



Molly Pomerance. *Flying Peppers*. Acrylic on canvas, 24" × 30".

The benefits of in utero transplantation in the early treatment of hematopoietic and other types of birth defects will continue to encourage development of this approach.

Stem Cell Transplantation in the Fetus

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Background: *In utero transplantation (IUT) of hematopoietic stem cells has the potential to treat a large number of hematologic and metabolic diseases amenable to partial replacement of the hematopoietic system.*

Methods: *A review of the literature was conducted that focused on the clinical and experimental experience with IUT and, in this context, the development of the hematopoietic and immune systems.*

Results: *Successful application of IUT has been limited to the treatment of various types of immunodeficiencies that affect lymphocyte development and function. Other congenital defects such as the thalassemias have not resulted in clinically significant engraftment. Recent efforts at understanding and overcoming the barriers to engraftment in the fetus have focused on providing a selective advantage to donor stem cells and fostering immune tolerance toward the donor cells. The critical cellular components of the graft that promote engraftment and tolerance induction are being evaluated in animal models. Improvements in engraftment have resulted from the inclusion of T cells and/or dendritic cells in the graft, as well as a strategy of combined prenatal and postnatal transplantation.*

Conclusions: *The advantages, necessity, and benefits of early treatment will continue to encourage development of IUT as a means to treat hematopoietic and other types of birth defects.*

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Submitted July 28, 2003; accepted December 23, 2003.

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No significant relationship exists between the authors and the companies/organizations whose products or services may be referenced in this article. This work was supported in part by NIH grant DK59301.

Introduction

A growing list of birth defects can be diagnosed before birth as a result of rapid advances in molecular and diagnostic medicine. Prenatal diagnosis offers the opportunity for early management of disease. Surgical intervention has been used to treat certain types of birth defects and cancers that would otherwise place the fetus or neonate at high risk for death or malformation.¹ Fetal gene therapy is also being studied as a means to treat a range of genetic birth defects. Although the lure of such a form of therapy is evident, concerns for the safety and efficacy of

genetic therapy need to be overcome before it can be applied. Fetal cellular therapy is another option for the treatment of a variety of birth defects and is the focus of this review.

There are several perceived advantages of in utero transplantation (IUT): (1) The rapid growth of the fetus provides opportunity for engraftment and expansion of donor cells, (2) the undeveloped immune system of the fetus cannot reject foreign tissues, and (3) early treatment of disease is beneficial or critical for effective treatment. These assumptions come from observations made half a century ago on cattle twins. Some cattle twins are dizygotic yet share placental circulation, a condition known as freemartinism. These freemartin cattle were shown to accept skin grafts despite the genetic differences between the twins.²⁴ Persistent chimerism was documented in freemartin cattle,⁵ indicating that a significant exchange of cells occurred during development, which can result in the acquisition of donor-specific immune tolerance that permits skin grafting. The goal of fetal transplantation is to mimic the effects of placental cross-circulation by directly transplanting cells into a fetus, thereby generating chimerism and tolerance to the donor tissue. Sufficient levels of chimerism could directly cure some diseases or, alternatively, immune tolerance specific for the donor tissue could be used as a foundation for further postnatal transplantation.

The limited success with IUT has resulted in a reevaluation of the underlying assumptions that have spurred research and clinical testing of IUT. This review explores the current understanding of the development of the human hematopoietic and immune systems as well as summarizes preclinical and clinical findings with IUT. Emphasis is placed on aspects of human ontogeny and IUT that are poorly understood, as well as on the design of methods at improving the outcome of IUT.

Development of the Human Hematopoietic System

IUT has been pursued as a treatment for a number of diseases primarily affecting the hematopoietic system. Recent findings that hematopoietic stem cells (HSCs) may be capable of forming nonhematopoietic tissues, as well as the discovery of other types of multipotent stem cells, suggest that IUT may find wider application.⁶ Nonetheless, understanding of the development of the hematopoietic system is important to all types of IUT as it provides a model for understanding the challenges associated with achieving engraftment and chimerism. Hematopoietic development also encompasses the development of the immune system, which affects the timing of IUT. Additionally,

unique aspects of fetal hematopoietic tissues have brought attention to the use of fetal tissues as a source of donor cells for IUT.

Migration of Hematopoiesis During Human Development

In adults, hematopoiesis takes place primarily in the bone marrow (Fig 1). However, no bones exist in the embryo, and until 12 weeks' gestation, the long bones are not of sufficient size to develop a marrow cavity that can support hematopoiesis.⁷ Prior to this time, hematopoiesis resides at first in the yolk sac outside the embryo and within the embryo in a region described as the paraaortic splanchnopleura and later as the aorta-gonad-mesonephros.⁸⁻¹¹ Hematopoiesis appears in the liver at approximately 5 weeks' gestation¹² and remains the primary site of hematopoiesis until mid-gestation, when bone marrow hematopoiesis exceeds that of the liver.^{13,14} Unlike in mice, the spleen in humans is never a major hematopoietic organ.^{15,16} Another hematopoietic organ is the thymus, which becomes anatomically mature by 15 weeks' gestation.¹⁷ Although T lymphopoiesis dominates the thymus, a range of other hematopoietic cell types can also be found in this tissue.¹⁸⁻²³

