Haematopoietic stem cell transplantation and gene therapy in the fetus: ready for clinical use?

D.V.Surbek1,2,3, W.Holzgreve2 and K.H.Nicolaides1

1Harris Birthright Research Centre for Fetal Medicine, King’s College Hospital, London SE5 9RS, UK and 2Department of Obstetrics and Gynecology, University Hospital of Basel, 4031 Basel, Switzerland

3To whom correspondence should be addressed at: Harris Birthright Research Centre for Fetal Medicine, King’s College Hospital, Denmark Hill, London SE5 9RS, UK. Phone: +44 171 924 0894; Fax: +44 171 738 3740; E-mail: surbek@hotmail.com

Allogeneic haematopoietic stem cell transplantation in utero has been successfully used for the prenatal treatment of severe combined immunodeficiency syndrome. However, this treatment has not been successful in the therapy of other conditions in which the fetus is immunologically competent. The main obstacles to success are lack of competitive advantage of donor versus host stem cells, preventing stable engraftment and graft rejection. Several strategies are being explored to overcome these problems, and some of them have been successful in animal studies. Prenatal gene therapy, using ex-vivo transduced autologous haematopoietic cells or direct gene targeting in utero, is another potential approach in the treatment of immunocompetent fetal recipients. Although this has been shown to be feasible in animal models, safety concerns regarding transduction of fetal germ cells or maternal cells should be addressed in preclinical experiments prior to initiation of clinical trials.

Key words: fetal gene therapy/haematopoietic stem cells/in-utero transplantation

TABLE OF CONTENTS

Introduction
Haematopoietic stem cell transplantation in utero
The gene therapy approach
Conclusions
References

Introduction

In the last 25 years, extensive progress has been made in the prenatal diagnosis of congenital diseases using non-invasive and invasive techniques (Holzgreve, 1997). Most genetic diseases of the lympho-haematopoietic system can now be diagnosed in the first trimester of pregnancy. In the near future, non-invasive screening of the population along with the development of molecular methods including DNA microarray technology will inevitably expand the number of conditions diagnosed prenatally with a consequent increase in the demand for therapeutic options.

TABLE OF CONTENTS

Introduction
Haematopoietic stem cell transplantation in utero
The gene therapy approach
Conclusions
References

Clinical success has been achieved recently with prenatal HSC transplantation in humans but, so far, is limited to diseases where severe immunodeficiency is present in the fetus. Several strategies to overcome engraftment failure in immunocompetent hosts are being developed. Additionally, recent advances in gene targeting open new perspectives for in-utero gene therapy as an alternative to transplantation of allogeneic stem cells. However, fetal gene therapy has not yet been attempted in humans.

Haematopoietic stem cell transplantation in utero

Advantages of intrauterine therapy

Post-natal stem cell transplantation from bone marrow or umbilical cord blood has the potential to definitively cure a range of genetic abnormalities including severe haemoglobinopathies, immunodeficiencies and storage diseases (Lucarelli et al., 1990, 1999; Issaragrisil et al., 1995; Krivit et al., 1998; Buckley et al., 1999). Although promising results have been achieved with allogeneic stem cell transplantation in some of these diseases, there are still several obstacles to overcome. A human leukocyte antigen (HLA)-compatible donor can be found only for about one third of the patients. The need for immunosuppression and marrow ablation in the recipient leads to substantial treatment-associated morbidity. Especially in non-related donor stem cell transplantation, graft versus host disease (GvHD) or graft failure are common. Additionally, the disease might lead to irreversible
D.V.Surbek, W.Holzgreve and K.H.Nicolaides

damage in the fetus already before birth (e.g. storage diseases or \(\alpha\)-thalassaemia) which adds to procedure-related toxicity. Finally, the costs for post-natal stem cell transplantation are exceedingly high.

