• BASIC RESEARCH •

Influence of intrauterine injection of rat fetal hepatocytes on rejection of rat liver transplantation

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Abstract

AIM: To investigate the influence of immune tolerance induced by intrauterine exposure to fetal hepatocytes on liver transplantation in the adult rat.

METHODS: LOU/CN rat fetal hepatocytes were injected into the fetuses of pregnant CHN rats (14-16 days of gestation). At 7-9 weeks of age, the surviving male rats received orthotopic liver transplantation (OLT) from male LOU/CN donors and the survival period was observed and monitered by mixed lymphocyte reaction assay and cytotoxicity test.

RESULTS: (1) A total of 31 pregnant CHN rats with 172 fetuses received fetal hepatocytes from LOU/CN rats via intrauterine injection. Among them, thirteen pregnant rats showed normal parturition, with 74 neonatal rats growing up normally. (2) The mean survival period after OLT in rats with fetal exposure to fetal hepatocytes was 32.1±3.7 days, which was significantly different from the control (11.8±2.3 days, $P<0.01$) in rats without fetal induction of immune tolerance. (3) Mixed lymphocyte proliferation assays yielded remarkable discrepancies between the groups of rats with- or without fetal exposure to fetal hepatocytes, with values of 8 411±1 361 and 22 473±1 856 (CPM±SD, $P<0.01$) respectively. (4) Cytotoxicity assays showed values of 21.2±6.5 % and 64.5±7.2 % ($P<0.01$) in adult rats with or without fetal induction of immune tolerance.

CONCLUSION: Intrauterine injection of fetal hepatocytes into rat fetuses can prolong the survival period of liver transplant adult male rats recipients, inducting immune tolerance in OLT.

INTRODUCTION

In both experimental study and clinical practice, rejection responses induced by organ transplants necessitate the use of potent immunosuppressive drugs[1-7]. It should be noted, however, that excessive dosage of immunosuppressive agents may result in severe side effects such as hypertension and hepatic and/or renal toxicity. Moreover, prolonged usage of immunosuppressants often leads to severe infection and increased susceptibility to malignancy, thus critically affecting the health of recipients. Particularly in juvenile patients, immunosuppressive drugs can lead to stunted growth. It is therefore imperative to assess, as an alternative to immunosuppressants, the protective effect of induced immune tolerance on organ transplantation. The ideal strategy is to induce a low responsiveness or irresponsiveness in the recipients toward donors' grafts, while preserving normal immunological functions for the recognition of tumor antigens and prevention of infection. Thus immunosuppressive agents can be avoided or be used at dramatically reduced dosage. The key steps toward successful transplantation therefore include either attenuated immune reactions or induced immune tolerance to grafts[8-15].

In utero, the stem cell transplantation represents a new therapeutic approach of experimental nature toward the treatment of hematopoietic diseases, immunodeficiency diseases, metabolic disorders and genetic diseases[16-19]. Some reports support the possibility of using this method to induce immunologic tolerance in both human subjects and animals receiving organ transplantation[20-22]. But the effect of *in utero* fetal exposure to fetal hepatocytes on liver transplantation in adulthood is unclear. In the present study, we injected fetal hepatocytes intraperitoneally into rat fetuses and then performed orthotopic liver transplantation (OLT) in surviving adult male rats. Some parameters were tested in order to confirm the anti-rejection effect of *in utero* injection of fetal hepatocytes.

MATERIALS AND METHODS

Animals
Male and female LOU/CN and CHN rats weighing 200-250 g were obtained from the Laboratory Animal Center of The Fourth Military Medical University, and fed with standard rat chow.

Timed pregnancy of CHN rats
Male CHN rats, or male LOU/CN rats, were housed individually in standard cages with a metal divider with 0.25-inch holes. One female CHN or LOU/CN rat was placed in the corresponding empty side. The cage divider was then removed 48 hrs later. Pairs of CHN or LOU/CN rats were allowed to mate overnight separately. The pairs were then separated with the females placed singly in cages. The presence of vaginal plug marked day 0 of gestation. Normal gestation is about 21 days.