Peripheral blood is another source of progenitors and HSCs in the fetus. The presence of HSCs in the fetal circulation is best demonstrated by the long-term engraftment that has followed transplantation of umbilical cord blood harvested at term.²⁴⁻²⁶ Analyses of pre-term fetal blood has indicated the presence of hematopoietic progenitors as early as 7 weeks' gestation.²⁷⁻³⁰ Indeed, the fre-

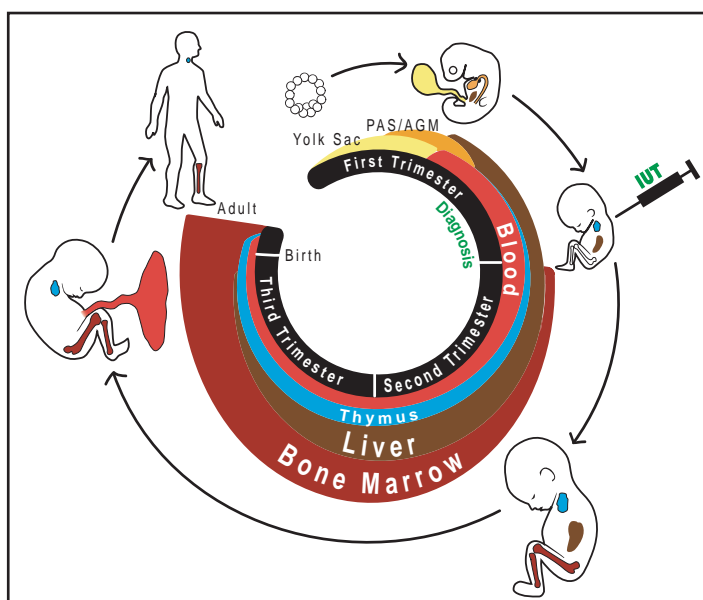


Fig 1. — Ontogeny of hematopoiesis in humans. Changes in the anatomical location of hematopoiesis during human development are shown on a circular timeline. In utero transplantation typically occurs at the beginning of the second trimester following prenatal diagnosis at the end of the first trimester.

quency of progenitors is higher in pre-term blood than at term. The CD38-CD34⁺⁺ subset, which contains HSCs, has also been observed in the blood of first trimester fetuses²⁹ as well as second trimester fetuses (M.O.M., A.B., unpublished data, 2003). The presence of a high frequency of circulating HSCs in the fetus presumably facilitates the migration of HSCs to new sites of hematopoiesis.

HSCs transplanted in utero presumably join the procession of HSCs in the fetal circulation before engrafting either the liver or bone marrow. However, the low rates of engraftment that have resulted from most attempts at IUT suggest inefficiency in the engraftment of donor HSCs (Fig 2). A study of circulating HSCs in adult mice has shown that these cells rapidly enter and leave the circulation.³¹ A bolus of donor HSCs may overwhelm the capacity of the fetal hematopoietic tissues to absorb the donor cells. However, transplants done on adult mice in the absence of any cytoablative conditioning do not support the contention that hematopoietic tissues have a finite capacity to accept donor HSCs. Engraftment levels closely match the ratio of donor-to-host HSCs.³² Thus, a high dose of HSCs should lead to high levels of engraftment in the fetus, yet this has not been observed. For instance, an estimated 30-fold excess of HSCs were transplanted into a fetal patient with chronic granulomatous disease and no chimerism was detected.³³ Therefore, either the dynamics of HSC homing differ in the fetus or other barriers to engraftment exist.

Homing of HSCs is multistep process that is in part affected by the cell cycle status and expression of adhe-

sion molecules by HSCs. Fewer cycling HSCs have been observed in mobilized peripheral blood compared with the bone marrow in adults.^{34,35} Moreover, analysis of mice in which progenitors were mobilized by treatment with granulocyte colony-stimulating factor and cyclophosphamide has indicated that mobilized progenitors are those that have recently undergone cell division.³⁶ This observation meshes with the ability of many early-acting growth factors to stimulate the mobilization of progenitors as well as the association between the presence of mobilized progenitors and the levels of hematopoietic activity. By extrapolation, it is likely that vigorous hematopoiesis in the developing fetus leads to high levels of circulating progenitors that are likely in the G₀/G₁ phase of the cell cycle. The heightened proliferation of fetal HSCs compared with adult HSCs³⁴ may confer a greater ability of fetal HSCs to engraft in the fetal recipient.

Sources of Donor Cells for In Utero Transplantation

Successful IUT has been performed with both fetal and adult HSCs, demonstrating that either source is suitable for IUT (Fig 2). Fleischman and Mintz³⁷ established that adult mouse bone marrow could engraft fetal mice, demonstrating that there is no developmental barrier that prevents adult HSCs from engrafting a fetus. Nonetheless, the first successful prenatal transplants to treat immunodeficiencies relied on fetal tissues from elective abortions as a source of donor cells. Fetuses afflicted with severe combined immunodeficiency (SCID) or bare lymphocyte syndrome received transplants of 7 to 8 weeks' gestation fetal liver and thymus by intravenous injection, which resulted in T-cell reconstitution in the recipients.^{38,39} Stringent quantitative comparisons of fetal vs adult HSCs in the IUT setting have not been performed, but improved engraftment using mixed populations of fetal liver cells have been noted in some animal studies. A higher number of chimeric goats were born when they received transplant using fetal livers obtained from sheep than when they received transplants with T-cell-depleted adult bone marrow.⁴⁰ However, the levels of chimerism in both groups were low (<1%), and thus in this study there was no notable advantage to using one type of tissue over the other. However, fetal liver was found to reconstitute murine SCID- or HSC-deficient fetuses (*W* locus mutants) nearly 10-fold better than an equal number of adult bone marrow cells.^{41,42} Murine fetal liver cells were also found to better engraft normal mice and mice with hemoglobinopathies.⁴³ Although these results may be partly due to differences in the cellular composition between the fetal and adult grafts, the clear superiority of the fetal grafts in some of these transplant models indicates that developmental factors may favor the engraftment of fetal tissues in a fetal recipient.

