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In utero haematopoietic stem cell transplantation

Experiences in mice, sheep and humans

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Summary

Early prenatal diagnosis and in utero therapy of certain fetal diseases have the potential to reduce fetal morbidity and mortality. The intrauterine transplantation of stem cells provides in some instances a therapeutic option before definitive organ failure occurs. Clinical experiences show that certain diseases, such as immune deficiencies or inborn errors of metabolism, can be successfully treated using stem cells derived from bone marrow. However, a remaining problem is the low level of engraftment that can be achieved.

Efforts are made in animal models to optimise the graft and study the recipient's microenvironment to increase long-term engraftment levels. Our experiments in mice show similar early homing of allogeneic and xenogeneic stem cells and reasonable early engraftment of allogeneic murine fetal liver cells (17.1% donor cells in peripheral blood 4 weeks after transplantation), whereas xenogeneic HSC are rapidly diminished due to missing self-renewal and low differentiation capacities in the host's microenvironment. Allogeneic murine fetal liver cells have very good long-term engraftment (49.9% donor cells in peripheral blood 16 weeks after transplantation). Compared to the rodents, the sheep model has the advantage of body size and gestation comparable to the human fetus. Here, ultrasound-guided injection techniques significantly decreased fetal loss rates. In contrast to the murine in utero model, the repopulation capacities of allogeneic ovine fetal liver cells are lower (0.112% donor cells in peripheral blood 3 weeks after transplantation). The effect of MHC on engraftment levels seems to be marginal, since no differences could be observed between autologous and allogeneic transplantation (0.117% donor cells vs 0.112% donor cells in peripheral blood 1 to 2 weeks after transplantation).

Further research is needed to study optimal timing and graft composition as well as immunological aspects of in utero transplantation.

Key words: in utero transplantation; stem cells; animal models

Introduction

Over the last two decades extensive efforts have been made to develop techniques for early prenatal diagnosis of certain genetic diseases. Today fetal material can be obtained for genetic analysis as early as in the first trimester by chorionic villous sampling (CVS) [1]. Prenatal diagnosis for chromosomal defects or single gene disorders is based on new molecular biology techniques such as PCR using either fetal material from an invasive test, such as CVS, or using fetal cells or fetal cell-free DNA from maternal blood [2, 3]. However, the early diagnosis should also allow early treatment, because some diseases would lead to manifest organ damage already during gestation. Though several prenatal treatment options are available for some diseases, such as intrauterine blood transfusion in cases of severe fetal anaemia, others are still experimental and prone to a high fetal loss rate [4].

It is known that some diseases, such as haemoglobinopathies (eg Fanconi's anaemia, thalassaemia), immunological defects (eg SCID) or certain inborn errors of metabolism (M. Hurler, M. Krabbe) can be treated by transplantation of stem cells [5]. If the stem cell transplantation is performed before symptoms of the disease occur, organ function can be preserved [6]. However, if transplantation is performed after delivery of the baby, intensive immunosuppression and myoablation have to be used to minimise the risk of Graft-versus-host disease and to empty the bone marrow. A suitable stem cell donor is not always available.

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Despite the established worldwide stem cell registries, approximately 40% of patients fail to find an HLA-matched stem cell donor [7].

Thus, in utero stem cell transplantation would be an alternative to the common postnatal transplantation. Its advantages are based on the unique opportunity provided by the normal haematological ontogeny. The early fetus is immunological immature and thus would theoretically accept foreign antigens [8]. The conditioning therapy prior to transplantation could be omitted. Using the advantages of early prenatal diagnosis in the first trimester, stem cells have to be transplanted before maturation of the fetal thymus and the accumulation of fetal T cells in early second trimester [9]. Naturally occurring intrauterine transplantation and following persistent microchimerism have already been described for dizygotic twins, which allows specific transplantation tolerance [10, 11]. During gestation, the developing fetal haematopoietic system undergoes rapid changes and expansion. Since the fetal bone marrow is becoming a niche in the beginning second trimester, it would then probably provide equal chances of homing for the circulating stem cells, either own or transplanted [12]. The small size of the fetus would obviate the transplantation of large cell numbers. All these factors would lead to an easier donor selection with less strict HLA-match and graft size. Additionally, once successfully transplanted, the intrauterine environment would protect the fetus during ongoing gestation from surrounding viral and bacterial infections. The major advantage would result from the early transplantation before definitive organ damage has occurred.

Clinical experiences

The first in utero transplantation of haematopoietic stem cells was performed in a 17-week old fetus with rhesus-isoimmunisation. Using maternal T-cell depleted bone marrow injected into the umbilical vein did however not result in postnatal engraftment [13]. Since then intrauterine

Table 1

Inherited disorders that might benefit from in utero stem cell transplantation (attempted in utero transplantation is indicated by bold font) [adapted from reference 44].

