Hematopoietic Stem Cells for the Treatment of Genetic Disease

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Hematopoietic stem cells (HSCs) from the bone marrow of postnatal donors and from the liver of fetal donors have been used for the treatment of genetic diseases. HSCs are hematopoietic organ-derived cells that have the capability of differentiating into a variety of progeny cells including erythroid, thromboid, myeloid, and lymphoid lines. Additionally, they can differentiate into cells of the reticuloendothelial system (e.g., Kupffer cells of the liver,\(^1\) alveolar macrophages,\(^2\) and osteoclasts—those cells capable of bone resorption). When HSCs are harvested and transferred from a donor to a recipient, the donor stem cells find their way to the hematopoietic stromal environment of the recipient where they implant, replicate, and differentiate. If conditions are favorable, these cells thrive in the recipient. When full self-renewal potential and differentiation are achieved, there is complete reconstitution of the recipient’s hematopoietic function. A genetic disorder that results from a defect in the function of cells derived from the HSC can be reversed if stem cells capable of producing normal progeny (differentiated cell lines) replace the existing HSC.

Since the observation of Lorenz and coworkers\(^4\) in 1951 that lethally irradiated mice could be reconstituted by donor bone marrow cells, there has been speculation that bone marrow transplantation (BMT) may have a role in the treatment of a wide variety of disorders. The earliest attempts at reconstitution of human hematopoiesis by BMT were for patients with leukemia, aplastic anemia, or severe combined immunodeficiency disease (SCID). These were the logical choices because they were diseases caused by stem cell aberrancies. However, the successes were initially limited to syngeneic transplantation between identical twins. The histocompatibility differences between donor and recipients in allogeneic transplantations frequently led to fatal graft-versus-host disease (GVHD). Researchers in the 1960s expanded the knowledge of histocompatibility differences and elucidated the HLA antigen system. It became clear that the major determinant of GVHD was the histocom-
apatibility of the D locus between the donor and recipient. Soon thereafter, allogeneic BMT was used to treat diseases of the HSC.

With the successes in the treatment of leukemia and SCID came the prospects that BMT might be useful as a form of replacement therapy for a variety of genetically determined diseases manifested in HSCs and their progeny. Defects in the synthesis of hemoglobin, granulocyte function, osteoclast function, and even more generalized metabolic errors that might be compensated for by replacing cells of the reticuloendothelial system became the targets of interest for clinicians and researchers. Indeed, many successes have been documented both in animal models and in the human (Fig. 1). However, BMT in postnatal recipients has three major problems: 1) preexisting sequelae to the disease state at the time of BMT, 2) GVHD, and 3) necessity of immunosuppression for recipient preparation.

A significant disadvantage of the current use of bone marrow HSC to treat genetic disease is that by the time the attempt to transplant occurs, there are almost always some sequelae to the disease state. SCID, a heterogeneous disorder of both T and B lymphocytes that can be genetically transmitted, is frequently treated by bone marrow HSC. However, SCID is often fatal if not treated early, and in the interim is accompanied by recurrent infections, chronic diarrhea, and multiple hospitalizations, all of which may interfere with growth and development. Some inherited hematologic disorders have their onset during fetal life (e.g., fetal hydrops from alpha-thalassemia). In this case the only opportunity to treat with HSC would be during fetal life.

GVHD is a significant obstacle to the use of bone marrow HSC to treat genetic diseases. It occurs because the graft inadvertently carries committed lymphocyte precursors that have the capability of recognizing the host as foreign and consequently attempt to reject the host. Some of these committed cells are mature lymphocytes and cause acute GVHD, while others are precursors of lymphocytes and require time for full differentiation into mature forms, at which time they can cause delayed GVHD.

