis coupled with high-quality ultrasonography and improvement in prenatal diagnostics has allowed the development of in utero interventions for diseases that were previously fatal or life altering. Patients with congenital hematologic diseases have the potential to benefit significantly from in utero stem cell transplantation (IUT), as current treatment protocols recommend postnatal bone marrow (BM) transplantation that carries varied long-term risks including chronic graft versus host disease, infertility, pulmonary complications, and solid cancers [1].

Stem cell therapy is the administration of living cells to patients with the intention of replacing diseased or damaged organs and tissues. The cells can be: autologous, meaning derived from the patient or allogenic, meaning derived from another individual (ideally a first-degree relative). The clinical effect results from donor cell engraftment and differentiation, which can replace and repair damaged tissue. Engraftment is defined as the successful growth of donor BM stem cells in the recipient [2]. In utero transplantation applies these stem cell therapy principles to the fetus by delivering cells using established clinical techniques like ultrasound-guided intraperitoneal or intravenous (umbilical vein) injection [3].

The advantage of transplantation before the fetus develops a functional immune system could remove acute and chronic graft–host disease, provide protection from infection through the sterility of the womb, all while the growth of the fetus leaves physical space to accept the graft without myeloablation [4]. IUT also takes advantage of regulatory cells (Treg)-mediated maternal immune tolerance in pregnancy [5]. Genetic screening now allows the diagnosis of many inherited disorders early in gestation, including immunodeficiency disorders, hemoglobinopathies, and enzyme storage disorders, all of which could be treated with IUT. Many of these diseases are life altering and potentially fatal,

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but IUT could ameliorate the effects of the disease before they can manifest [6].

Although in theory the fetus is a perfect specimen for stem cell transplantation, clinically, IUT has experienced limited success. Therapeutic levels of engraftment have been reported in a number of cases of IUT using hematopoietic stem cells (HSCs) in humans. These were reported in 10 cases, mainly treating severe combined immunodeficiency (SCID) [6–10]. Only one attempt of IUT in hemophilia A has been made using allogenic fetal liver cells, resulting in a milder phenotype without the development of Factor VIII antibodies [11]. Attempts to treat other disorders such as Neimann Pick disease and chronic granulomatous disease have been unsuccessful owing to low levels of chimerism and engraftment [12]. These unsuccessful attempts at IUT suggest that the assumptions of immune physiology in the fetus that were originally made might be incorrect. IUT not only has the ability to provide curative treatment to severe disorders but in its development, sheds light on fetal physiology and calls into question long-held assumptions that have previously gone unconfirmed.

Fetal Hematopoietic Compartment Provides Space for Homing and Engraftment of Donor Cells

One unique aspect of fetal development that some protocols of IUT reportedly takes advantage is the large-scale migration of stem cells occurring during the early stages of hematopoiesis, and the potential space for engraftment the natural migration of stem cells from niches provide [13]. A niche is defined as a local tissue microenvironment that preserves and maintains a stem cell population or progenitor [14].

Hematopoiesis begins in the yolk sac and aorto-gonadal-mesonephric region, before moving to the fetal liver where it expands, and then finally settling in the BM where hematopoiesis will take place until the end of life (Fig. 1) [15]. Theoretically, it has been accepted that stromal support must be developed before the movement of a niche, implying stromal compartments must stand empty before stem cells migrate. Since there is a large migration of stem cells from the yolk sac to the fetal liver in gestation, there may be empty compartments to take advantage of [16]. By performing IUT while these stromal compartments are naturally empty, myeloablation can be circumvented. Because myeloablation in postnatal stem cell transplant is associated with high morbidity and mortality, this would be a particularly attractive advantage to IUT therapeutically [17].

This theory originated with Brecher et al. [18] in 1982, who used the syngeneic nonmyeloablated mouse model to demonstrate that the creation of ''space'' is not required in BM transplant. This model is analogous to IUT in that no myeloablation was used and the recipients' hematopoietic compartment was intact, the difference being that transplantation occurs postnatally. Researchers used mice and transplanted BM cells from male mice into female mice in