The physiology of the ontogenetic development of the haematopoietic and immunological system in the human fetus offers a theoretical opportunity to circumvent these problems by prenatal stem cell transplantation (Zanjani et al., 1997). There is convincing evidence that early in gestation, until the end of the first trimester, the fetus is immunologically naive (Stites et al., 1974; Rayfield et al., 1980; Thilaganathan et al., 1992, 1993a), before the fetal thymic microenvironment regulates the determination as to whether an antigen is recognized as self or foreign through positive and negative selection by clonal deletion or expansion (Blackman et al., 1990). Foreign cells transplanted early enough would therefore be recognized as ‘self’ and would not be rejected, obviating the need for HLA-matching of transplanted cells and immunosuppression of the recipient. Theoretically, long-term donor-specific tolerance could be induced.

Developmental ontology of the haematopoietic system is characterized by a chronological sequence of change in the primary haemopoietic site from the yolk sac and the embryonic aorta–gonadal mesonephron region to the fetal liver and finally the bone marrow (Tavassoli, 1991; Medvinsky and Dzierzak, 1996; Tavian et al., 1996). Before and during the second trimester of gestation, the fetal bone marrow is still relatively empty, and haematopoietic niches are rapidly expanding, allowing marrow population by donor cells without the need for marrow ablation prior to transplantation.

According to these assumptions, physiological development could be exploited by using the ‘window of opportunity’ early in gestation to transplant HSCs without HLA matching or myeloablation (Blau and Stamatoyannopoulos, 1996). If successful, prenatally developing irreversible organ damage could be prevented and the disease could be corrected. Additionally, specific advantages compared to post-natal bone marrow transplantation, e.g. reduced GvHD tissue damage and more rapid immune reconstitution, could be exploited (Blazar et al., 1998). In general, every disease which is treatable by post-natal bone marrow transplantation, and which can be diagnosed prenatally, is potentially amenable to in-utero HSC transplantation, including haematopoietic, immunological and metabolic diseases (Surbek et al., 1999).

Experimental evidence in animals

The concept of prenatal induction of chimerism is derived from observations in twin pregnancies. Naturally-occurring chimerism in dizygotic twins sharing transplacental circulation has been described in cattle (Owen, 1945), primates (Picus et al., 1985) and humans (Van Dijk et al., 1996). Based on the concept of acquired neonatal tolerance to foreign antigens (Billingham et al., 1953), initial experiments have been performed in a mouse model using intraplacental injection of haematopoietic stem cells and showed the potential of this method to cure genetic anaemias prenatally (Fleischmann and Mintz, 1979). Later, a sheep model was described in which fetal liver cells were successfully injected into the peritoneal cavity of another fetus (Flake et al., 1986). Subsequently, several different animal models have been introduced, including mice (Blazar et al., 1995; Archer et al., 1997), and monkeys (Harrison et al., 1989; Cowan et al., 1996). In these animal studies, it became evident that apart from the sheep (Zanjani et al., 1992; Colas et al., 1999) and immunodeficient mice, e.g. non-obese diabetic/severe combined immune deficiency (NOD/SCID) mouse (Archer et al., 1997), the level of engraftment detected at birth or soon afterwards is <1%. Furthermore, in a sheep model of ceroid–lipofuscinosis, no beneficial effect on central nervous system disease regarding clinical and histological parameters could be demonstrated despite an average donor cell engraftment level of 9% after in-utero transplantation of allogeneic fetal liver cells (Westlake et al., 1995). Recently, the mechanism and kinetics of engraftment after in-utero transplantation has been investigated, with the aim of defining specific barriers to and possible improvements of engraftment (Shaaban et al., 1999).

In summary, animal studies have demonstrated that the principal limitation of in-utero stem cell transplantation is the low level of engraftment, precluding clinical efficacy in most diseases. Still, many of these models have proven to be useful in the study of the mechanisms and kinetics of engraftment of transplanted cells in the bone marrow, effects of donor cell dose or graft manipulations.