Currently, the most efficient method of limiting the expression of GVHD is the use of the mixed lymphocyte reaction (MLR). The MLR is a test that cocultures the lymphocytes of the donor and recipient. Either the donor's cells or the recipient's cells are pretreated with irradiation or mitomycin C to arrest DNA synthesis. When the two cell lines are cocultured, evidence of DNA synthesis indicates a stimulation of growth of the untreated cell line by the pretreated cell line. This occurs only when there is a difference at the HLA-D locus and means that GVHD probably would follow transplantation. However, even when donor-recipient pairs are matched by MLR, there still remains a 50% incidence of GVHD, and 50% of those cases are fatal.

The recipient of a bone marrow HSC graft must be immunotolerant in order to accept the graft. If there is a preexisting immunodeficiency as in SCID, nothing more needs to be done. However, most genetic diseases that might be treatable with HSC are in immunocompetent hosts. These potential recipients must be immunosuppressed before the transplantation. To do this, clinicians have employed the use of X-irradiation and, more commonly, chemotherapeutic agents like cyclophosphamide. These measures effectively remove the host's competent lymphocytes that could interfere with engraftment and have the added advantage of ablating the recipient's bone marrow stroma of existing HSCs. Unfortunately, having lost the ability to mount an immunologic response, the host becomes predisposed to infections.

It is because of the inherent problems of BMT that fetal liver HSC transplantation has been investigated. Fetal liver HSCs
Disorders of the Erythrocyte
  Sickle cell disease
  Thalassemia major
  Hereditary spherocytosis (murine)
  Diamond-Blackfan syndrome
  Fanconi anemia
  Pyruvate kinase deficiency (canine)

Disorders of the Lymphocyte
  Sex-linked severe combined immunodeficiency disorder
  Severe combined immunodeficiency secondary to adenosine deaminase deficiency
  Severe combined immunodeficiency secondary to purine nucleoside phosphorylase deficiency
  Wiskott-Aldrich syndrome

Disorders of the Granulocyte
  Chronic granulomatous disease
  Chediak-Higashi
  Infantile agranulocytosis (Kostmann)
  Cyclic neutropenia (canine)
  Lazy leukocyte syndrome (neutrophil actin deficiency)
  Neutrophil membrane GP-180 deficiency
  Cartilage-hair syndrome

Metabolic Errors of Lysozomes of Reticuloendothelial Cells
  Mucopolysaccharidoses
    Hurler disease (MPS I) (alpha-iduronidase deficiency)
    Hurler-Scheie
    Hunter disease (MPS II) (iduronate sulfatase deficiency)
    Sanfilippo B (MPS IIIB) (alpha-glycosaminidase deficiency)
    Morquio (MPS IV) (hexosamine-6-sulfatase deficiency)
    Maroteaux-Lamy (MPS VI) (aryl sulfatase B deficiency)
  Mucolipidoses
    Gaucher disease (glucocerebrosidase deficiency)
    Metachromatic leukodystrophy (aryl sulfatase A deficiency)
    Krabbe disease (galactosylceramidase deficiency) (murine)
    Niemann-Pick disease (sphingomyelinase deficiency)
    Beta-glucuronidase deficiency (murine)
    Fabry disease (alpha-galactosidase A deficiency)
    Adrenal leukodystrophy

Disorder of the Osteoclast
  Infantile osteopetrosis

FIG. 1. Genetic disorders treated by bone marrow transplantation.
have the major advantage of being immunotolerant of the recipient. Also, when used in a fetal recipient, they would obviate the other two main objections to BMT as it is now practiced: they would preempt the expression of the disease state, and the recipient would require no immunosuppression.

Fetal Liver HSC Transplantation

Fetal liver HSCs are derived from the liver of a fetus at a time when that organ is the primary organ of hematopoiesis. The yolk sac is the first primary hematopoietic organ of fetal life. In the human, hematopoiesis switches from the yolk sac to the liver at about the 6th–7th week of gestation. The proposed mechanism is by "seeding" of the liver by HSCs from the yolk sac. Likewise, the bone marrow is later seeded by HSCs from the liver at about 20 weeks of gestation. Certain factors orchestrate these events. They accomplish a variety of things: they prepare the HSCs for reimplantation, stimulate their departure from their familiar stromal environment, allow the HSCs to move to the bone marrow by the vascular system and to reimplant themselves. Simultaneously, the bone marrow increases its available stromal space and prepares its architecture to function as a hematopoietic organ. At its zenith (mid–2nd trimester), 60% of the liver functions as a hematopoietic organ. At 40 weeks' gestation there are negligible numbers of hematopoietic cells in the liver, unless diseases like erythroblastosis fetalis have necessitated continued extramedullary hematopoiesis.