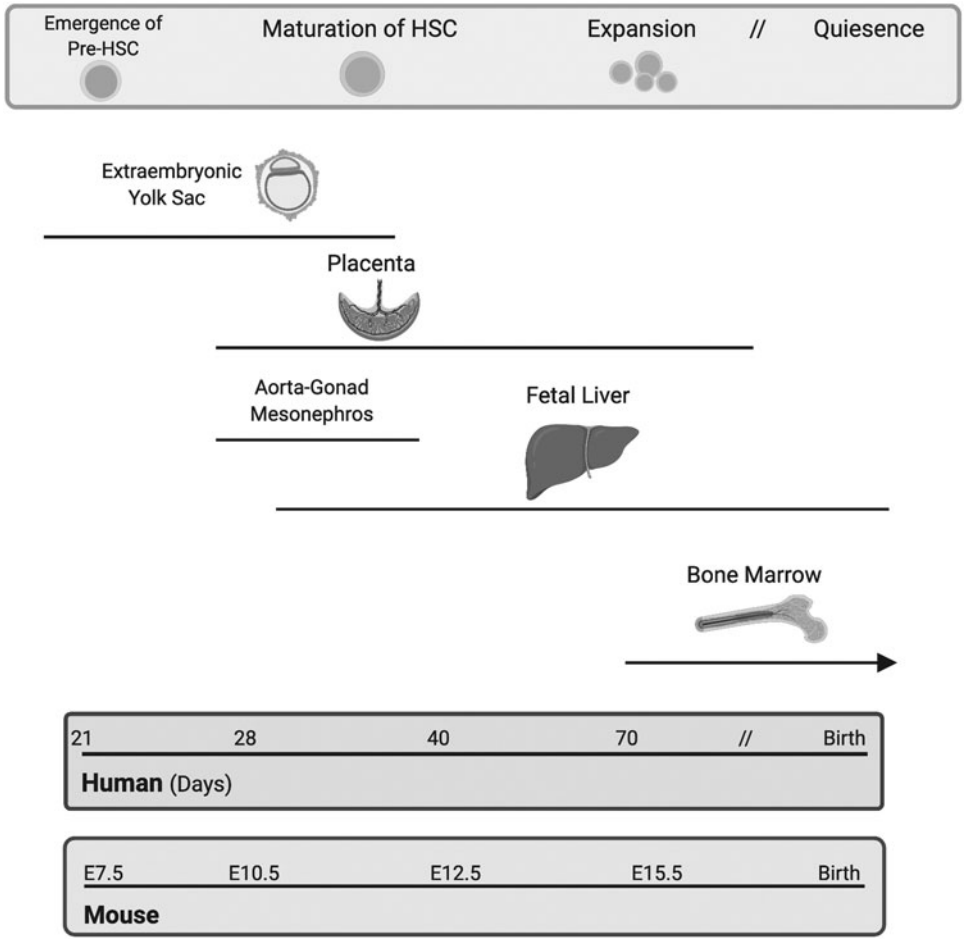

FIG. 1. Illustrates when hematopoietic stem cells colonize different fetal tissues in gestation and after birth (Bárcena *et al.*, 2009). Created with BioRender software.

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lots of 40 million at a time. It was found that with the largest transfusions of 200 million cells taking place over 5 days, donor cell presence was 20% after 13 weeks. Donor cell presence being obtained without prior myeloablation could imply a high proportion of empty niches in normal BM.

This original study is bolstered by recent research from Shimoto et al. [19], evidencing after transplantation of a large number of HSCs into mice unexposed to myeloablation, engraftment was high. It was found that HSC engraftment in BM increased at large doses (~390% of the total number of endogenous HSCs). In these transplanted mice the number of endogenous HSCs was similar, but the total number of HSCs was twofold higher than animals that were not transplanted at 24 weeks old, indicating significant engraftment in an unmyeloablated mouse. This argument for IUT has not been effectively proven clinically or experimentally. Brecher's research implies the availability of space but is limited in the use of the analogous model in which transplant occurs postnatally, not accounting for the complex fetal environment amend to [18,20,21].

Although, theoretically, fetal stem cell development creates stromal space for engraftment, the physiologic advantage may be overstated. In studies that do not use myeloablation, a large number of donor cells are required to achieve engraftment, and myeloablation seems to provide an advantage to engraftment in comparison. The few studies that provide evidence of sufficient space are weakened by use of the postnatal transplant experimental model. The cycling kinetics of HSCs in the fetus are not well established and relative excess of HSCs and progenitors in peripheral blood has been observed prenatally compared with after birth [22]. The speed at which hematopoietic activity increases within each new compartment suggests that the newly created niches are occupied quickly [23,24]. Therefore, the expectation that niches formed remain available for long enough to provide engraftment opportunity might be unfounded. This presents a significant barrier as no myeloablative conditioning to create space in any stem cell niche can be used safely in the human fetus [25]. It is worth noting all IUT attempts to date have been made after the fetal liver has been first seeded, meaning this is a potentially ''empty niche'' that is yet to be explored clinically [20,26].