Immunodeficiency disorders	**Bare lymphocyte syndrome**
	Cartilage-hair hypoplasia
	Chediak-Higashi syndrome
	Chronic granulomatous disease
	Kostman's syndrome
	Leukocyte adhesion defiency
	Omenn syndrome
	Severe combined immunodeficiency syndrome
	Wiskott-Aldrich syndrome
	X-linked immunodeficiency with hyperimmunoglobin M
	X-linked Bruton agammaglobulinaemia
Haemoglobinopathies and Rh disease	Congenital erythropoietic porphyria
	α-Thalassaemia
	β-Thalassaemia
	Sickle cell disease
	Rhesus isoimmunisation
Enzyme storage diseases	α-Mannosidosis
	Adrenoleukodystrophy
	Gaucher disease
	Globoid cell leukodystrophy
	Metachromatic leukodystrophy
	Mucopolysaccharidoses
	Niemann-Pick disease
	Wolmans disease
Others	Dyskeratosis congenital
	Familial haemaphagocytic lymphohistiocytosis
	Haemophilia A
	Infantile osteopetrosis
	Osteogenesis imperfecta
	Shwachman-Diamond syndrome

stem cell transplantation has been attempted in cases of haematopoietic and non-haematopoietic diseases (table 1). It has been most successful in cases with immunodeficiencies. Touraine and co-workers used fetal liver and thymic epithelial cells for transplantation in a 30-week old fetus with bare lymphocyte syndrome [14]. At delivery, the infant showed a 10% chimerism in peripheral blood. Interestingly, the patient developed split chimerism where only T cells were reconstituted, whereas other immune competent cells were still missing. Such split chimerism has also been observed in cases of severe combined immunodeficiency (SCID), where donor derived T-cells reconstituted whereas only low levels of donor myeloid and B-cell chimerism have been noted [15,16]. In patients with B^+SCID (B cells present) a normalisation of T cell number and function with a high T cell receptor diversity could be observed, whereas in patients with B^-SCID (B cells missing) a significantly restricted T cell repertoire could be detected [17]. These differences might be due to different selection advantages of donor-derived early T-cell precursors and competition with autologous precursors for intrathymic differentiation.

In the cases with erythroid disorders, such as α or β-thalassaemia, sickle cell anaemia or Rh isoimmunisation no significant chimerism could be detected [18–20]. The main problem here might be related to the present fetal cells in the bone marrow, since the transplanted cells have no proliferation advantage. However, in cases of thalassaemia, already low-level engraftment at 10–20% could be sufficient to diminish the symptoms of the disease [21]. To achieve such engraftment levels other strategies have to be applied. One key issue seems to be the composition of the graft. From animal models it is known that co-transplantation of haematopoietic and mesenchymal stem cells, tandem transplantation of two HSC samples, third-party transplantation of MSC or postnatal booster transplantation could increase engraftment levels [22–25]. Successful engraftment might be a matter of composition of the graft, but also of cell numbers, as can be concluded from the case reported by Bambach and co-workers [26]. In a fetus with globoid cell leukodystrophy 5×10^9 $CD34^+$ paternal bone marrow cells per kg were transplanted at 14 weeks gestation. 7 weeks later the fetus died with overwhelming donor cell engraftment and leukostasis. Extramedullary hematopoiesis occurred in the epicardium, serosa of the bowel, and the interstitium of the lungs and kidneys.

From these clinical experiences it can be concluded that further animal studies are necessary to study technical and immunological issues related to in utero stem cell transplantation in detail.

Experiences with the murine model

Initial experiments have been performed using transplacental injection of haematopoietic stem cells to cure anaemic mice [27]. 43% of recipient mice were engraftable for more than 6 months. Blazar and co-workers optimised the injection technique by using the intraperitoneal route [28]. They also could show that multilineage long-term engraftment can be achieved (day 141: 57 to 80% donor T cells, 10 to 15% donor B cells, 27 to 43% donor granulocytes). Additionally, they could prove that adult bone marrow stem cells engraft longer than 141 days by transferring bone marrow from an engrafted primary recipient (141 days after in utero transplantation) into lethally irradiated secondary recipients. It can be speculated that in utero transplantation in anaemic mice is successful because of proliferation advantages of the healthy donor cells. In immune competent, non-anaemic mice limited engraftment after in utero transplantation is due to the competition between host and donor cells for a limited number of niches. This might be increased by a postnatal booster injection as has been shown by Milner and co-workers [29]. After an initial in utero transplantation of 10^6 nucleated cells, booster injections of 5×10^6 nucleated cells at day 2, 4, and 7 after delivery resulted in a significantly elevated multilineage engraftment with granulocyte predominance (3.30% donor cells after booster vs 0.69% donor cells in control cases). Another strategy to increase engraftment in non-defective mice makes use of pretreated cytotoxic, non-proliferative T cells. After co-injection with T cell-depleted bone marrow a significantly better engraftment could be observed (13.3%) presumably due to a destruction of host haematopoietic cells without causing Graft versus-host disease [30].