In 1958, Uphoff discovered that fetal liver HSCs had a tremendous advantage over bone marrow HSCs in their ability to reconstitute the hematologic system of mice. They did not produce running disease. Until that time, running disease was the major obstacle in the development of BMT as a clinical tool. It was known that running disease could be avoided by the use of syngeneic transplantation, but to the clinician this meant that donor–recipient matched pairs would have to be identical twins. When researchers attempted the transplantation of bone marrow HSC from an allogeneic donor in animals, the recipient always died from a disease characterized by poor growth, splenomegaly, and dermatitis-running disease. In 1959 Billingham and Brent demonstrated that this disease was caused by the transmission of immune-competent T lymphocytes from the donor to the host. The disease appropriately came to be known as graft-versus-host disease. Interestingly, the answer to the problem of GVHD, then, already had been answered by the time the cause of the problem was determined. Why did Uphoff's use of fetal liver HSC circumvent the problem of GVHD? The answer is that the fetal liver HSC graft was devoid of competent T lymphocytes and their precursors. When the more immature T lymphocyte precursors are engrafted, they are tolerized by the host's antigenic determinants during maturation. Consequently, they see the host as "self." The end result is frequently no GVHD, or mild disease.

The advantage of using the fetal HSC for transplantation, therefore, is that fetal immunocompetency is not fully developed. In theory, fetal lymphocytes should be totally naive because the immunocompetent lymphocytes must "learn" what is "self" so that they can distinguish what is foreign. While this is true early in gestation, it is not true later in gestation. Human fetal lymphocytes show the capacity to proliferate in response to mitogens by the 11th to 12th week of gestation. By the 14th week of gestation, thymic lymphocytes are capable of responding to allogeneic cells in MLR. Postthymic T lymphocytes are those capable of causing fatal GVHD, and these are detected in the liver after the 18th to 20th week of gestation. This is also the class of T lymphocytes that is responsible for graft rejection and prevents the engraftment of
maternal HSCs that pass from the maternal into the fetal circulation during gestation. Ideally, the fetal recipient of HSCs should be no older than 20 weeks of gestation. An initial attempt to transplant before 20 weeks' gestation would have a better chance of engraftment because of fetal immunotolerance, and if there was no engraftment, such an attempt might have the benefit of tolerizing the recipient so that later attempts at transplantation might have a greater chance of engraftment. In humans, fetal liver HSCs have been used for transplantation when a matched donor was not available. However, because postthymic lymphocytes can be found in the fetal liver after 20 weeks' gestation, clinicians who have used fetal liver HSCs have taken them from fetuses before 14 weeks' gestation. Indeed, there has never been a case of fatal GVHD in any such recipients, although mild cases have been reported.\textsuperscript{10}