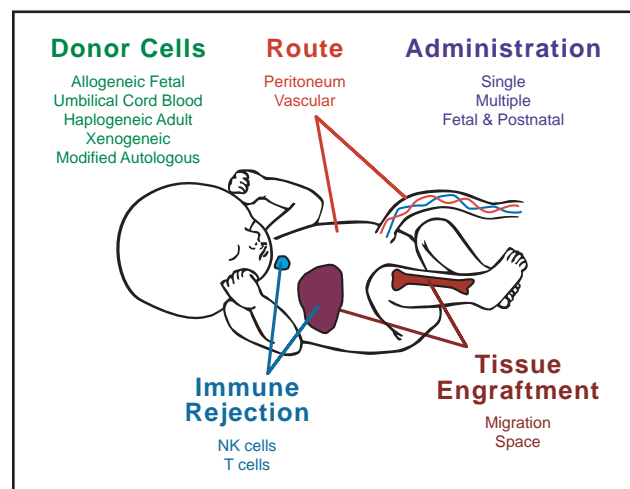


Fig 2. — Variables and potential obstacles to fetal transplantation. A variety of donor cell sources have been used for in utero transplantation, but in most cases fetal tissues obtained from elective abortions or parental bone marrow have been used. Future transplants may even use xenogeneic sources of cells or autologous cells that have been modified ex utero to express desired gene products. Cells are generally administered as a single intraperitoneal injection, but multiple injections have also been used. Combined prenatal and postnatal transplants have also been shown to be effective in some human and animal transplants. Potential barriers to fetal transplantation include rejection by the emergent fetal immune system, inefficient migration of donor cells to the host tissues, and limited displacement of the host stem cells.

Safety and availability will continue to govern the choice of which tissue to use in future prenatal transplants. The threat of graft-vs-host disease (GVHD) initially favored the choice of fetal tissues for IUT since these tissues contain relatively few mature T cells when harvested prior to 15 weeks' gestation.^{33,44} Several groups have reported procedures and methods for obtaining, processing, and banking fetal tissues, making them suitable for IUT.^{13,14,44-48} Varying legal restrictions on the availability and maximum age of abortions dictate the accessibility to fetal tissues in different countries. Methods of processing adult hematopoietic tissues that enrich HSCs and deplete T cells has made adult bone marrow transplantation another safe alternative for IUT.⁴⁹⁻⁵¹ Some success in transplanting human CD34⁺ cells isolated from umbilical cord blood into fetal pigs has also been reported, but a high rate of intrauterine deaths was noted.⁵² These deaths were possibly due to GVHD resulting from the remaining T cells in the processed grafts.

Development of the Human Immune System

The human fetus is protected from most pathogens. The womb provides a physical barrier between the fetus and the outside world, and the maternal immune system guards against infection of the mother and fetus. If this arrangement were to suffice, then it might be conceivable that the development of the immune system could be delayed until early in the third trimester of pregnancy, leaving several months for the generation of lymphocytes. However, it is clear that a fetus is vulnerable to infection when the physical barriers to its environment are breached or when the maternal immune system has failed to rapidly clear an infection. Perhaps for this reason it is advantageous that lymphocytes are found in the fetus as early as the end of the first trimester. Little is known about the ontogeny of the human immune system other than a general picture of when the different lineages of lymphocytes begin to appear in development. Understanding the functional capacity of the immune system throughout fetal development is important to understand the barriers that prevent engraftment of foreign cells.

Natural Killer Cells

Natural killer (NK) cells are the early arrivals among lymphocytes. Indeed, before the development of T cells, NK cells may constitute the earliest and sole bastion of cellular defense in the embryo and fetus. NK cells in the liver appear as early as 6 weeks of gestation.^{53,54} Analysis of NK cell frequencies in fetal blood at different gestational ages has indicated that NK cells are most frequent early in gestation (29% at 13 weeks) and decrease exponentially until

term (6% at 38 weeks).⁵⁴ The functional capacity of these fetal blood NK cells is unknown.

The fetal liver is not the only site at which NK cells are generated. NK cells and their progenitors have also been detected in the fetal thymus.^{19,55,56} Immunohistochemical studies of thymic rudiment have indicated that there is a lymphoid population in the perithymic mesenchyma between 7 to 9.5 weeks of gestation. This lymphoid population expresses markers associated with NK cells and immature T cells.^{53,57-59} Analyses of thymi at later time points in gestation have unequivocally demonstrated the presence of CD56⁺CD3⁻ NK cells.^{19,56} Thus, NK cells are generated in at least two different organs in the fetus, the liver and thymus, at the time when these organs become hematopoietic. The early development of NK cells suggests an important role for these cells in fetal immunity.