As in clinical experiences, murine SCID recipients show better engraftment than normal mice, suggesting that immune effector cells prevent higher levels of sustained long-term engraftment [31]. To study this issue, several groups used NOD/SCID mice recipients, where functional T, B and NK cells are missing and macrophage function is defective [31, 32]. Although the majority of circulating donor cells after in utero transplantation of haematopoietic stem cells consists of lymphocytes, reconstitution of both lymphoid and myeloid cell lineages could be observed.

Whereas long-term engraftment is well studied, only few data are available on early homing of haematopoietic stem cells after in utero transplantation, although these early events are crucial for later long-term repopulation. We have therefore studied the engraftment kinetics up to 48 hours after transplantation, and 4 to 16 weeks after de-

Table 2

Postnatal long-term engraftment levels of allogeneic donor cells in peripheral blood, bone marrow and spleen of NOD/SCID mice at various time points (n = number of samples, SD = standard deviation, na = not assessed).

Time postnatal	Peripheral blood			Bone marrow			Spleen		
	n	mean	SD	n	mean	SD	n	mean	SD
4 weeks	24	22.7%	13.1%	8	0.9%	1.1%	8	37.4%	18.8%
8 weeks	14	41.6%	15.7%	na			na		
12 weeks	11	47.2%	20.7%	na			na		
16 weeks	9	50.0%	17.0%	9	5.1%	4.8%	9	87.7%	7.3%

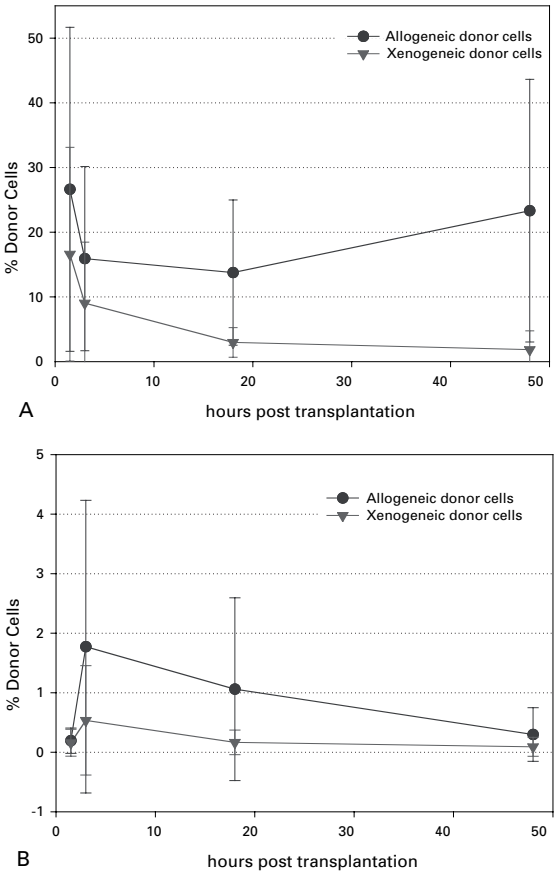

Figure 1

Prenatal kinetics of engraftment of donor cells from cord blood (circles) and fetal liver (triangles) found in fetal liver (figure 1A, above) and peripheral blood (figure 1B, below) at different time points after in utero transplantation. The donor cell frequency is calculated as the percentage of human CD45 (in case of xenogeneic donor) or CD45.2 (in case of allogeneic donor) expressing cells among the total of CD45.1 (mean, ±SD).

livery [33]. NOD/SCID fetal mice were injected with either 10^5–10^6 murine fetal liver cells or human cord blood-derived CD34$^+$ cells at day 13.5 of gestation. Both donor cell types homed rapidly and could be detected in peripheral blood and fetal liver soon after intraperitoneal injection (see figure 1). The frequency of xenogeneic stem cells however rapidly diminished, whereas the allogeneic stem cells expanded over time and led to multilineage reconstitution (see table 2). The failure of human cord blood-derived CD34$^+$ cells to engraft in the long term might be due to a barrier in the definitive homing process. or a missing supportive microenvironment in the host's bone marrow. Our results are in accordance with the findings of Archer et al. who transplanted allogeneic HSC from bone marrow in utero to NOD/SCID mice and noticed similar engraftment levels 4 weeks postnatally (30% in peripheral blood) [31]. Other groups using immune competent mouse models achieved much lower engraftment levels (0.69–10%), which indicates that the fetal immune system has alloreactivity already at that early stage of gestation [28, 34].