Early Gestational Fetus Is Immunologically Tolerant of Foreign Antigens

Another important assumption is that the fetus is immunologically tolerant to foreign antigens, presenting a unique ''window of opportunity,'' which is not present in a mature immune system [27]. As the immune system develops in utero, the thymus is colonized by HSCs between weeks 7 and 9.5. Immature T cells, detectable beginning in week 8, undergo thymic education—a process also known as the development of central tolerance (Fig. 2) [28,29]. In the thymus, different microenvironments will direct T cell development. This involves negative selection (deletion) of prelymphocytes with high recognition of self-antigens in association with self-major histocompatibility complex (MHC) antigen, and positive selection for prelymphocytes that moderately or weakly recognize and bind to self-MHC. This combined with Treg-mediated peripheral tolerance leaves the fetus with lymphocytes that appropriately recognize only

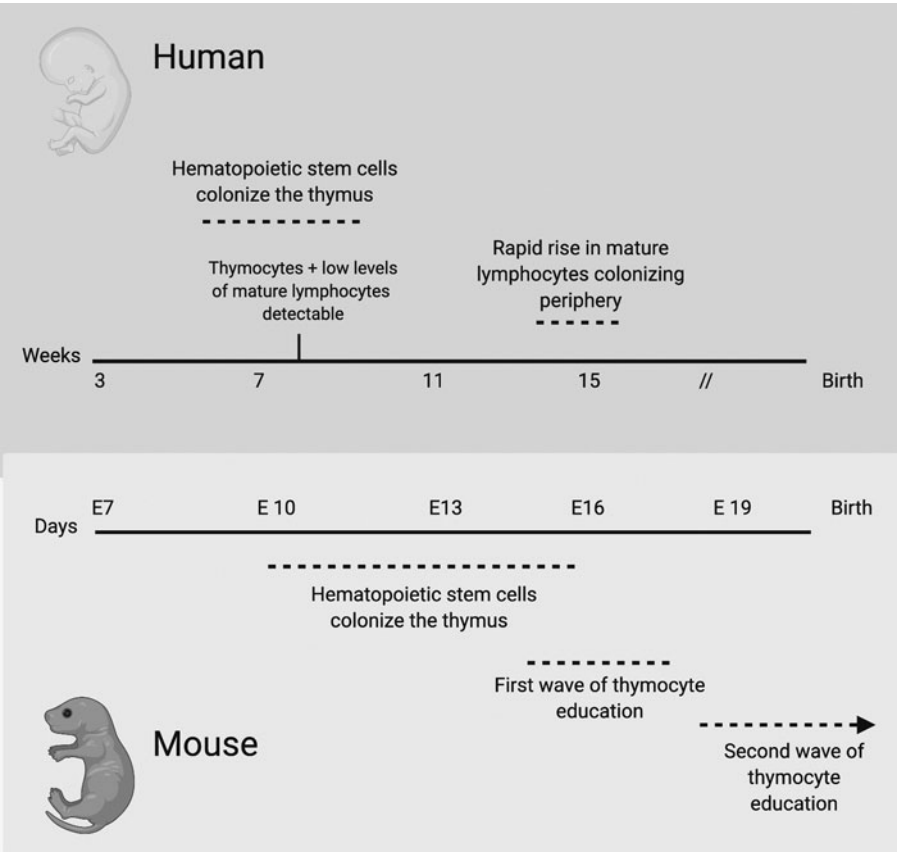

FIG. 2. Comparison of timeline of thymic development, colonization, and education in fetal humans and mice. Human, colonization of primordial thymus begins at weeks 7.5–9.5. T lineage progenitors observable by week 8. Some evidence suggests mature T cells are also observable in low levels at week 8. Between 14 and 16 weeks, mature lymphocytes leave the thymus to seed the peripheral immune system in large numbers. Mouse (BALB/c), lymphoid progenitors colonize the thymus epithelium rudiment E10–E12. Differentiation of thymocytes are observed to occur in stages pre and postnatally—the first occurring E14–E17, the second occurring E18—1 day after birth. Based on works by Muench [12] and Farley et al. [28], Created with BioRender software.

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foreign antigens [30–32]. The developing fetus then becomes equipped to recognize and eliminate pathogens and recognize ''self'' cells, forming the basis of immunity.