Evidence from clinical experience

To date, up to about 30 cases of in-utero HSC transplantation have been reported, one third being performed for immunodeficient disorders including SCID or chronic granulomatous disease (Flake and Zanjani, 1999). The others included fetuses affected by Rhesus isoimmunization, haemoglobinopathies (primarily \(\alpha\)- or \(\beta\)-thalassaemias), or storage diseases (Hurler syndrome, Krabbe’s disease). Different sources of donor stem cells were used (maternal, paternal or sibling bone marrow and fetal liver cells) with or without T cell-depletion/CD34+ cell enrichment. The gestational age of the recipients ranged from 11 to >30 weeks, and in some cases, multiple transplants were performed. Significant engraftment has been achieved in fetuses affected by a severe deficiency of the immune system. Two recent reports in fetuses with X-linked SCID showed successful treatment; both have persistent split chimerism [donor T and natural killer (NK) cells, host B-cells] after birth (Flake et al., 1996; Wengler et al., 1996). In contrast, in fetuses with no immunodeficiency, no clinically significant, stable engraftment could be achieved, although microchimerism and donor-specific tolerance could be shown in some cases (Thilaganathan et al., 1993b).

To summarize, stable engraftment in non-myelo-ablated immunologically competent host fetuses has not yet been achieved. This might be due to the lack of competitive advantage of donor haematopoietic cells over host cells in bone marrow niches or due to graft rejection by the host.

Strategies to overcome engraftment failure

Current strategies to improve donor cell engraftment are based on improved understanding of the biology of in-utero transplantation obtained from experiments in animals and experience in humans.
These include:

**Timing of transplantation**

This is an important issue for non-immunodeficient fetuses. The 'window of opportunity' is rather small, because the stage of gestation at which the fetus is believed to be immunoincompetent (and thus not able to reject foreign antigens) is prior to 14 weeks.

**Source of haematopoietic stem cells**

Some evidence suggests that stem cells from fetal liver may be superior to adult sources, e.g. bone marrow or peripheral blood, especially for the in-utero therapy of haemoglobinopathies, e.g. thalassaemia, although this is not supported by clinical experience (Westgren et al., 1996). There are, however, major drawbacks in the use of fetal liver or bone marrow cells from aborted fetuses, including ethical issues, the risk of infection in the recipient, and the limited amount of cells available from the same donor. Cord blood is another important source of fetal cells with favourable characteristics which might be used for in-utero transplants (Surbek et al., 1998).

**Dose of donor cells**

In the fetal sheep model there is a dose-dependent increase in the level of engraftment, which seems to reach a plateau above a certain dose (Zanjani et al., 1997). However, data from post-natal transplantation experiments in mice suggest that large donor cell doses may be beneficial because they can even displace host cells from niches in the bone marrow, providing an overall competitive advantage (Bachar-Lustig et al., 1995; Reisner and Martelli, 1999, 2000). Repeated transplants 1–2 weeks apart have been used in humans (Flake et al., 1996) and provide further support in favour of homing and proliferation of donor haematopoietic cells within the host stromal microenvironment.

**Route of administration**

It is uncertain whether i.v. rather than i.p. injection is associated with a higher frequency of donor cells in the target organ haematopoietic microenvironment in the fetal liver and marrow (Westgren et al., 1997). However, even if the intravascular route was more favourable, it may be technically difficult and risky at <14 weeks of gestation (Surbek et al., 2000) and therefore the i.p. route would be preferred by most investigators. Nevertheless, it is unknown to what extent the transplanted cells enter the circulation to reach the target organ.

**Graft modification by co-transplantation of donor-specific stromal cells and/or growth factors**

This strategy is based on the assumption that the interaction between haematopoietic cells and stromal cells, which is necessary for homing as well as for proliferation and 'expression' of haematopoietic cells, is restricted between HLA-disparant cells. Co-transplanted stromal cells from the same donor would therefore improve donor haematopoietic cell support. It has now been confirmed in both the allogeneic and the xenogeneic fetal sheep models that stroma co-transplantation persistently increases donor cell engraftment and the level of circulating donor cells in the recipient (Almeida-Porada et al., 1999, 2000). Especially in diseases which lead to early organ damage the fetus might benefit from early donor cell presence in the peripheral blood. Growth factor administration during or after in-utero transplantation (Tarantal and Cowan, 1999) might further support donor cells, but it remains to be shown whether this approach improves the long-term engraftment level. Nevertheless, growth factors could be administered pre- or post-natally to mobilize donor cells into the periphery (Carrier et al., 1997).