**The Fetus: A Potential Recipient of HSC**

The fetus is an ideal host for transplantation of HSC cells for two reasons: ontologically it is prepared for engraftment and immunologically it is tolerant and should permit foreign grafts without rejection. A natural-occurring chimera illustrates this—the freemartin, a masculinized female bovine twin. In 1916, Lillie\textsuperscript{11} used a mathematical construct to argue that vascular anastomoses between male and female bovine twins permitted the exchange of humoral factors that resulted in the female's masculinization. It was 29 years later in 1945 that Owen\textsuperscript{12} demonstrated that these vascular connections permitted intratropical HSC transfusions between the bovine twins that led to blood type chimeras—an intratropical HSC transplantation. Indeed, Jolly and associates\textsuperscript{13} in 1976 published that in Angus cattle there occurs an "experiment of nature" that demonstrates that intratropical transfer of HSCs between nonidentical co-twins alters the pathology of mannosidosis in the affected twin. Although the clinical outcome was not changed, histologic examination of tissues revealed a significant decrease in pathology. It was argued that the transplanted hematopoietic cells that had normal mannosidosis activity were unable to compensate for the absence of mannosidosis in the brain tissue of the affected calf. In the human, an inadvertent experiment demonstrates the immunotolerance of the fetus. In 1973 Turner\textsuperscript{14} reported that 5 of 65 infants who had had a fetal intratropical transfusion for Rh isoimmunization demonstrated the persistence of circulating donor white blood cells beyond 1 year of age.

Fetal immunotolerance permits the formation of these chimeras. Chimeras occur because the fetal host does not recognize the allogeneic graft as foreign and subsequently becomes tolerant of the antigenic differences of the foreign graft. From a practical standpoint, this immunotolerance would allow the use of allogeneic stem cell grafts from unmatched donors, and possibly even pooled donor cells, thus obviating the need for HLA antigen typing and a specific donor search as is generally required for postnatal BMT. It also would remove the need for pretreatment with radiation or chemotherapy, as is necessary with immunocompetent recipients who have the capacity to reject an allogeneic graft. The pretransplantation preparation can have significant morbidity and can be fatal. An additional reason for pretreatment of the recipient is to rid the bone marrow stroma of its occupant cells to make room for the implantation of injected HSC cells. Before the 20th week, the human fetus not only has a depleted stroma but also is programmed for the reception of HSCs. Fetal immunotolerance and stromal preparedness obviate the need for host preparation with chemotherapy and irradiation, a previously mentioned disadvantage of postnatal BMT.
Animal Research

Evidence in animal research supports the notion that the use of fetal liver HSCs to correct genetic disorders in the human fetus is more than just a vision for the future. There exists a mouse model for a hematologic disorder that causes a hypoplastic, macrocytic anemia—the W mouse, a frequently fatal, autosomal recessive disorder. There are varying degrees of severity of this disorder, and the most often used mouse model is the W<sup>−</sup>, W<sup>−</sup> for viable. A number of researchers have used this model to see if the anemia can be corrected by the implantation of normal HSCs in the diseased animal. In 1966, Sellers and Polani<sup>15</sup> were the first to publish the successful engraftment of HSC in newborn W<sup>−</sup> pups. These mice were homozygous for the W<sup>−</sup> gene. The engrafted HSCs persisted throughout the life of the recipients and cured them of their anemia. There was no mention of GVHD, and the mice survived to the same age as their healthy, unaffected siblings. This was landmark research because it was the first time that allogeneic HSCs were used to treat genetic disease, in animals or humans. (Good and his associates<sup>16</sup> published the first bone marrow transplantation in humans in 1968 for the treatment of immunodeficiency.) Further work with fetal liver HSCs demonstrated that these cells had the capability of differentiating into myeloid, erythroid, thromboid, and lymphoid cell lines,<sup>17</sup> as well as implanting into all of the hematopoietic organs of the recipient, including bone marrow, lymph glands, thymus, and spleen. The fetus was not used as a recipient for fetal HSCs in an animal model until 1979 when Fleischman and Mintz<sup>19</sup> published that intraplacental transfusions of fetal liver HSCs into midgestation fetal W<sup>−</sup> mice led to the permanent cure of their anemia. In extension of this research they later published that the engraftment frequency of fetal liver HSCs was higher in the more severe forms of the W anemia data, which may have implications for future human recipients of HSC for treatment of genetic disorders in utero.<sup>20</sup> The W anemia is a stem cell defect; one interpretation of the data is that engraftment may depend on the competition for available space in the stromal environment of the bone marrow.