Naturally occurring cases of chimerism such as Owen's bovine twins [33], or the cotton-top tamarin [34] brought about the concept of ''actively acquired tolerance.'' Chimerism in IUT is the state in which donor cells have engrafted effectively to the recipient. This can occur to varying degrees—full donor chimerism would imply 100% of the BM and blood cells are of foreign or donor origin [35]. Owen [33] studied dizygotic cattle twins, remarking those who shared placental circulation were partially chimeric postnatally. This chimerism remained observable for life. With this knowledge, it was theorized that if a transplant were to occur at the time before self-antigens are defined, donor antigens could be presented in the thymus, and alloreactive T cells could be deleted facilitating allogenic transplants without the need for intense conditioning regimens [27]. Billingham et al. [36] classically evidenced this phenomenon of actively acquired tolerance in his study of mice. When a donor antigen was introduced to the fetal environment, the mice were tolerant of a postnatal skin graft from the same donor [37]. Billingham et al. [38] achieved similar results with similar studies, solidifying this theory for a number of years in IUT research.

Strong et al. [39] wanted to further examine the development of allospecific tolerance and characterize the interaction between donor-specific Treg and donor-specific host effector cells (Teff) in IUT. Prenatal allogenic chimera mice were created through in utero transplant of liver cells into allogenic recipients at E14, at the beginning of thymic selection in fetal mice (shown in Fig. 2). Kinetic analysis of donor-specific T cell populations in peripheral blood was performed over 24 weeks. Results showed a decrease of donor-specific Teff corresponding with a rise in donor-specific Treg at 4 weeks. The lowered Teff suggests that donor antigens can be recognized as ''self'' by the fetal immune system if transplanted early in thymic education. This supports a theory of the immunologically tolerant maternal–fetal environment, and the theory of transplants occurring in the correct time frame giving rise to actively acquired tolerance.

Research from Barker et al. [40] and Carrier et al. [41] evidencing a lack of advantage for engraftment of congenic versus allogenic cells provided compelling evidence of the immunologically tolerant fetus. Congenic organisms are organisms bred with a small genetic region or a single gene from another strain, but are otherwise identical to the original strain. If transplanting cells, which are as close to self as possible, provide the same level of engraftment as foreign cells, it implies a lack of immune response from the fetus. Although this is true, these studies [40,41] were limited by globally low engraftment for congenic and allogenic transplants, which could obscure differences between the groups [13].

Immunological Barriers

Conversely, more recent studies in mice suggest the immune barrier to allogenic transplantation is stronger than previously thought. Peranteau et al. [42] re-examined the engraftment of congenic versus allogenic stem cells in utero using an injection methodology allowing for the administration of higher numbers of cells compared with other research. This allowed for broadly successful levels of engraftment and more accurate results than previous comparison studies. Although at week 1 there was 100% engraftment, researchers found that every mouse injected with congenic stem cells sustained engraftment through the 6 months of follow-up, whereas only 19% of mice injected with allogenic stem cells sustained engraftment. This pattern suggests the presence of an immune response occurring with allogenic transplant that is not present in the congenic transplant [42].

Shangaris et al. [43] bolster this research by similarly comparing congenic versus allogenic IUT in mice. It was observed that all congenic transplanted animals (18 of 18) achieved engraftment compared with only 29% (5 of 17) of the animals receiving allogeneic cells. The work of Peranteau et al. [42] and Shangaris et al. [43] implies that congenic stem cells appear to have a significant engraftment advantage over allogenic stem cells, which may be mediated by a fetal immune response calling into question the assumption of fetal tolerance and strongly evidence the presence of a fetal immune response.

The presence of the fetal immune barrier is now generally accepted, although the composition of the barrier is still debated. Effectors of the adaptive and innate immune system have been proposed, and some research suggests a significant role for natural killer (NK) cells. The delayed graft rejection evidenced in Peranteau et al. [42] coincides temporally with the maturation of NK allorecognition [44]. Alhajjat et al. [45] evidenced experimentally that the outcome of engraftment or rejection after IUT could be predicted by chimerism thresholds. All mice exhibiting <1.8% chimerism experienced NK-mediated rejection, and all mice with >1.8% chimerism engrafted fully. Rejection could be avoided in mice under the threshold by depletion of NK host cells, but abruptly returned on withdrawal of the NK-depleting antibody. Although the fetal immune barrier is likely multifaceted, an understanding of fetal NK cells is important to clinical IUT success.

Maternal Antibody Response

This proposed immune barrier might not be exclusively fetal. Evidence of engraftment resistance in the preimmune fetus despite the existence of measurable chimerism [41,46] has led to an investigation of the role the maternal immune system plays in engraftment.