The cytolytic activity of purified fetal CD56⁺CD3⁻ NK cells from fetal livers older than 15 weeks' gestation has been demonstrated.^{53,60} The cytotoxic activity of the fetal NK cells appeared to be somewhat less than that observed with adult NK cells. Direct comparisons of fetal and adult NK cells are needed to definitively demonstrate any decreased functional capacity of the fetal cells. There may be a link between the gestational age of the NK cells and their functional activity, with younger NK cells appearing to exhibit less cytolytic and lymphokine-activated killer activity than older cells. Moreover, NK cells isolated from term umbilical cord blood are reported to have reduced cytotoxic activity, further suggesting that fetal NK cells may be somewhat functionally deficient.⁶¹

T Cells

Anatomic maturation of the thymus gland at 14 to 15 weeks' gestation is the developmental milestone that determines the timing of most transplants that have been attempted in fetuses (Fig 1).⁶² After the 15th week of gestation, T cells begin to rapidly accumulate in tissues outside of the thymus, thus the likelihood for rejection of foreign tissue is thought to increase.⁵⁸ However, even before complete maturation of the thymus, T cells have been observed in the late-stage embryo.⁶³ Intrathymic CD3⁺ T cells have been detected at 8 weeks' gestation.^{58,64} T cells were also observed in the liver as early as 7 weeks' gestation and in peripheral blood at 9 weeks' gestation.^{33,65} Nevertheless, it is important to consider that $<1 \times 10^6$ peripheral T cells are estimated to exist before 14 weeks' gestation.^{33,44} Although this number is likely insufficient for a vigorous antigen-specific response, a higher proportion of the T-cell pool can invoke a response to foreign major histocompatibility antigens. Of further concern is the period of time required for donor cells to take part in T-cell selection in the thymus. Donor HSCs and progenitors may require several weeks to colonize the thymus and contribute to the selection of T cells.^{20,21} During this peri-

od of engraftment, T cells could be generated that are reactive to the donor cells and therefore contribute to graft rejection (Fig 2). A greater understanding of the ontogeny of T-cell immunity, specifically an assessment of the functional capacity of T cells, during the early second trimester is needed to understand the threat that these cells pose to transplanted foreign cells.

Diversity within the T-cell pool develops rapidly following thymic maturation. A similar frequency of CD56⁺ natural cytotoxic T cells has been observed in the mid-gestation fetus as in the adult,⁶⁶ whereas a higher frequency of fetal T cells has been found to express the γ/δ T-cell receptor.⁶⁷ These γ/δ T cells are capable of mediating graft rejection.⁶⁸ However, graft rejection is primarily associated with α/β T cells. The V β chain repertoire of fetal T-cell receptors has been analyzed, and a diverse repertoire comparable to that of adults was observed as early as 16 weeks' gestation.⁶⁶ This suggests that sufficient diversity to respond to a range of antigens develops in the T-cell receptor repertoire soon after thymic maturation. However, reduced diversity within the V β chain families of late-gestation fetal and neonatal blood samples has been observed with analysis techniques sensitive to minor sequence differences.^{69,70} Thus, not surprisingly, the diversity of the fetal T-cell receptor repertoire is still less than that observed in adults, although it should be sufficient to mount a response to allogeneic stimuli.

A few studies have addressed the functional capacity of T cells in the human fetus. Mixed lymphocyte cultures have measured proliferation of T cells from livers as young as 9 weeks' gestation in response to stimulation by allogeneic cells.^{65,71-73} However, reduced proliferation of allostimulated neonatal T cells has been observed when compared with adult responses.⁷⁴⁻⁷⁷ Cultured fetal T cells have further exhibited some defects in cytotoxic responses, which could be reversed by prestimulation with cytokine.^{72,78} T cells from umbilical cord blood also have reduced perforin expression.⁷⁹ Other proteins important in the functioning of T cells, such as CD3 and CD28, are expressed at similar levels on neonatal T cells compared with adult cells, but signaling through these proteins is attenuated in neonatal T cells. Neonatal T cells failed to increase CD25, CD154, and CD178 (fas ligand) expression when stimulated through CD3 and CD28.⁸⁰ Moreover, the peripheral T-cell pool at birth is composed of primarily CD45RO⁻CD45RA⁺ naive T cells with few CD45RO⁺CD45RA⁻ memory T cells, common in adult blood.^{74,81-84} Surprisingly, a much higher frequency of CD45RO⁺CD45RA⁻ T cells has been found in mid-gestation blood and spleen.^{66,85} Higher expression of a panel of markers associated with T-cell activation was also observed on fetal T cells compared with newborn T cells.⁶⁶ The reason for the expression of cell-surface antigens associated with activation on fetal T cells remains unclear, but it indi-

Table 1. — Summary of Clinical Experience With In Utero Transplantation*

Indication	No. of Cases	Outcome and Comments	References
Immunodeficiencies:			
Bare lymphocyte syndrome	1	Alive and well with reconstitution of T cells after fetal liver and thymus transplant.	38, 86
Severe combined immunodeficiencies	9	8 cases with lymphoid engraftment and 1 case electively terminated without engraftment.	49, 50, 87-93
Chediak-Higashi syndrome	1	Born with no engraftment.	93
Omenn syndrome	1	T-cell engraftment.	92
Chronic granulomatous disease	3	2 cases born with no engraftment and 1 procedure-related death.	33, 86, 102
Erythroid Disorders:			
α -Thalassemia	2	No chimerism in 1 case and microchimerism with donor-specific tolerance noted in 1 case.	51, 96
β -Thalassemia	12	No alleviation of disease in surviving births. Evidence of chimerism ($\leq 4\%$) in 3 recipients.	86, 93-101
Sickle cell anemia	3	No engraftment.	99, 101
Rh isoimmunization	3	Donor-specific T-cell tolerance observed in 1 recipient.	103-105
Storage Diseases:			
Globoid cell leukodystrophy	3	No engraftment in 2 cases. Hyperengraftment and fetal death in 1 case.	106, 107
Hurler's syndrome	1	No engraftment.	108
Niemann-Pick disease (type A)	1	Patient alive and well, no engraftment data reported.	86
Metachromatic leukodystrophy	2	No chimerism.	94