Conclusion

The treatment of genetic disorders has become the territorial imperative for the geneticist. It is only now that we have glimpses of the technology that may be the key to such therapy. In the most exact employment of such technologies, the defective gene would be precisely repaired. Short of that, and not inconceivable, is the probability that a gene coding correctly for the same sequence as the defective gene may be inserted into the DNA of the patient. This technology exists, but it is far from practical. The vectors for such gene insertion are highly nonspecific for their insertion sites, they could be oncogenic, and there is a major problem in achieving appropriate regulation of such an inserted gene. Also, such inserted DNA would be vertically transmissible to progeny of recipients if the insertion was into the germ cells as well as the somatic cells. This may be desirable, because the progeny of these individuals might then have a normally functioning gene; however, in less than optimal circumstances it might be deleterious to the progeny of the patient receiving such therapy. The use of HSCs for the treatment of genetic disease also offers the recipient a form of gene replacement therapy. Instead of specific gene insertion, however, this offers the replacement of the complete genome from normal donors. The DNA of the HSC comes in the most favorable form for normal function: its whole cell environment. Of course, this therapy is limited by the fact that the genetic diseases curable are those that are manifested as a result of defects in the hematologic system.
However, even diseases such as phenylketonuria might benefit from such gene therapy because the normal gene for phenylalanine hydroxylase would be available in limited amounts, which might help reduce the levels of phenylalanine in the host.

There is a recent technologic breakthrough that makes the consideration of treatment of genetic disorders in the fetus a therapeutic alternative. This is chorionic villus sampling (CVS). CVS allows the diagnostician to obtain fetal cells during the first trimester. These cells are capable of dividing in tissue culture and can be harvested for analysis. An ever-increasing number of disorders can be prenatally diagnosed with this method. The diagnosis of these diseases, therefore, can be determined late in the first trimester, a large advantage over amniocentesis, which allows for diagnosis probably too late for intrauterine transplantation without immunosuppression of the recipient. Thus a diagnosis can now be made, and treatment considered, in the first trimester, which is the critical time during hematologic ontogeny for transplantation.

The technology for intrauterine fetal liver stem cell transplantation already exists. Probably the most likely technique will be the same as for intrauterine transfusions for erythroblastosis. Ultrasonographically guided needles will be used to penetrate the abdominal wall of the less-than-20-weeks' gestation fetus, and the stem cell preparation will be injected into the intraperitoneal cavity. Engraftment can be determined by documenting a blood type shift or leukocyte antigen shift from recipient to donor type. If there is no engraftment, a repeat transfusion may be attempted because the fetus may have been tolerized by the first transfusion, giving a second transfusion of HSCs an opportunity to engraft without rejection by the host. If engraftment occurs, the major potential complication would be GVHD. It is expected that such a reaction would be of low incidence and mild; however, should GVHD be severe it might well be fatal to the growing fetus. Intrauterine diagnosis would be extremely difficult and GVHD would likely be untreatable. Even after delivery GVHD would carry a poor prognosis because almost all infants born with GVHD have died. This scenario is a grim possibility if in-utero correction of genetic disease is attempted by the use of HSC. However, it may be an acceptable risk given its low probability and the potential benefit.

The possibility of HSC transplantation for the treatment of genetic disease portends an optimistic future. GVHD is the major problem associated with the use of bone marrow HSC for the correction of genetic disorders. However, investigators are devising reliable methods for the purification of bone marrow HSC, which will rid the cell preparation of competent T lymphocytes and their committed precursors and retain those cells with pluripotential capacity. Fetal liver HSCs are a possible alternative to bone marrow because they cause much less GVHD. Fetal liver HSCs also have a theoretic appeal because during ontogeny this is the source from which the bone marrow is seeded. The fetus may be the ideal recipient of HSC transplantation because of its ontologic readiness for engraftment and its immunotolerance and because it represents the earliest possible time a genetic disease could be corrected. The time is ripe for such adventurous therapy. The technology is available, the technique has shown itself effective in animal models, and genetic disease has been cured by the transplantation of HSC in postnatal recipients.

References

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