Merianos et al. [38] found evidence of a postnatal adaptive immune response in fetal murine recipients of IUT causing failure of engraftment that was dependent on maternal sensitization to the donor. IUT was performed with allogenic murine donors at E14. Although all recipients initially engrafted, 70% of recipients lost chimerism 2–4 weeks after birth (5 weeks post-transplant).

Analysis of the maternal humoral response was performed, finding alloantibodies against the injected donor stem cells in the serum significantly increasing at 2–4 weeks after birth. This overlap of loss of chimerism in the pup and increase in donor-specific alloantibodies in the mother suggests a postnatal maternal barrier to engraftment, perhaps mediated through murine breast milk. The magnitude of maternal humoral response corresponded to the loss of chimerism as the recipients that lost chimerism were exposed to

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significantly higher levels of maternal alloantibodies than those that retained chimerism. Merianos et al. [38] also postulated that the immune barrier was entirely maternal, as they found that if mothers who had not undergone IUT fostered and breastfed the pups, 100% of the pups retained their chimerism. Therefore, without immune influence from the mother, perhaps there is no immune barrier at all.

Riley et al. [47] further investigated the maternal barrier theory. Researchers chose a murine IUT model, using pregnant mice that were either sensitized to donor cells by injection or unsensitized to investigate if donor-specific antigens (DSAs) can cause graft rejection in the fetus. If engraftment in fetus born to mothers sensitized to donor cells was lower, it could imply a maternal immune response-mediating rejection. Fifty-two of 54 pups (96.3%) born to unsensitized mothers achieved engraftment, with a mean chimerism level of 16.3% ($P < 0.001$). By contrast, no pups born to sensitized mothers achieved engraftment ($P < 0.001$). Humoral response assays showed that in sensitized mothers, levels of IgG and IgM were significantly elevated compared with naive controls, and there was a higher fold change detected for IgG compared with IgM (70-fold vs. 10-fold at serum-to-target cell ratio 1:2, $P < 0.05$). To demonstrate that maternal DSAs were being transferred to the fetus and could potentially modulate the immune response, naive pregnant mice were injected with sensitized or unsensitized serum, and no IUT was performed. The humoral response assay showed that pups born to sensitized mothers had significantly higher levels of IgG, but IgM was not different between the two groups. These results not only imply the presence of maternal–fetal transfer of DSAs mediating the immunological barrier to transplant, but that IgG are transferred preferentially to the fetus than IgM [47].

The assumption of the maternal–fetal environment being immunologically tolerant to foreign cells seems to have been disproven. Evidence suggests that pre- and postnatal maternal–fetal transfer of antibodies may be a significant barrier to IUT success. Coupled with evidence implying the presence of the fetus' own immune response, it appears there could be immune barriers at multiple levels. The ability to overcome these immune responses is necessary to improve the clinical application of IUT.

Donor HSCs Can Home and Engraft in the Presence of Host HSCs

If the immunological barrier was to be overcome, and sufficient niches are provided, the transplanted stem cells must have the ability to survive and expand to provide any therapeutic benefit. Engraftment of a relatively small number of donor cells has the potential to heal the whole recipient if allowed to proliferate [27]. A proposed barrier to enhanced engraftment and proliferation is competition with endogenous HSCs for limited fetal hematopoietic niches. With the previously mentioned evidence that space is limited in the fetal hematopoietic compartment, it is clear that donor HSCs and endogenous HSCs must compete for that space.

Competition of Host HSCs

Donor cells would be able to compete effectively with host cells to achieve expression of therapeutic proteins such as hemoglobin after IUT. Fleischman and Mintz (reviewed in [13]) used mice with genetic anemia owing to stem cell deficiency and found that transplanting allogenic BM cells into the placenta of fetal mice reversed that anemia. With their success, the assumption of donor cells competing effectively with host cells was made. Only 20 years later, would Blazar et al. [48] extend the findings of Fleischman and Mintz to lineage deficiency. Researchers found in the case of severe combined immune deficiency that affects T cell survival, only lymphoid reconstitution occurred. This was the first study to suggest that host cell competition had the ability to limit donor cell engraftment. As discussed previously, the only successful human trials of IUT that have achieved therapeutic levels of chimerism have been in cases of X-linked SCID [7,49]. In cases where host cells are present, fetal liver and cord blood HSCs have been shown to have a competitive advantage over donor cells [22,50–52].