* Information on unpublished cases obtained from published reviews.^{104, 164}

cates that the study of neonatal T cells can be misleading when the aim is to understand the functional status of the fetal immune system early in the second trimester. Our limited understanding suggests that fetal T cells are functional and can take part in rejecting transplanted cells, even if there is some attenuation of T-cell function and despite the naiveté of the fetal immune system.

Clinical Experience With In Utero Transplantation

To date, we are aware of 42 transplants that have been performed on fetuses to treat various ailments (Table 1). Successful outcomes have been achieved only in cases of

immunodeficiency such as bare lymphocyte syndrome and SCID cases.^{38,49,50,86-91} The SCID cases successfully transplanted include the X-linked form of the disease that affects the common γ chain of the interleukin-2 receptor (CD132), SCID T-B⁺NK⁻, and other forms of the syndrome including one that is characterized by the presence of NK cells, SCID T-B⁺NK⁺.⁸⁹ These findings are of particular interest since they indicate that engraftment can occur, at least in some cases, in fetuses with functional NK cells.⁹⁰ It is important to note, however, that although T-cell engraftment occurs readily in all SCID patients transplanted, a lack of B-cell engraftment has been noted in a case of SCID T-B-NK⁺.⁹² Similarly, in a case of Omenn syndrome, which is characterized by T- and B-cell defects,¹⁰⁹ T-cell engraftment was achieved by IUT but not B-cell reconstitution.⁹²

Table 2. — Selected Publications of Syngeneic (Congenic) and Allogeneic In Utero Transplantation in Animals

Recipient	Donor	Outcome and Comments	References
Dorset-Merino sheep	FL	10%-29% chimerism of erythrocytes and leukocytes in 3 of 4 recipients. No GVHD.	112
Rhesus monkeys	FL	3%-20% multilineage chimerism in 4 of 5 recipients for up to 2 years. No GVHD.	113-115
Normal mice*	FL or ABM	Engraftment in 1%-50% of recipients but low donor cell levels (<1%). Donor-specific immune tolerance observed in some mice.	116, 117
Normal mice*	ABM or enriched HSCs from mobilized peripheral blood	Microchimerism leading to either donor-specific immune tolerance or immune sensitization. Evidence of clonal deletion and anergy of alloreactive T cells.	118-123
Normal mice*	ABM with dendritic cell precursors	Increased incidence of allogeneic engraftment with co-transplantation of dendritic cell precursors, but higher incidence of GVHD also noted.	124
Normal mice*	T-cell-depleted allogeneic ABM in utero and postnatal splenocytes or ABM	Postnatal transplant of donor lymphocytes or ABM resulted in conversion from low-level engraftment to complete hematopoietic replacement with minimal incidence of GVHD.	125-127
Normal mice*	ABM with attenuated T cells	Co-transplantation of host reactive T cells that have been photochemically treated to prevent GVHD increases chimerism levels to 13%.	128
SCID and NOD/SCID mice	ABM	High incidence and levels of multilineage engraftment. Substantial but not complete immune reconstitution.	129-131
β -Thalassemic and sickle cell disease mice	FL or ABM	Levels of erythroid chimerism up to 50%.	43, 132, 133
Anemic mice with HSC defects (<i>W</i> locus mutants)	FL or ABM	High incidence and levels of multilineage engraftment.	37, 42, 117, 134
Mice with muscular dystrophy	FL or ABM	Evidence of low-level myocyte engraftment.	135
Mice with factor X deficiency	FL	Multilineage engraftment, including engraftment of hepatocytes and partial reconstitution of factor X levels.	136
Mice with mucopolysaccharidosis type VII	Gene-modified FL expressing β -glucuronidase	Microchimerism with delayed onset of disease.	137
Dogs with α -L-iduronidase deficiency	Gene-modified ABM expressing iduronidase	Microchimerism and immune tolerance to iduronidase, but no alleviation of the disease.	138
Goats	FL	Persistent chimerism noted in one study but no sustained engraftment in another.	139, 140

* Normal mice refer to various inbred wild-type mouse strains that do not have a well-characterized hematopoietic defect.
 SCID = severe combined immunodeficient
 NOD = nonobese diabetic
 FL = fetal liver
 ABM = adult bone marrow

The measured success in engrafting SCID fetuses is not unexpected since SCID patients have also been transplanted postnatally with minimal or no cytoablation.¹¹⁰ Even with minimal HSC engraftment,⁵⁰ the profound cellular deficiencies and the ability of normal mature lymphocytes to rapidly divide provide an overwhelming advantage to the donor lymphoid cells, resulting in split chimerism. Given that postnatal transplantation is an option for SCID patients, the need to treat this disease by IUT has been questioned. Certainly, IUT carries some level of risk due to the procedure itself and there is a risk of infection or GVHD. These risks are also present after birth, and the data are insufficient to make a meaningful comparison of these risks between prenatal and postnatal transplantation. We argue that prenatal treatment is favored given the findings that clinical signs of GVHD were observed in a high number of SCID patients owing to in utero engraftment by maternal lymphocytes.¹¹¹