Preparation of LOU/CN fetal rat hepatocytes
Pregnant LOU/CN females were killed by cervical dislocation at 15 to 16 days of gestation. All subsequent handling was done under sterile conditions. The uterine horns were removed and dissected in phosphate-buffered saline (PBS). Fetuses were separated from decidua and extraembryonic membranes and transferred to 35 mm petri dishes filled with PBS. Fetal livers were removed, with 5 to 10 livers placed in 1 to 2 ml RPMI 1640 medium (GIBCO BRL, Grand Island, CA) with 15 % fetal calf serum (FCS, GIBCO BRL, Grand Island, CA). Cell suspensions were prepared by repeated pipetting of the livers

through a 23-gauge needle attached to a 1ml pipette. The mononuclear cells were separated by centrifugation at 500 g for 20 minutes and filtered through a Nitex filter with a 41 μm pore size (Huamei chemical company, Henan province, China). Cell viability counting (usually greater than 90 %) was done using trypan blue exclusion test, and the final cell suspension was prepared in PBS/15 % FCS at a concentration of 5×10^7/ml. The cell suspension was used for intrauterine injection within 4 hours of preparation.

In utero intraperitoneal injection into CHN rat fetuses with LOU/CN fetal rat hepatocytes

Micropipettes were prepared from capillaries using a Brown and Flaming micropipette puller. The end of the needle was cut under a microscope with ophthalmological forceps, and the tip was sharpened on a micro-grinding wheel. Pregnant (11 to 13 days of gestation) CHN rats were anesthetized i.p. with sodium pentobarbital, and the uterine horns were exteriorized with ophthalmological forceps. Fetal hepatocytes from LOU/CN rats were injected into CHN fetuses through a hand-drawn glass micropipette with a beveled edge. The placenta was penetrated at an oblique angle. The number of cells injected per fetus was 10^6 (about 5×10^{10} cells/kg of recipient weight), except in experiments in which the effects of different cell concentrations and volumes of cell suspension used were examined. Muscle layers were closed with 4-0 silk suture, and 1-0 silk thread was used for skin suture.

Orthotopic liver transplantation (OLT)

Orthotopic whole-liver transplantation was performed[23-26] using the simplified cuff technique for portal and intrahepatic vena cava anastomosis, whereas the hepatic artery was not reconstructed. The twenty male CHN rats, aged 7 to 9 weeks that survived intraperitoneal injection with fetal liver cells from LOU/CN rats, were taken as graft recipients, with normal male LOU/CN rats as liver donors. Both donors and recipients were anesthetized with methoxyflurane. After explantation, livers were stored at 0-4 ℃ for 1 hr in UW solution. Grafts were connected to suprahepatic vena cava with a running 7-0 prolene suture. We insert cuffs into the corresponding vessels, and anastomose the bile duct and hepatic artery over an intraluminal polyethylene splint. Transplantation required less than 40 min, while the portal vein was clamped for 12-15 min during this period. After transplantation, the recipients had free access to standard laboratory chow and tap water. Animals that died within 3 days were considered technical failures and were excluded from data collection. The rate of success of liver transplantation was more than 90 % in this study. Five rats were sacrified at 5 days after OLT. Immediately before sacrifice, the livers were removed for histological investigation. Liver samples from 10 normal male CHN rats were taken as controls.

