Present and future applications of hepatic cell transplantation

E.M. Sokal and M. Najimi

Université Catholique de Louvain & Cliniques St-Luc. Laboratory of Pediatric Hepatology & Cell Therapy. Brussels. Belgium.

INTRODUCTION

After validation in several animal models, liver cell transplantation (LCT) is actually in its clinical phase development. As documented in more than 20 human case reports, the technique is well tolerated, feasible and safe. LCT is moreover less invasive than orthotopic liver transplantation as the native liver remains preserved avoiding early technical complications and consequences of long term progressive graft damages. LCT addresses ideally patients with inborn errors of metabolism, either as a full treatment option, or in more severe situations, as a bridge to transplantation. In these patients, it can bring metabolic control for variable period of time, up to 18 months following infusions. The technique is still facing many hurdles and efforts are currently focused on its improvement. This includes the use of alternatives cell sources-essentially stem or progenitor cells- that may have a higher repopulation potential in the recipient liver, while helping also to solve the problem of organ shortage thanks to *in vitro* expansion. In parallel, efforts aim at improving the viability and quality of cryopreserved mature hepatocytes as well as to prevent the rejection and/or loss of transplanted hepatocytes.

PHYSIOLOGY OF LCT

LCT procedure supposes the transfer of isolated hepatocyte suspension into the diseased liver via an intact portal vein system (fig. 1). Hepatocyte isolation was performed on a liver or a liver segment non used for orthotopic liver transplantation. Hepatocyte isolation is carried out by qualified people in clean rooms according to GMP (Good Manufacturing Practices) guidelines. Hepatocytes are obtained from cadaveric donor livers using the two-step collagenase perfusion technique[1], modified by Seglen[2]. The first liver perfusion is performed with a prewarmed buffer containing EGTA, which aids to the dissolution of intercellular junctions between the hepatic cells whereas the second perfusion buffer contains calcium and collagenase. Recovered hepatocytes are submitted to a series of quality control tests for sterility, viability and metabolic activity. Isolated hepatocytes can be infused immediately after isolation, being kept in a cold solution of University of Wisconsin, or cryopreserved for further use. Transplantation efficiency is evaluated by engraftment, in situ metabolic functionality of the transplanted cells and repopulation of the recipient parenchyma at the long term level. Hence, the quality of the transplanted cells and in particular their ability to recover after isolation, cold storage and cryopreservation remain key and limitant factors for the success of the technique.

DONOR ORGANS

Liver cells are often isolated from resected liver segments obtained after reduction hepatectomy for size matching of orthotopic liver transplantation donor and recipients[3]. Such liver segment allows to recover sufficient hepatocyte mass for a transplantation series in newborn infants and children. Since 2003, 64 livers or liver segments were used in our centre for hepatocyte isolation with a mean viability and efficiency of plating of 83% and 65% respectively. Supply of donor livers remains a major problem in LCT programs. Traumatic livers unsuitable for surgical procedures are perfect sources to be used for hepatocyte isolation. On the contrary, hepatocytes isolated from fatty livers, disregarded for transplantation, are of poorer viability. Living donor liver transplantation is another alternative source of organs for orthotopic liver transplantation[4] but cannot be ethically considered for LCT as long as the procedure has not been clinically validated. Hence and because of the high costs of organ procurement, hepatocyte isolation and banking, close collaboration of all centers active in the field is mandatory in order to accelerate knowledge, standardization of the protocols and performance of LCT.

Correspondence: E.M. Sokal.
Université Catholique de Louvain. Faculty of Medicine. Laboratory of Pediatric Hepatology & Cell Therapy.
Avenue Hippocrate 10/1301. 1200 Brussels. Belgium.
E-mail: etienne.sokal@uclouvain.be

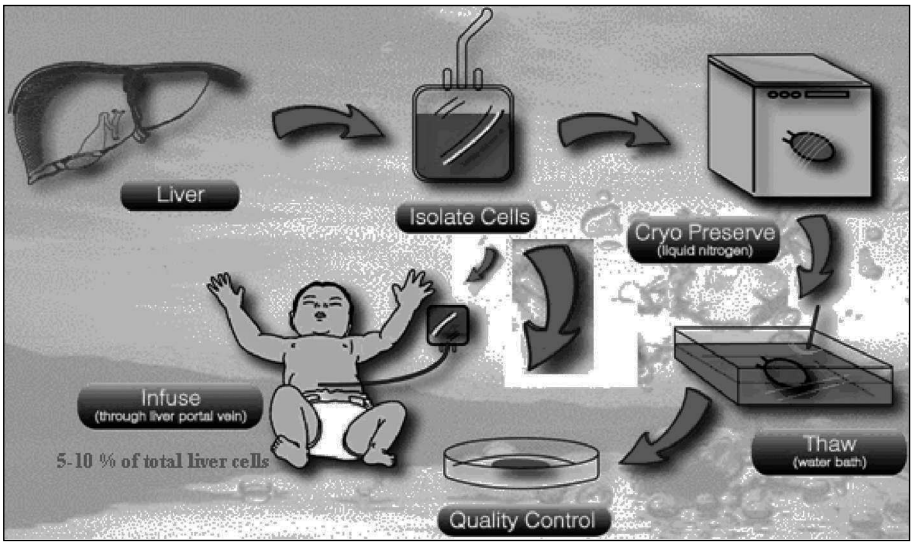

Fig. 1. Steps of liver cell transplantation procedure.