IUT has been attempted in a number of other diseases with no significant clinical impact (Table 1). Hematologic diseases characterized by a defect in the function of mature cells, such as hemoglobinopathies and chronic granulomatous disease, have not been durably engrafted. Attempts at treating a number of storage diseases by IUT have also failed. All of these diseases are similar in that the host's hematopoietic precursors are unaffected by the inherited disease. Only thalassemic patients have shown a survival advantage of normal erythrocytes compared with abnormal erythrocytes. This advantage manifests itself only in the final stages of erythropoiesis, yet durable erythroid engraftment has not been observed in patients receiving transplants for α - or β -thalassemia.^{51,86,93-101} Since lymphoid, but not myeloid or erythroid, engraftment occurs in SCID patients following IUT, these findings indicate that one major barrier to engraftment is a lack of space and competitive advantage for the donor HSCs (Fig 2). An immunologic barrier to

engraftment owing to fetal NK cells or T cells may also exist and further limit engraftment, but it is not the sole reason for the failure of donor cells to engraft in the fetus.

In Utero Transplantation in Animals

IUT has been studied in a variety of animal models with varying degrees of success. Encouraging results were reported for allogeneic transplants performed in sheep and rhesus monkeys. Persistent engraftment was demonstrated, with the levels of chimerism reaching 29% in the sheep¹¹² and over 15% in the monkey¹¹³ (Table 2). The fetal sheep is also a permissive recipient for xenogeneic cells, having been engrafted with human, goat, and swine hematopoietic cells (Table 3).^{141,151,152} Despite the genetic differences between sheep and humans, maintenance of human HSCs in the sheep was demonstrated by harvesting human cells from primary recipients and successfully transplanting them into secondary sheep.^{142,153-155} These studies demonstrate that IUT can result in hematopoietic chimerism, even across widely disparate species barriers.

Many aspects of IUT have also been studied in the mouse. Normal wild-type mice are generally resistant to engraftment by IUT (Table 2), suggesting they are good models for IUT in humans. IUT is effective when the donor cells have some proliferative advantage over the recipient's cells, underscoring the advantage the resident HSCs have over the donor cells. For instance, high levels of chimerism were obtained when normal HSCs were transplanted into mice with a defect in HSCs resulting from mutations in *c-kit*, the gene encoding the receptor for stem cell factor.³⁷ When normal HSCs are transplanted into these mice with *W* locus mutations, they effectively replace the host's hematopoiesis due to their competitive advantage.¹⁵⁶ Immunodeficient SCID or nonobese diabet-

Table 3. — Selected Publications of Xenogeneic In Utero Transplantation in Animals

Recipient	Donor	Outcome and Comments	References
Sheep	Human FL or ABM, including enriched populations of HSCs	High incidence of engraftment with up to 28% chimerism in the BM.	141-146
Baboons	Human FL	1.5% chimerism in 1 of 3 animals transplanted.	147
Normal mice	Human FL	27% of mice had evidence of human cells (0.2%-0.5%).	148
Anemic mice with HSC defects (<i>W</i> locus mutants)	Human FL or FBM	50% of mice had evidence of human cells ($\leq 1.0\%$).	149
NOD/SCID mice	Human FL or FBM CD34+ cells	High incidence of engraftment and levels of chimerism.	150
Sheep	Swine ABM	11% of sheep had evidence of swine cells (1%-3%).	151
Sheep	Goat FL	16% of sheep had evidence of goat cells (0.4%-6%).	152
Goats	Sheep FL or T-cell-depleted ABM	High incidence of microchimerism in the peripheral blood.	40

FL = fetal liver
 FBM = fetal bone marrow
 ABM = adult bone marrow
 HSCs = hematopoietic stem cells

ic (NOD)/SCID mice provide further examples of permissive recipients due to the vacancies in the lymphoid compartments in these animals.¹²⁹⁻¹³¹ Lymphoid engraftment dominates in these recipients, but moderate levels of myeloid chimerism have been observed.

NOD/SCID mice have also been successfully engrafted by human fetal cells.¹⁵⁰ Erythrocyte chimerism was achieved in mice with β -thalassemia.^{45,132} Although these mice do not have an HSC or even a notable progenitor defect, normal erythrocytes have a distinct survival advantage. The presence of a high frequency of donor-derived blood cells in these different murine disease models is likely not to be due to increased HSC engraftment, but rather is likely to be due to the competitive advantage that the few HSCs that did engraft has over the host's cells. The fact that significant chimerism can be achieved in these mice provides hope that IUT may yet prove to be effective in treating diseases in which normal hematopoietic cells have a proliferative or survival advantage over the patient's own cells.

Future Directions

Achieving clinically relevant levels of engraftment by IUT requires a more complete understanding of the barriers that limit engraftment. Evidence suggests that engraftment is curtailed by the presence of host HSCs (Fig 2). The fetal immune system, specifically NK cells and emerging T cells, may further limit engraftment by a host-vs-graft mechanism. It is not difficult to imagine a situation in which competition by the host's own hematopoietic cells initially limits engraftment to a small number of HSCs and then these cells are eventually rejected before any significant chimerism and immune tolerance can be established. A threshold effect may occur in which immune rejection is avoided and immune tolerance ensues if a sufficient level of chimerism is achieved early after transplantation. The composition of the graft may play an important role in dictating its success since various cell types may help to facilitate engraftment and/or tolerance induction.