**Fetal conditioning**

The aim of fetal conditioning is to provide a selective advantage to donor cells by a suppression of the interaction between haematopoietic and stromal cells and by ''minimal' haematopoietic cell support prior to transplantation. Experimental evidence suggests that the addition of specific blocking antibodies to support engraftment of donor cells might be one option to improve the competitive capacity of donor cells (Zanjani et al., 1999). Immunosuppressants, e.g. glucocorticoids or cyclosporin, low-dose cytotoxic regimens or irradiation, could be successful regarding engraftment. However, one of the main potential advantages of in-utero transplantation is that recipient conditioning, which is usually performed in the post-natal stem cell transplantation setting, can be avoided. This advantage would be lost, and toxic or teratogenic side-effects might prohibit their use in the rapidly developing fetus, especially in the first trimester. There might be other innovative strategies such as the administration of an attenuated parvovirus B19, which is known to suppress fetal bone marrow leading to severe anaemia (Shields et al., 2000).

**Microchimerism, tolerance induction and post-natal boosting**

Haematopoietic microchimerism has recently been identified in recipients of solid-organ transplants and is thought to be essential for maintenance of immunological unresponsiveness to donor organs. The concept of tolerance induction and mixed chimerism is currently being investigated clinically using simultaneous transplantation of bone marrow-derived cells and solid organ transplantation from the same donor to induce donor-specific tolerance (Elwood et al., 1998). After in-utero stem cell transplantation, persistent microchimerism has been achieved in several animals models as well as in humans (Thilaganathan et al., 1993b). Donor-specific tolerance has been shown in vitro in humans and in vivo in mice (Carrier et al., 1995) and primates using a later kidney graft (Mychaliska et al., 1997) from the same donor. As data from other preclinical studies suggest, prenatally-induced tolerance can be used for a post-natal boost-transplantation of stem cells from the same donor with or without minimal conditioning (Milner et al., 1999).

**The gene therapy approach**

Fetal gene therapy is a further strategy to circumvent the limitations of prenatal allogeneic stem cell transplantation.

**Gene therapy targeting haematopoietic stem cells**

Haematopoietic stem cells are attractive targets for somatic cell-based gene therapy, because they have the potential to produce progeny cells containing a therapeutic gene lifelong. Current clinical protocols of post-natal gene therapy in paediatric patients with genetic diseases are based on ex-vivo retroviral transduction...
of lymphocytes (Bordignon et al., 1995) or haematopoietic stem/progenitor cells from cord blood or bone marrow, followed by autologous transplantation of the engineered cells back into the patient (Kohn et al., 1995). Initial trials showed the feasibility and safety of gene therapy using cord blood cells in patients with ADA-deficiency, although only very limited clinical efficiency has been achieved as reported in a follow-up study (Kohn et al., 1998). Recently, however, clinical success has been reported 10 months after gene therapy in X-SCID disease using autologous retrovirally transduced bone marrow CD34+ cells (Cavazzana-Calvo et al., 2000). Nevertheless, several obstacles must be overcome before gene therapy can gain broad clinical application (Blau and Khavari, 1997). The main obstacles concern transduction efficiency, random integration of vector-gene-construct into host genome, duration of expression of the therapeutic gene (`gene silencing'), host immune response against vector, gene or gene product, and reproducible production of safe replication-free high-titre vectors (Verma and Somalia, 1997). Gene expression can be severely impaired by spontaneous cessation of regulatory sequence activity that control gene expression, by inactivation of promoters (e.g. by methylation) in the transduced host cell, by specific host defence mechanisms or by elimination of the transduced cells by the host immune system recognizing the foreign gene product (Bestor, 2000). Inflammatory cytokines, e.g. tumour necrosis factor (TNF)-α or interferon-γ have been shown to be involved in the host immune response towards the ‘foreign’ gene or gene product by direct inhibition of the expression of transgenes (Qin et al., 1997). Although some success has been achieved in mouse models (Bunting et al., 1998), recent results in large animal models reveal that gene expression in vivo can still be severely impaired despite successful engraftment of genetically modified autologous haematopoietic progenitor/stem cells (Lutzko et al., 1999a). A further difficulty is the identification of the target for gene transfer, i.e. haematopoietic stem cells, because definitive markers for undifferentiated, quiescent haematopoietic stem cells are still lacking. Recent reports suggest that there is no single stem cell marker, leading to the assumption that the stem cell compartment is heterogeneous (Goodell et al., 1997; Bhatia et al., 1998; Ziegler et al., 1999).