Studies that attempt to give donor cells a competitive advantage or disadvantage host cells illustrate the level to which competition is a barrier to engraftment. Kim et al. [53] observed in mice that donor engraftment could be enhanced through maternal administration of a pharmacological blockade of pathways critical to endogenous fetal HSC engraftment. The *CXCR4|SDF-1a* and *α4β1|VCAM-1* pathways during normal embryogenesis direct the migration of hematopoietic cells from the fetal liver to BM. Through pharmacological augmentation with AMD3100 (a *CXCR4* antagonist) and firategrast (an α4β1/7 integrin antagonist), researchers were able to increase the total donor chimerism at 6 months, by 15% and 20% more than controls ($P < 0.05$). These pharmacological antagonists have been evidenced to mobilize endogenous HSCs from adult BM hematopoietic niches, and have now been observed to do the same in fetal BM through maternal administration. This mobilization allowed for enhanced short- and long-term engraftment in mice, supporting the theory that donor cells are only competitive in scenarios where the host is disadvantaged [53].

Loukogeorgakis et al. attempted to improve donor engraftment by providing donor cells with a competitive advantage [54]. Donor cells were made competitive using a cell engineering strategy in which encapsulated glycogen synthase kinase-3 inhibitor nanoparticles were conjugated to donor hematopoietic cell surfaces to improve proliferation kinetics. It was reported that 48.5% of mice treated achieved levels of stable, long-term hematopoietic engraftment at 24 weeks post-IUT, compared with 3.4% engraftment in controls.

Conversely, evidence of successful homing, engraftment, and proliferation in animals with no hematopoietic pathology would suggest donor cells compete effectively without modifying the competitive balance in allogenic recipients. Loukogeorgakis and colleagues [43,55] managed to achieve long-term engraftment of congenic amniotic fluid stem cells in healthy mice without changing the advantage of donor or recipient [43]. This suggests that without an immune barrier, perhaps donor cells are competitive. Vrecenak et al. [56] reported successful engraftment in a healthy canine model of allogenic IUT without conditioning or immunosuppression—21 of 24 pups were chimeric at 2 months of age. Experimentally it is important to note that these studies used purified stem cells in their IUT protocols, whereas other studies like Kim et al. [53] used BM cells and low-density

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mononuclear cells together. This alters the effectiveness of IUT and perhaps skews results [57].

Alternatively, there is evidence for the optimization of receptive HSC niches through cotransplantation of human mesenchymal stem cells (MSCs). It was hypothesized that MSCs would improve the affinity of the stroma for transplant, and its ability to support definitive hematopoiesis. Early results demonstrated that cotransplantation of adult MSCs and HSCs in fetal sheep increased long-term engraftment, and the number of receptive sites for homing and engraftment [58]. More than optimizing the stroma, it has been shown that transplantation of BM-derived endothelial progenitor cells (EPCs) before HSC transplantation could optimize the recipients' vascular niche that supports long-term engraftment [59]. Mokhtari et al. evidenced that administration of allogenic MSCs and EPCs 3 days before allogenic IUT improved engraftment. Through confocal analysis it was shown that EPCs expressed more *CXCL12* than endogenous EPCs, which was thought to be responsible for the significant enhancement in engraftment as *CXCL12* is implicated in directing migration of hematopoietic cells from liver to BM [60].

Thus, post-IUT hematopoiesis appears to be restricted by limited engraftment ability owing to lack of space and receptive sites, and inability of the cells that do engraft to expand in the compartment because of competitive disadvantage and a suboptimal stromal or vascular support. Studies that artificially disadvantage endogenous cells or advantage donor cells illustrate the capacity to which competition limits engraftment, although some other studies refute these claims. Perhaps this could be attributed to study design including method of administration of cells and the purity of those cells. Alternatively, improving the receptive affinity of the niche by cotransplantation of MSCs and EPCs for stromal and vascular support improves engraftment and is an avenue through which clinical results might be improved.

Prenatal Therapy for Thalassemia

With these engraftment barriers in mind, it would seem the most favorable target disorders for IUT involves a natural selective advantage for donor cells prenatally. History of clinically therapeutic IUT for SCID, a disorder resulting in a lack of T and NK cells, points to this assumption being true. Flake et al. [7] used congenic transplants and achieved split chimerism—all T lymphocytes were of donor origin, whereas all remaining lineages originated from the host. SCID reduces numbers of host T lymphocytes that not only provides competitive advantage and space, but also immunosuppression, allowing for engraftment and proliferation of donor T cells.