Mixed lymphocyte reaction assay

Mixed spleen cells were used for setting up lymphocyte cultures. Briefly, the spleens were aseptically removed from experimental CHN rats that survived in utero intraperitoneal injection of fetal LOU/CN rat liver cell, and from control CHN rats. Then they were separately disrupted mechanically using a pair of sterile forceps. LOU/CN spleen cells were treated with 25 μg/ml mitocin-C (Kyowa Hakko Kogyo, Tokyo, Japan) at 37 ℃ for 45 min. Then the two groups of cells were washed by PBS, followed by lysis of erythrocytes in Tris-NH₄Cl solution (pH 7.2). After further washes, the cells were finally resuspended in RPMI 1640 with 10 % FCS. The numbers of two groups of spleen cells were adjusted, and 5×10^5 spleen cells from each of the two sources were mixed together (final

volume 0.2 ml) and added to triplicate wells of 96-well round-bottomed microtiter plates. Cells were cultured for 120 hr, and 1 u Ci [3H]-thymidine was added into wells 18hr before cell harvesting. Radioactive thymidine incorporation rate was measured by a liquid scintillation counter (1205 Betaplate), with data expressed as CPM±SD[27,28].

Complement-dependent cytotoxicity test

Recipient CHN rats were tested one week after OLT for complement-dependent cytotoxicity test against lymphocytes suspensions from LOU/CN donor rats. Sera from recipients were prepared and added into the wells of 96-well round-bottomed microtiter plates (1 μl per well). Then 1 μl lymphocytes (about 2 000 cells per 1 μl volume) were added into the same wells. They were gently mixed and incubated for 30 min at 22 ℃±2 ℃, then 5 μl rabbit complement (Huamei chemical company, Henan province, China) was added, and the mixture incubated for 60 min at 22 ℃±2 ℃. Afterwards, the mixtures were stained with eosin (50 g/L) for 5 min. The cells were fixed in 36 % formalin for 2 hr and observed with an inverted phase-contrast microscope (Olympus, Tokyo, Japan). Cytotoxicity was determined by eosin exclusion and percentage enumeration of cells killed by sera from OLT[29,30].

Histology

Livers from CHN rats that underwent OLT were fixed in 10 % neutral formalin for five days and then embedded in paraffin. Five micron sections were cut and stained with hematoxylin and eosin for histological examination. Sections from normal rat livers served as controls.

Diagnostic criterion of graft rejection

Several diagnostic criteria were used to determine the existence of rejection response following hepatic transplantation. The first is survival of recipients over 7 days post-transplantation. The second entails the occurrence of acute hepatic dysfunction as typified by inappetence, weight loss, auricle or nail jaundice, depressed mood, with incidental diarrhea or ascites in some cases. The third criterion involves typical histological changes, such as enlarged liver bulk, yellowish white appearance of the liver, and the presence of liver conglutination to muscles or abdominal walls, and viscera. Also frequently encountered are apparent degeneration or necrosis of hepatic cells, and infiltration of numerous lymphocytes, especially at liver sinusoidal area. The fourth denotes the presence in good condition of the recipients plus good appetite, accompanied with normal weight 3 days after successful transplantation. The recipients that died from post-surgical complications such as necrosis of bile duct, intra-abdominal hemorrhage and infection were excluded from data collection.

Statistics

All the data were analyzed by Student's t test and expressed as mean ±S. The statistical difference $P<0.05$ was considered significant and $P<0.01$ as very significant.

RESULTS

Influence of intraperitoneal injection into fetus in utero on survival of neonatal CHN rats

Thirty-one pregnant CHN recipients who later delivered 172 fetal CHN rats were injected into their fetuses with isolated fetal hepatocytes from LOU/CN rats. Among them, eleven pregnant females died from various surgical complications, and seven females aborted or consumed their litters. There were 17 pregnant rats with normal parturition, and 74 neonatal rats grew up normally.

Suppression of rejection response to rat liver transplantation after intrauterine injection

The mean survival period of rats with fetal intraperitoneal injection of fetal hepatocytes prior to OLT was 32.1±3.7 day, in comparison to control values of 11.8±2.3 day (*P*<0.01) obtained from rats without this procedure.

Inhibitory effect of intraperitoneal injection in utero on mixed lymphocytes reaction assay

Mixed lymphocytes reaction assay showed significant differences in [3H]-thymidine incorporation rates in groups of CHN rats that were either fetally exposed (8411±1361, CPM±SD) or not (22473±1856, CPM±SD) to intraperitoneal injection of fetal hepatocytes in utero (*P*<0.01).