Gene delivery systems to haematopoietic stem cells include retroviral and adenoviral vectors, adeno-associated vectors, lentiviral [human immunodeficiency virus (HIV)-based] vectors and non-viral (liposome) vectors (for review, see Verma and Somalia, 1997). New generation adeno-associated viral vectors have recently been reported as leading to successful long-term gene expression and correction of haemophilia B in canine and mouse models after intrahepatic (Snyder et al., 1999) or i.m. (Herzog et al., 1999) injection. Retroviruses (as opposed to adenoviruses) have the property to stably integrate into the host genome, if the host cells are actively dividing and they are therefore preferentially used to deliver genes into haematopoietic stem cells. However, one major problem is the inefficiency of retroviral gene transfer into non-dividing cells, e.g. haematopoietic stem cells (Brenner, 1996). This problem has not been fully overcome by new strategies for efficient and stable retroviral transduction such as prestimulation with novel growth factors, e.g. thrombopoietin (megakaryocyte growth and development factor) and flt3 ligand, centrifugation (`spinoculation') or transduction on fibronectin fragments (Van Hennik et al., 1998).

Recently, replication-deficient lentiviral HIV-based vector systems have been developed, which stably integrate into the host genome in dividing as well as non-dividing cells (Miyoshi et al., 1999), including fetal haematopoietic stem cells (Luther-Wyrsh et al., 1999). They hold great promise to achieve the goal of stable long-term transduction of haematopoietic stem cells and their progeny.

Fetal gene therapy: experience in animal models

Several characteristics of the developing fetal haematopoietic system may prove beneficial for gene transfer and may render it highly susceptible to genetic transduction (Coutelle et al., 1995). These include the high proliferative status and expansion of the stem cell pool, the relatively small amount of necessary gene-engineered stem cells and the presumed lack of immune response towards the vector after gene transfer into the preimmune fetus, avoiding cessation of gene expression and/or tissue damage (Zanjani and Anderson, 1999). In addition, as we have shown recently, fetal umbilical cord stem cells have a high expansion potential (Wyrsch et al., 1999) and a superior retroviral transduction potential compared to adult cells (Ektherae et al., 1990; Luther-Wyrsh et al., 1999). Furthermore, recent data from experiments using stem cell transplantation into mouse blastocysts show that gene expression of donor cells largely depends on the developmental stage of the host microenvironment (Geiger et al., 1998), which could mean that gene expression is enhanced after prenatal gene transfer compared with post-natal gene therapy.

Different in-utero gene transfer models targeted to various cells and organs have been developed, including pulmonary epithelial cells (Holzinger et al., 1995; Sekhon and Larson, 1995; Vincent et al., 1995; Larsen et al., 1997), hepatocytes (Wang et al., 1998), skin (Hayashi et al., 1996), intestine (Wu et al., 1999) heart (Woo et al., 1997) and ductus arteriosus (Mason et al., 1999). The models used intra-amniotic, intratracheal, intraperitoneal, intrahepatic, intravascular, intraplacental or in situ (Ductus arteriosus region) administration of the vector–gene construct. Transplacental transfer of the vector–gene construct after injection into the maternal circulation has also been shown to be possible (Tsukamoto et al., 1995). This route, however, is unlikely to be feasible for use in humans because of the high risk for maternal transduction. An alternative approach is targeting of placental tissue instead of haematopoietic cells. In a small animal model, autologous trophoblast cells of the rodent placenta were removed, genetically altered in vitro and after re-transplantation into the placenta in utero; the cells survived and the gene product was expressed and detected in the fetal circulation (Senut et al., 1998).