Donor T cells have been one focus of efforts to improve engraftment. Despite the known risk of GVHD,¹⁵⁷ T cells and their subsets are being studied as a possible means to provide space and to counter rejection of donor HSCs. The number of allogeneic T cells that can be safely transplanted into a human fetus is unknown. A standard, based on experience in adult transplantation, of 1 to 2×10^5 /kg has been used. Based on an approximate fetal weight of 50 to 100 g, this allows for transplant of only 1×10^4 T cells. However, the rapid growth of the fetus and the poorly understood fetal immune system raise the possibility that the number of T cells that may be transplanted safely could be higher.

Many examples point to a beneficial role for donor T cells in the IUT setting. Early work indicated improved

engraftment in fetal sheep with co-transplantation of a low number of T cells.¹⁵⁷ In murine studies, a correlation between high levels of engraftment and a high risk of GVHD due to the presence of T cells in the graft has also been observed.^{124,128} Bhattacharyya et al¹²⁸ more recently tested the effects of co-transplanting attenuated host-reactive T cells. These investigators stimulated donor T cells using recipient cells and then rendered the donor T cells nonproliferative by treatment with S-59 psoralen and UVA light. Transplantation of these T cells together with T-cell-depleted bone marrow resulted in over 10% engraftment with minimal incidence of GVHD.

Donor NK cells also deserve attention in the IUT setting. The beneficial effects of NK-cell-mediated graft-vs-host activity was highlighted in a report by Ruggeri et al.¹⁵⁸ These investigators demonstrated in cancer patients a high frequency of donor-derived NK cell clones that showed lytic activity against host lymphocytes and leukemic cells. Moreover, patients receiving transplants from donors expressing incompatible killer-cell immunoglobulin-like receptors (KIRs) had higher rates of engraftment without clinical evidence of GVHD. Transplanting alloreactive NK cells was also shown to aid donor cell engraftment in adult mice with minimal cytoablation.¹⁵⁹ Thus, donor NK cells may also prove useful to aid engraftment in utero.

Prenatal Tolerance Induction

Another approach to circumventing the problem of limited engraftment has been to focus on the in utero induction of donor-specific immune tolerance to foster a milder transplant regime following birth. Different approaches have been tested. IUT of fetal liver in mice followed by postnatal donor cell infusion, without any cytoablation or immunosuppression, was found to increase chimerism from 0.2% to approximately 5%.¹¹⁶ In another murine study, IUT of T-cell-depleted bone marrow was followed by postnatal donor lymphocyte infusion using splenocytes.¹²⁵ These investigators achieved a remarkable conversion of low-level chimerism following IUT to complete engraftment by donor cells. GVHD was rarely observed.

The importance of various mature cell types in the initial graft to the process of tolerance induction is being investigated. Donohue et al¹¹⁸ transplanted large numbers of highly purified murine HSCs that led to microchimerism but also sensitized the recipient's immune system toward donor cells. Sensitization of the recipients was also associated with higher levels of interleukin-2 production and expansion of CD4⁺ T cells, suggesting a role for type 1 helper (Th1) T cells in graft rejection.¹¹⁹ Additionally, higher levels of interferon- γ production, another indication of a Th1 response, were observed from sensitized mice, whereas Th2 cytokine production was favored in tolerant mice.¹²⁰ In one human case, a high dose of

purified CD90⁺CD34⁺ HSCs failed to induce T-cell tolerance in a fetus with chronic granulomatous disease, but there was also no evidence of immune sensitization.³³ In contrast to the findings with enriched HSC grafts, tolerance induction has been reported in mice transplanted with minimally purified or unpurified grafts.^{116,121,122,125,160} Tolerance induction was also suggested in a human transplant done for α -thalassemia using CD34⁺ cells containing about 1% CD3⁺ cells.⁵¹ Similarly, partially T-cell-depleted bone marrow transplanted in swine resulted in chimerism and T-cell tolerance in 1 of 7 recipients, but no chimerism in fully T-cell-depleted grafts and lethal GVHD in grafts without T-cell depletion.¹⁶¹ The presence of T cells in murine grafts was further found to improve the incidence of tolerance induction in a model system that did not permit GVHD but did permit graft reaction.¹⁶² Thus, donor T cells might not only improve engraftment by providing space for HSC engraftment, but also contribute to the induction of immune tolerance.

Co-transplantation of immature dendritic cells (DCs) has been tested as another means for inducing immune tolerance. Chou et al¹²⁴ showed that DC progenitors, generated in vitro from bone marrow, could increase the level of chimerism following IUT in mice. Despite donor cell frequencies averaging 29%, tolerance was not observed except in infrequent cases of complete engraftment by donor cells. A high incidence of GVHD was also noted in this study. The DCs transplanted expressed low levels of CD80 and lacked CD86 expression, indicating their immaturity. DCs employ these molecules in stimulating T cells. Thus, the limited expression of CD80 and CD86 was hoped to induce anergy rather than stimulate host T cells. The fact that tolerance was not achieved with regularity was attributed to the possible maturation of the DCs in vivo. Indeed, the peritoneum has been shown to act as a reservoir for transplanted bone marrow cells for many months and may have provided the proper environment for the maturation of the DCs.¹⁶² Interestingly, in this study, there was a significant correlation between the presence of donor cells in the peritoneum and donor-specific T-cell tolerance. Further study of the types of donor cells supported in the peritoneal cavity and their influence in the process of tolerance induction may lead to reliable and safe methods of prenatal tolerance induction.