Suppressive effect of fetal intraperitoneal injection in utero on cytotoxicity test after rat liver transplantation in adulthood

Cytotoxicity test revealed percentage values of dead cells to be 21.2±6.5 % *vs* 64.5±7.2 % (*P*<0.01) respectively from the two groups of CHN rats with or without fetal intraperitoneal injection in utero prior to OLT in the adult rats, the difference being statistically significant.

DISCUSSION

With rapid advances in antenatal diagnostic technology, many prenatal diseases (congenital metabolic liver disorders, congenital heart disease and hemophilia, e.g.) can be diagnosed. Therefore organ transplantation becomes a very useful choice for treating these diseases in afflicted children. However, routine regime of immunosuppression in use today suffered from many serious side effects, including stunted growth seen in children using most immunosuppressive drugs[31-34]. If we can induce low responsiveness or even irresponsiveness in the recipients to the donors' grafts, immunosuppressive drugs can then be used at much reduced dosage. Until now, low responsiveness or irresponsiveness to the graft has not been achieved in clinical practice. Therefore, induction of immunologic tolerance becomes imperative in clinical transplantation[35-42].

Clonal deletion theory maintains that burst of cell proliferation during embryonic period is very frequent, with resultant formation of multiple specific clones of lymphocytes capable of responding to respective antigens[43,44]. During embryonic period, the lymphocyte clones encounter internal antigens or artificially introduced antigens, with consequent damage to, or suppression of these clones, the so-called abstinence clones. In postnatal life, the abstinence clone remains inactive to an internal antigen or an artificially introduced foreign antigen that has been present in utero, a status of immunologic tolerance in the adult to an antigen following prior exposure during the embryonic period. On the contrary, if a lymphocyte clone meets an antigen that has never been introduced during the embryonic period, the specific immunological response would ensue. According to the theory, intrauterine injection is considered a useful tool for the induction of immunologic tolerance, especially for clinical transplantation. Some advantages of intrauterine injection for evoking immunologic tolerance should be noted. Firstly, because the host at fetal stage can't recognize a foreign substance and thus display immunologic tolerance to a xenogen (the earlier the fetal stage of the host, the stronger the tolerance effect to the antigen), no immunologic reaction is seen between the host and ecdemic grafts. Therefore intrauterine injection spares tissue-matching work needed in routine transplantation and makes easier graft transplantation. Meanwhile, immunologic tolerance induced by intrauterine injection makes unnecessary pretreatment with immunosuppressive drugs, thus avoiding possible side effects of immunosuppressive agents on the body. Secondly, fewer fetal liver cells are needed for transplanting into the recipients during embryonic period because of their small body size and weight. The latter assures little influence and gentle torture for the recipients. Thirdly, in addition to exempt from infection due to microorganism, the maternal body offers adequate nutrition and energy to the fetuses, thus the uterus can be seen as an ideal ' isolation room' [45-51].

We also compared the effect of using rat spleen or bone marrow cells, with that of fetal liver cells, for intraperitoneal injection in utero into fetuses in pregnant rats. It was observed that the survival birth rate using fetal liver cells was much greater than that with spleen or bone marrow cells. Several factors may help to explain the discrepancy. The most important one may be that the response against the host is induced by active lymphocytes located in the spleen or bone marrow cells. In contrast, the main components of fetal liver cells are hematopoietic stem cells possessing two effects, with the first reducing graft versus host reaction, and the other for the maintenance of chimerism because hematopoietic stem cells are characterized by self-renewal and multipotential for differentiation[52,53].

As evidenced by both *in vivo* and *in vitro* data in the present study, the method of fetal hepatocytes in utero injection can prolong the survival period of rat liver transplants. Although complete immune tolerance can not be induced, partial immune tolerance observed in our study is sufficient for liver tranplantation. Further work will be needed to reveal if chimerism is induced by transplantation itself, as well as its possible influence on the immune system in the grafted recipients.