Experiences with the sheep model

In contrast to the small animal models, sheep have the advantage of body size. This allows studying technical issues, such as the ultrasound-guided collection of stem cells from the early ovine fetus or the injection techniques [35, 36]. Additionally immunocompetence develops at similar gestational age compared to human gestation. Though the development of the immune system is not identical, the fetal lamb is tolerant to xenogeneic (eg human) stem cell transplantations before day 60 of gestation [37]. Furthermore, the relatively long gestation allows the study of different application strategies, eg injections at different time points [22, 38]. In a previous study we were able to decrease fetal loss rate to 24% (compared to approximately 50% in surgical procedures), although ultrasound-guided transplantation was performed as early as 45 to 60 days of gestation [38, 39].

As stated above, successful in utero transplantation in human fetuses is still limited to cases with immune defects. To further study the role of foreign MHC recognition by the fetal immune system, we compared allogeneic versus autologous stem cell transplantation in the established sheep model [39]. Autologous fetal liver stem cells were collected by ultrasound-guided puncture of the fetal liver at day 50 to 57 of gestation. Allogeneic fetal liver stem cells were obtained by a surgical procedure at similar gestational age. In utero transplantation revealed no significant differences between allogeneic and autologous short-term stem cell engraftment (see table 3). However, due to the more invasive approach, autologous transplantation yielded a higher fetal loss rate (73% versus 29%). Either the very early transplantation, before cell-mediated immune response is elicited,

Table 3

Short-term engraftment (1–2 weeks post partum) of allogeneic versus autologous transplantation in White Alpine sheep by FACS analysis (PKH26-labelling). Mean % donor cells; t-test (mean, p) or Mann-Whitney Rank Sum test (median, p; if normality test failed, italics).

Tissue	Transplantation group	n	% Donor cells	p
Blood	Allogeneic	11	0.112	0.928
	Autologous	4	0.117	
Bone marrow	Allogeneic	11	0.560	0.174
	Autologous	4	0.160	
Liver	Allogeneic	11	0.233	0.041
	Autologous	4	0.647	
Spleen	Allogeneic	11	1.140	0.935
	Autologous	4	1.067	
Thymus	Allogeneic	11	0.395	0.396
	Autologous	4	0.053	

resulted in similar early engraftment, or MHC recognition is not important in the sheep model [40]. The later theory is supported by experiments in swine, where in utero transplantation of fully HLA-mismatched bone marrow-derived stem cells led to stable multilineage engraftment [41]. The level of engraftment in our studies is low (below 1%), which is in agreement with the results of other groups using xenogeneic in utero transplantation [42, 43]. Only few report higher engraftment levels in sheep, but with a different setting [22].

In summary, most immune competent animal models show only low levels of engraftment, as is the clinical experience. Further research should address unresolved questions, such as the optimal time point of transplantation, composition of the graft and dose of injected stem cells, the role of immune effector cells in the host and as a co-transplant, and methods to induce immune tolerance in the fetus.

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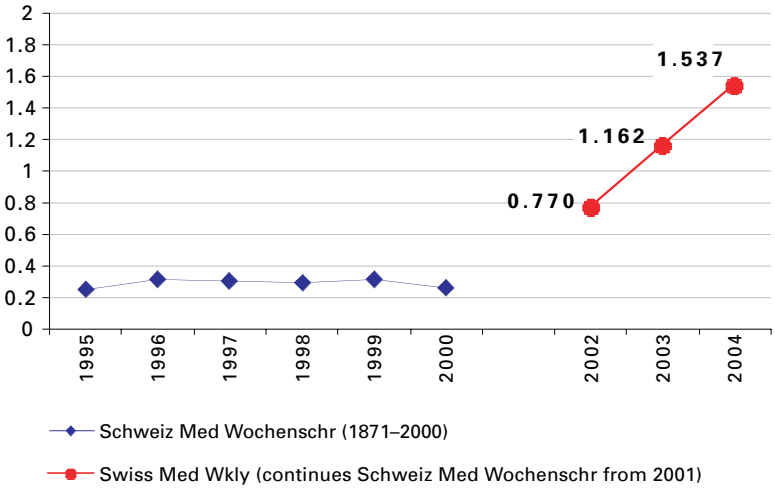

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