Successful generation of immune tolerance by IUT has also been envisioned as a means toward safer and more readily available solid-organ transplantation after birth. A number of genetic and developmental defects that affect kidney function can be diagnosed in utero. A transplantation strategy has been envisioned in which a living relative may become a kidney donor after IUT has been performed to promote immune tolerance specific for the donor. This strategy was tested in the sheep¹⁶³ and rhesus monkey¹⁶⁴ but with only delayed rejection of the kidney being the best result. More encouraging results have come from a study performed by Mathes et al¹⁶⁵ in the

swine. Tolerance was induced by IUT of T-cell-depleted bone marrow that still contained 1.5% to 1.9% T cells and was assessed by mixed lymphocyte reaction and cytotoxicity assays. Four tolerant swine received kidney transplants from donors with major histocompatibility antigens matching the original bone marrow donors, and the grafts were accepted without the need for long-term immunosuppression.

The possibility of using IUT to enable xenogeneic solid-organ transplantation is being studied since IUT has been successful in generating chimerism across species barriers. Such a strategy is of particular interest in the area of pediatric heart transplantation because living donors of human hearts are not possible. Rats have been the preferred recipients for these studies. At first, allogeneic IUT was performed using splenocytes, which led to prolonged survival or even complete tolerance of grafted hearts.¹⁶⁶ A measure of success was also observed with IUT of allogeneic fetal liver followed by neonatal skin grafting and then heterotopic heart transplantation at 8 to 10 weeks of age.¹⁶⁷⁻¹⁶⁹ Prenatal xenotransplantation with hamster splenocytes or thymocytes was at first found to result in hyperacute rejection of donor heart tissue in rats.¹⁷⁰ However, prolonged xenograft survival was reported in another study after bone marrow transplantation with hamster cells in utero.¹⁷¹ Xenotransplantation poses a greater number of obstacles to success than allogeneic transplantation, but improvements in the methods of immune tolerance induction in utero may lead to successful implementation of xenotransplantation in the future.

In Utero Transplantation of Nonhematopoietic Cells

Fetal cellular therapy need not be limited to hematopoietic cells. Stem cells and committed progenitors exist for most tissues and may someday be successfully transplanted. Human mesenchymal stem cells (MSCs) have been transplanted into fetal sheep where they differentiated into chondrocytes, adipocytes, myocytes, cardiomyocytes, bone marrow stromal cells, and thymic stromal cells.^{143,172-175} Moreover, bone marrow and fetal liver, both of which contain MSCs as well as HSCs, were shown to engraft the skeletal muscle of mice with muscular dystrophy.¹⁵⁵ As with HSC transplantation, obtaining sufficiently high levels of chimerism to have a clinical impact is the greatest challenge facing MSC transplantation. Recent reports also question if some of the engraftment observed is due to cell fusion rather than to actual engraftment, growth, and differentiation of the transplanted MSCs.^{176,177}

Efficient IUT of hepatocytes could offer the opportunity for treatment of a number of inherited metabolic diseases. The relatively large and fragile hepatocytes have been successfully transplanted in a rat model,¹⁷⁸ and even human hepatocyte engraftment was achieved in normal

rats by a combination of in utero and postnatal transplantation.¹⁷⁹ Rosen et al¹³⁶ investigated the potential for prenatal hepatocyte transplantation in factor X-deficient mice. IUT of fetal hepatocytes achieved engraftment in approximately half of the recipients, and the plasma levels of factor X were 1% to 6% of normal levels in these mice. Furthermore, liver engraftment was observed in some nonhuman primates transplanted with fetal hepatocytes.^{155,180,181} For the purpose of producing a specific liver gene product, hepatocytes might not be the only cells that can be transplanted. Takahashi et al¹⁸² used amniotic epithelial cells that were transfected with adenoviral vector and transplanted into fetal rat liver to show that other cell types can be used to deliver gene products to a fetus.

Conclusions

Currently, IUT of stem cells is a viable clinical option for the treatment of a number of immunodeficiency syndromes that affect the development and function of lymphocytes. Wider application of IUT to treat other inherited diseases awaits the development of safe and reliable methods of increasing the levels of donor cell engraftment. Early assumptions that donor cells are likely to engraft a rapidly growing fetus that lacks a functional immune system are being reevaluated. In the absence of any cytoablation, stem cell engraftment in the fetus may be no more favorable than in adults. The emergent fetal immune system may also pose some threat to donor cells. Nonetheless, recent animal studies have provided encouraging examples of how the barriers to stem cell engraftment may be breached. Further progress in the field of IUT will likely be aided by research aimed at minimizing the preparatory regimen used for cytoablation and immunosuppression in the adult transplant setting. Continued efforts at fostering fetal transplantation are warranted by the need and benefit afforded by early treatment of disease.

Appreciation is expressed to our many colleagues who have shared their efforts and enthusiasm with us in the pursuit of developing the practice of fetal transplantation. Special thanks go to Drs. Jeng-Chang Chen, François Golfier, Michael R. Harrison, and Yuet-Wai Kan.

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