REFERENCES

1 **Karim M**, Steger U, Bushell AR, Wood KJ. The role of the graft in establishing tolerance. *Front Biosci* 2002; **7**: 129-154

2 **Riordan SM**, Williams R. Transplantation of primary and reversibly immortalized human liver cells and other gene therapies in acute liver failure and decompensated chronic liver disease. *World J Gastroenterol* 2000; **6**: 636-642

3 **Tang ZY**. Hepatocellular carcinoma-cause, treatment and metastasis. *World J Gastroenterol* 2001; **7**: 445-454

4 **Zhu XF**, Chen GH, He XS, Lu MQ, Wang GD, Cai CJ, Yang Y, Huang JF. Liver transplantation and artificial liver support in fulminant hepatic failure. *World J Gastroenterol* 2001; **7**: 566-568

5 **Platell CF**, Coster J, McCauley RD, Hall JC. The management of patients with the short bowel syndrome. *World J Gastroenterol* 2002; **8**: 13-20

6 **Ouyang EC**, Wu CH, Walton C, Promrat K, Wu GY. Transplantation of human hepatocytes into tolerized genetically immunocompetent rats. *World J Gastroenterol* 2001; **7**: 324-330

7 **Waldmann H**, Cobbold S. Approaching tolerance in transplantation. *Int Arch Allergy Immunol* 2001; **126**: 11-22

8 **Yu X**, Carpenter P, Anasetti C. Advances in transplantation tolerance. *Lancet* 2001; **357**: 1959-1963

9 **Qian YB**, Cheng GH, Huang JF. Multivariate regression analysis on early mortality after orthotopic liver transplantation. *World J Gastroenterol* 2002; **8**: 128-130

10 **Shi LB**, Peng SY, Meng XK, Peng CH, Liu YB, Chen XP, Ji ZL, Yang DT, Chen HR. Diagnosis and treatment of congenital choledochal cyst: 20 years' experience in China. *World J Gastroenterol* 2001; **7**: 732-734

11 **Bramhall S**, Minford E, Gunson B, Buckels JA. Liver transplantation in the UK. *World J Gastroenterol* 2001; **7**: 602-611

12 **Womer KL**, Lee RS, Madsen JC, Sayegh MH. Tolerance and chronic rejection. *Philos Trans R Soc Lond B Biol Sci* 2001; **356**: 727-738

13 **Zavazava N**, Kabelitz D. Alloreactivity and apoptosis in graft rejection and transplantation tolerance. *J Leukoc Biol* 2000; **68**: 167-174

14 **Hong JC**, Kahan BD. Immunosuppressive agents in organ transplantation: past, present, and future. *Semin Nephrol* 2000; **20**: 108-125

15 **Bartlett A**, McCall J, Munn S. Co-stimulatory blockade and tolerance induction in transplantation. *BioDrugs* 2001; **15**: 491-500

16   **Jones DR**. In utero stem cell transplantation: two steps forward but one step back? *Expert Opin Biol Ther* 2001; **1**: 205-212

17   **Hayashi S**, Flake AW. In utero hematopoietic stem cell therapy. *Yonsei Med J* 2001; **42**: 615-629

18   **Flake AW**. In utero transplantation of haemopoietic stem cells. *Best Pract Res Clin Haematol* 2001; **14**: 671-683

19   **Cowan MJ**, Chou SH, Tarantal AF. Tolerance induction post in utero stem cell transplantation. *Ernst Schering Res Found Workshop* 2001; **33**: 145-171

20   **Kim HB**, Shaaban AF, Milner R, Fichter C, Flake AW. In utero bone marrow transplantation induces donor-specific tolerance by a combination of clonal deletion and clonal anergy. *J Pediatr Surg* 1999; **34**: 726-729