For gene transfer to haematopoietic cells in the fetus, two different strategies are under investigation:

Ex-vivo gene therapy

This strategy implies autologous transplantation of in-vitro transduced stem cells, which has already been done with partial success in human neonates with aminodeaminase (ADA) deficiency using cord blood stem cells (Kohn et al., 1998) or later using autologous bone marrow (Cavazzana-Calvo et al., 2000). Animal studies have demonstrated the feasibility of ex-vivo gene therapy with circulating haematopoietic stem cells.
which were obtained from the fetal circulation by cordocentesis, subjected to ex-vivo expansion and transduction, and transplanted back into the fetus (Kantoff et al., 1989; Lutzko et al., 1999b; Omori et al., 1999; Winkler et al., 1999). The major advantage of this method is that the risk for germline transduction is substantially smaller as compared with in-vivo gene delivery where the whole fetus is directly exposed to a high titre gene-vector construct. On the other hand, major technical difficulties in obtaining enough stem cells from the fetus during pregnancy remain a challenge and must be overcome.

In-vivo gene therapy

In this approach the gene-containing vector (with or without producer cells) is transferred directly to the fetus, leading to in-vivo transduction of fetal haematopoietic stem cells. Recently, successful long-term gene expression in a sheep model after i.p. gene transfer to haematopoietic cells has been reported (Porada et al., 1998; Tran et al., 2000), and in utero vector–gene administration has proven feasible and effective in different animal models (Baumgartner et al., 1999; Lipshutz et al., 1999; Schachtner et al., 1999; Themis et al., 1999). While this approach is technically much easier, a higher risk regarding germline transduction must be expected.

Safety aspects of prenatal gene transfer

Important safety concerns exist whenever gene transfer is used to treat a fetus (Billing, 1999; King et al., 1999). One major concern is the risk of transduction of gonadal cells which might result in genetic germ line transduction. There is also a potential risk for the mother because transduction of her somatic or germinal cells through transplacental migration of the vector–gene construct could theoretically occur. An additional important safety aspect is the possibility of insertional mutagenesis in fetal cells resulting in a functional gene defect leading to a ‘genetic’ disease or to the formation of a malignant tumour. As mentioned above, ex-vivo gene therapy might be safer compared with in-vivo vector–gene administration in utero regarding these risks. However, it will never be possible to fully exclude these possible complications if fetal gene therapy is performed. Studies are now underway to determine the risk for germline or maternal cell transduction; some of them suggest this risk to be very low (Ye et al., 1998; Tran et al., 2000). However, a large number of treated animals will be necessary to determine the exact magnitude of this risk, which may depend on vector systems, age of the fetal recipient, route of administration, titre of replication-deficient viral particles used, or cells targeted.

Conclusions

Prenatal haematopoietic stem cell transplantation holds a great deal of promise for the treatment of a variety of severe congenital diseases. Although the preimmune fetus is theoretically amenable to stem cell transplantation in general, this promise has so far been partly fulfilled only in fetuses with immunological diseases. Successful treatment of other conditions requires a better understanding of the physiology and disease-specific impairment of the fetal immunological development and of the molecular mechanism of microenvironment–stem cell interaction during the haematopoietic homing process in the fetal bone marrow after haematopoietic stem cell transplantation. Although fetal gene therapy might be the only way for successful prenatal treatment of some of these diseases, major hurdles inherent to gene therapy in general have to be overcome, predominantly concerning efficient HSC transduction and long-term gene expression. In addition, germline transduction or maternal cell transduction can theoretically occur and must be excluded. Prior to the use of fetal gene therapy in humans, these issues must be resolved, and major ethical questions must be addressed regarding immediate and long-term consequences (Fletcher and Richter, 1996; Schneider and Coutelle, 1999; Caplan and Wilson, 2000). The motto might be ‘proceed with caution’.

References


Carrier, E., Hae, T., Busch, M.P. et al. (1995) Induction of tolerance in nondefective mice after in utero transplantation of major


Prenatal stem cell and gene therapy


Received on May 24, 20000; accepted on September 8, 2000.