Treatments for other immune deficiencies that affect the function rather than production of hematopoietic cells have been less successful. IUT for chronic granulomatous disease have seen low levels of engraftment—even using large quantities of congenic donor cells was not sufficient to compete with the hosts' hematopoietic system [12]. Instead of altering the IUT protocol, researchers have begun to accept the relatively low levels of chimerism achieved in nonmyeloablative, noncompetitive scenarios and use IUT in conjunction with other treatments like postnatal BM transplants [61]. Ideally, postnatal BM transplant is performed with a matched sibling donor, but when that is not available, options for therapy are limited. IUT that results in low-level chimerism could improve donor tolerance to allogenic

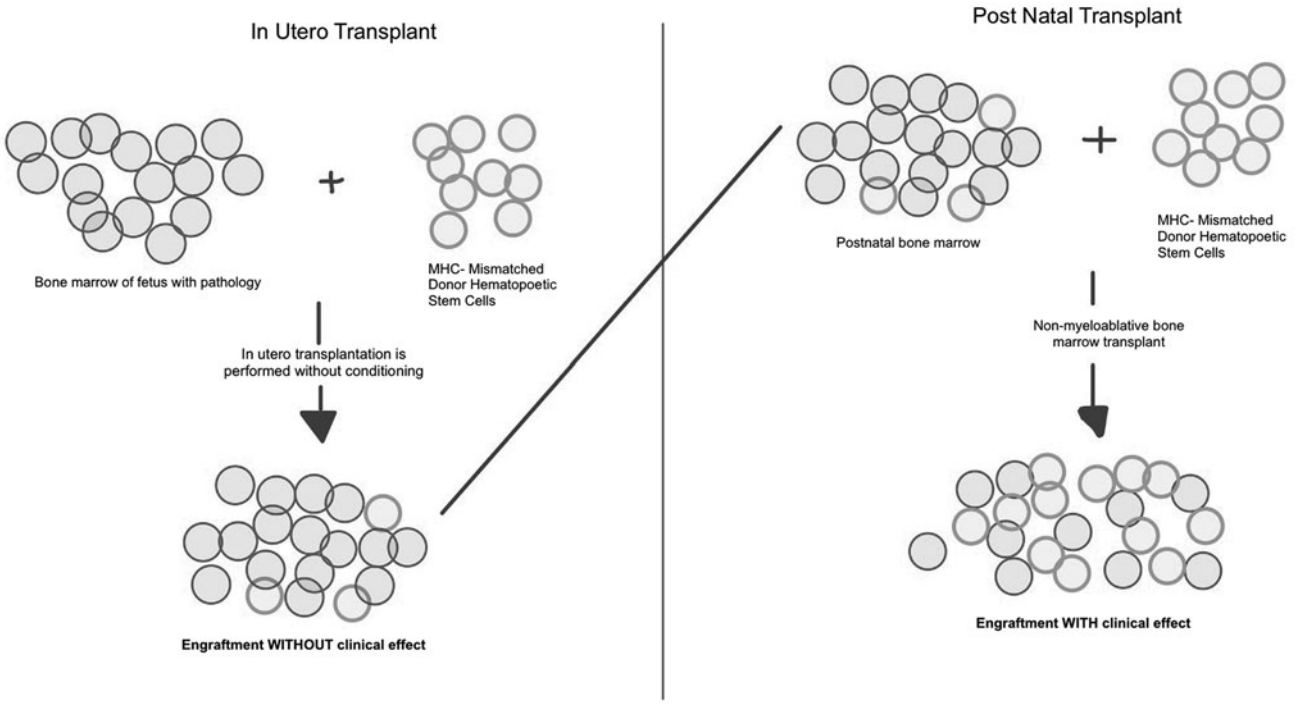

FIG. 3. Schematic illustrating the induction of tolerance after IUT followed by a second transplant after birth with the same donor to establish donor–host mixed chimerism. The level for chimerism after the ''booster'' is sufficient for a clinical effect. Based on work by Walters et al. [61]. Created with BioRender software IUT, in utero stem cell transplantation.

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postnatal transplants and significantly reduce antigenicity after birth (Fig. 3) [61]. The hemoglobinopathies are currently the primary candidates for this combined approach. Postnatal BM transplants are the only curative treatment for hemoglobinopathies, although transplant complications are a significant risk. Diagnosis of hemoglobinopathies early in gestation is well established, which allows prenatal therapies like IUT to be considered in conditions like α- or β-thalassemia (Thal), which are the disorders at the forefront of current research [12]. The hemoglobinopathies are the most prevalent monogenetic disorders, meaning they are costly for health services to treat and burden families across the world, making curative treatment that much more valuable [62].