21   **Rubin JP**, Cober SR, Butler PE, Randolph MA, Gazelle GS, Ierino FL, Sachs DH, Lee WP. Injection of allogeneic bone marrow cells into the portal vein of swine in utero. *J Surg Res* 2001; **95**: 188-194

22   **Takahashi T**, Kakita A, Sakamoto I, Takahashi Y, Hayashi K, Tadokoro F, Yamashina S. Immunohistochemical and electron microscopic study of extrinsic hepatic reinnervation following orthotopic liver transplantation in rats. *Liver* 2001; **21**: 300-308

23   **Boros P**, Tarcsafalvi A, Wang L, Megyesi J, Liu J, Miller CM. Intrahepatic expression and release of vascular endothelial growth factor following orthotopic liver transplantation in the rat. *Transplantation* 2001; **72**: 805-811

24   **Okano S**, Eto M, Tomita Y, Yoshizumi T, Yamada H, Minagawa R, Nomoto K, Sugimachi K, Nomoto K. Cyclophosphamide-induced tolerance in rat orthotopic liver transplantation. *Transplantation* 2001; **71**: 447-456

25   **He XS**, Huang JF, Chen GH, Zheng KL, Ye XM. A successful case of combined liver and kidney transplantation for autosomal dominant polycystic liver and kidney disease. *World J Gastroenterol* 1999; **5**: 79-80

26   **Zhang SG**, Wu MC, Tan JW, Chen H, Yang JM, Qian QJ. Expression of perforin and granzyme B mRNA in judgement of immunosuppressive effect in rat liver transplantation. *World J Gastroenterol* 1999; **5**: 217-220

27   **Genden EM**, Mackinnon SE, Yu S, Hunter DA, Flye MW. Pretreatment with portal venous ultraviolet B-irradiated donor alloantigen promotes donor-specific tolerance to rat nerve allografts. *Laryngoscope* 2001; **111**: 439-447

28   **Horimoto H**, Nakai Y, Nakahara K, Nomura Y, Mieno S, Sasaki S. HMG-CoA reductase inhibitor cerivastatin prolonged rat cardiac allograft survival by blocking intercellular signals. *J Heart Lung Transplant* 2002; **21**: 440-445

29   **Hehmke B**, Schroder D, Kloting I, Kohnert KD. Complement-dependent antibody-mediated cytotoxicity (CAMC) to pancreatic islet cells in the spontaneously diabetic BB/OK rat: interference from cell-bound and soluble inhibitors. *J Clin Lab Immunol* 1991; **35**: 71-81

30   **Tange S**, Scherer MN, Graeb C, Weiss T, Justl M, Frank E, Andrassy J, Jauch KW, Geissler EK. The antineoplastic drug Paclitaxel has immunosuppressive properties that can effectively promote allograft survival in a rat heart transplant model. *Transplantation* 2002; **73**: 216-223

31   **Reyes J**, Mazariegos GV, Bond GM, Green M, Dvorchik I, Kosmach-Park B, Abu-Elmagd K. Pediatric intestinal transplantation: Historical notes, principles and controversies. *Pediatr Transplant* 2002; **6**: 193-207

32   **Bassas AF**, Chehab MS, Al-Shahed MS, Djurberg HG, Al-Shurafa HA, Jawdat MT, Al-Hussaini HF, Zuleika MA, Al-Hebby HA, Wali SH. Pediatric living-related liver transplantation in Saudi

Arabia. *Saudi Med J* 2002; **23**: 640-644

33   **Robinson LG**, Hilinski J, Graham F, Hymes L, Beck-Sague CM, Hsia J, Nesheim SR. Predictors of cytomegalovirus disease among pediatric transplant recipients within one year of renal transplantation. *Pediatr Transplant* 2002; **6**: 111-118

34   **Samsonov D**, Briscoe DM. Long-term care of pediatric renal transplant patients: from bench to bedside. *Curr Opin Pediatr* 2002; **14**: 205-210

35   **Rela M**, Dhawan A. Liver transplantation in children. *Indian J Pediatr* 2002; **69**: 175-183

36   **Fine RN**. Growth following solid-organ transplantation. *Pediatr Transplant* 2002; **6**: 47-52

37   **Bucuvalas JC**, Ryckman FC. Long-term outcome after liver transplantation in children. *Pediatr Transplant* 2002; **6**: 30-36

38   **Shneider BL**. Pediatric liver transplantation in metabolic disease: clinical decision making. *Pediatr Transplant* 2002; **6**: 25-29

39   **He XS**, Huang JF, Chen GH, Fu Q, Zhu XF, Lu MQ, Wang GD, Guan XD. Orthotopic liver transplantation for fulminant hepatitis B. *World J Gastroenterol* 2000; **6**: 398-399

40   **Guan WX**, Li KZ, Dou KF. Hepatocellular carcinoma and liver transplantation. *Shijie Huaren Xiaohua Zazhi* 2001; **9**: 1292-1295

41   **Song WL**, Wang WZ, Wu GS, Dong GL, Ling R, Ji G, Zhao JX. Evaluation of perioperative serum cytokine level in acute rejection in human living-related small bowel transplantation. *Shijie Huaren Xiaohua Zazhi* 2001; **9**: 401-404

42   **He XS**, Huang JF, Chen GH, Zheng KL, Ye XM. A successful case of combined liver and kidney transplantation for autosomal dominant polycystic liver and kidney disease. *World J Gastroenterol* 1999; **5**: 79-80

43   **Prevot A**, Martini S, Guignard JP. Exposure in utero to immunosuppressives. *Rev Med Suisse Romande* 2001; **121**: 283-291

44   **Tanaka SA**, Hiramatsu T, Oshitomi T, Imai Y, Koyanagi H. Induction of donor-specific tolerance to cardiac xenografts in utero. *J Heart Lung Transplant* 1998; **17**: 888-891

45   **Mackenzie TC**, Shaaban AF, Radu A, Flake AW. Engraftment of bone marrow and fetal liver cells after in utero transplantation in MDX mice. *J Pediatr Surg* 2002; **37**: 1058-1064

46   **Taylor PA**, McElmurry RT, Lees CJ, Harrison DE, Blazar BR. Allogenic fetal liver cells have a distinct competitive engraftment advantage over adult bone marrow cells when infused into fetal as compared with adult severe combined immunodeficient recipients. *Blood* 2002; **99**: 1870-1872

47   **Barker JE**, Deveau S, Lessard M, Hamblen N, Vogler C, Levy B. In utero fetal liver cell transplantation without toxic irradiation alleviates lysosomal storage in mice with mucopolysaccharidosis type VII. *Blood Cells Mol Dis* 2001; **27**: 861-873

48   **Casal ML**, Wolfe JH. In utero transplantation of fetal liver cells in the mucopolysaccharidosis type VII mouse results in low-level chimerism, but overexpression of beta-glucuronidase can delay onset of clinical signs. *Blood* 2001; **97**: 1625-1634

49   **Surbek DV**, Holzgreve W, Nicolaides KH. Haematopoietic stem cell transplantation and gene therapy in the fetus: ready for clinical use? *Hum Reprod Update* 2001; **7**: 85-91

50   **Surbek DV**, Gratwohl A, Holzgreve W. In utero hematopoietic stem cell transfer: current status and future strategies. *Eur J Obstet Gynecol Reprod Biol* 1999; **85**: 109-115

51   **Flake AW**, Zanjani ED. In utero transplantation for thalassemia. *Ann N Y Acad Sci* 1998; 850: 300-311

52   **Pschera H**. Stem cell therapy in utero. *J Perinat Med* 2000; **28**: 346-354

53   **Stanworth SJ**, Newland AC. Stem cells: progress in research and edging towards the clinical setting. *Clin Med* 2001; **1**: 378-382

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