Thalassemias are a group of genetic hematologic disorders affecting the synthesis of hemoglobin protein chains. In their most severe phenotypes, they can be fatal. α-Thalassemia major, which results from defects in all four alleles coding for α-globin, can be detected through ultrasound at 10 weeks gestation as α-globin-dependent hemoglobin production occurs at 8 weeks [63]. By 12–14 weeks of gestation, high output cardiac failure (fetal hydrops) can be observed, a condition incompatible with life [63]. Erythropoiesis in the fetal liver will grow to be ineffective, and sites of abnormal extramedullary hematopoiesis will develop to compensate. α-Thalassemia is almost always lethal in utero. Often parents choose elective termination at diagnosis [64]. β-Thalassemia major, resulting from defects in all four alleles coding for *β-globin*, can be diagnosed through fetal DNA sampling and PCR techniques as early as 10 weeks gestation [63]. Because *β-globin* dependent hemoglobin production does not occur until after birth, individuals affected may present in the first 2 years of life with severe anemia and complications including growth retardation and skeletal changes from marrow expansion [65]. Thalassemia major possesses the combination of possible early detection with severe or fatal clinical manifestation making them ideal for IUT.

Experimental data supporting the efficacy of IUT in thalassemia comes from Peranteau et al. [66] who demonstrated in an animal model of mice with Thal that a combination of IUT and nonmyeloablative postnatal transplant therapy with allogenic donor BM provided engraftment in the therapeutic range. IUT was performed through intraperitoneal injection of impure BM cells at E14 to establish low-level chimerism ($\sim$2%). This did not correct the phenotype, but donor-specific tolerance was compared in IUT mice and non-IUT mice using responder splenocytes from chimeric Thal mice. Responder populations proliferated in response to third-party stimulators and demonstrated minimal stimulation to donor splenocytes. After further postnatal transplant, engraftment was improved in all mice with chimerism levels >1% compared with non-IUT mice. As for correction of the hemoglobinopathy, in those transplanted at 4 weeks with IUT, donor hemoglobin constituted >90% of all hemoglobin in Thal recipients, which is sufficient to correct the hemoglobinopathy phenotype. Of interest, Thal mice that had received postnatal transplant and nonconditioned mice both demonstrate clinically significant donor hemoglobin levels, which might suggest that the addition of postnatal treatment is unnecessary [66]. Opposing this theory, Hayashi et al. [67] performed IUT in a Thal murine model and found that although hematologic parameters (hemoglobin, hematocrit, and mean cell volume) were improved in chimeric Thal mice, the improvement was not sustained despite stable levels of donor leukocyte engraftment.

At present, IUT for treatment of α-thalassemia major is being tested in humans. Mackenzie et al. [64] are recruiting for a phase 1 clinical trial to demonstrate the safety of stem cell transplantation for treatment of α-thalassemia major. With the understanding that the fetal immune system should be tolerant to maternal antigens, the HSCs will be harvested from maternal BM. This study will evaluate any risks to the mother or fetus, and the feasibility of a combined approach as if there are low levels of chimerism, postnatal transplantation of maternal cells will be performed. As a curative option, this could be truly revolutionary in the treatment of thalassemias and has implications in the treatment of other hemoglobinopathies.

IUT and the Future

Challenges facing the clinical success of IUT seem to be primarily related to competitive barriers to engraftment, and the adaptive and maternal immune responses. Further understanding of stem cell biology and fetal hematopoietic/immune ontogeny is vital to propel IUT to clinical success. Combined therapy shows promise, but a single-step treatment achieving therapeutic effects would be ideal. Areas of future research could include ways of competitively disadvantaging host cells, for example, through antibodies, myeloablative options that are safe in the human fetus and investigations providing better characterization of the maternal immune response. It would be pertinent for research groups to eventually develop common guidelines and programs regarding indication and protocol, and a structured follow-up for IUT for ease of comparison.

Conclusion

IUT is a rapidly growing potential therapy that has the capacity to change the lives of families globally. Its clinical advantages are clear, and in the development of these new techniques, aspects of fetal and maternal physiology are clarified. Plenty of the assumptions about fetal physiology made by those who originally pioneered IUT are being questioned or disproven. Evidence suggests the fetus may not be immunologically tolerant, potentially experiencing both endogenous and maternal immune modulation. Sufficient space in the hematopoietic compartment may not be as available as once assumed, and host HSCs are likely differentially advantaged compared with donor cells. Despite these discoveries and barriers to engraftment, insights from animal and human studies suggest that IUT is both possible and incredibly valuable clinically. IUT for treatment of thalassemia in particular shows promises, suggesting the barriers to donor engraftment can be breached.

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