LCT FOR METABOLIC DISEASES

Inborn errors of metabolism are rare diseases which affect around 1/900 life birth. Such diseases can lead at the long term level to intellectual impairment and damage of other organs as for urea cycle disorders. Management of these patients requires severe diet restrictions and special education leading to a poor quality of life due to anorexia, nasogastric feeding, poor variability of diet, social eviction and frequent hospitalizations. For these indications, LCT was proposed as an alternative to orthotopic liver transplantation especially if the liver is intact but with extra-hepatic damage. Other "metabolic" patients are also in a such unstable condition that major surgery is contra-indicated since it may lead to severe or even fatal metabolic crisis.

Up to now, more than 20 patients with inborn errors of metabolism were treated by LCT. The number of isolated hepatocytes (fresh, cryopreserved or both) used allowed to reach 5 to 10% of the theoretical liver mass. No side effects related to LCT were documented.

In most published cases, a metabolic effect was observed even using exclusively cryopreserved hepatocytes (table I). After hepatocytes infusion, an immediate and transient effect is often observed and followed by a stable metabolic and clinic effect, which duration can be different depending on the disease etiology.

For most LCT treated patients, the technique was proposed as a bridge to transplantation during the waiting time for a graft. In urea cycle disorders patients, an ongoing benefit of LCT was maintained at the time of transplantation. In other diseases such as Crigler Najjar or Factor VII deficiency, the metabolic effect was lost after 3 to 6 months post-LCT due probably to rejection or death of the transplanted cells (table I).

CRYOPRESERVATION OF HUMAN HEPATOCYTES

Cryopreservation remains the practical and appropriate option for hepatocyte long-term storage and planned availability for clinical use. Cryopreserved hepatocytes have been demonstrated to behave similarly than freshly isolated cells after transplantation[11] and have variable ability to repopulate the damaged liver and to correct the deficient function in the various animal models tested[12]. In humans, even if cryopreserved hepatocytes have been successfully used in an ornithine transcarbamylase and factor VII deficient patients while waiting orthotopic liver transplantation[8,9], the quality of isolated hepatocytes remains deeply altered after cryopreservation/thawing[13].

We recently reported that cryopreservation induces a dramatic drop of ATP levels as well as a decreased oxygen consumption rate in suspended hepatocytes. These alterations were correlated to structural damages of mitochondria as demonstrated using electron microscopy (probably due to ice crystal formation). Specific dysfunction of the mitochondrial respiratory chain complexes 1 & 5 activity was also evidenced[14]. Mitochondrion to cytoplasm release of cytochrome c is one of the consequences of these cryopreservation-induced alterations. Nevertheless, no association with caspase activation, DNA fragmentation and apoptosis was observed[14].

Efforts are currently focused of the improvement of both preservation protocols and cryopreservation medium such as the use of vitrification (or ice free preservation) or the supplementation of substances that are able to stabilize hepatocyte cell membranes and proteins[15,16]. The impact of these strategies must also be evaluated *in vivo* at the engraftment and *in situ* survival of cryopreserved/thawed hepatocytes level.

DO STEM/PREGENITOR CELLS MAY EFFICIENTLY REPLACE MATURE HEPATOCYTES FOR LIVER CELL THERAPY?

To face the growing shortage of human livers, *in vitro* hepatocyte alteration (after culture and cryopreservation), and side effects of lifelong immunosuppression, research is currently focused on the evaluation of the stem/proge-

TABLE I. **LCT and inborn errors of metabolism**

Disease	Age of the patient (months)	Effect post LCT	Remarks (references)
Crigler Najjar	120	Up to 11 months	First LCT human published trial[5]
Glycogenosis 1a	564	Up to 18 months	Adult patient[6]
Refsum disease	48	Up to 18 months	First European pediatric LCT[7]
Factor VII deficiency	3	Up to 6 months	Only cryopreserved cells used[8]
OTC deficiency	14	Up to 6 months	Only cryopreserved cells used[9]
ASL deficiency	42	Up to 18 months	Direct demonstration of functional donor cells in the recipient liver parenchyma[10]

nitor cells potential. Their Self-renewal, differentiation capacity and the easy access to these cells may help to enlarge significantly the pool of cell sources for LCT and to overcome these limitations.

Intra-hepatic stem cells

Beside fetal liver, evidence has been accumulated to point out the presence of stem/progenitor cell compartment into the mammalian adult liver. Oval cells, a well described intra-hepatic stem cell type, are located at the canals of Hering[17] and participate to liver regeneration whenever proliferation of parenchymal cells is impaired as during chronic or extensive damage of the liver[18]. We and others have demonstrated the possibility to isolate non parenchymal epithelial stem/progenitor cell candidates from healthy and diseased human livers respectively[19,20]. In our laboratory, flow cytometry immuno-phenotype analysis revealed that these liver epithelial cells (LECs) expressed CD90 but were negative for CD117 and CD34 (markers of oval cells). As demonstrated using immuno-cytochemistry, LECs expressed not only immature (α-fetoprotein and transcription factor Oct-4) but also differentiated hepatocyte markers such as albumin and cytokeratin-18 and biliary markers (cytokeratins 7 and 19), whereas they were negative for OV-6, an oval cell marker, as well as for vimentin. RT-PCR assays confirm the immuno-cytochemistry data and revealed that LECs did not express mature hepatocyte markers such as CYP2B6, CYP3A4 and tyrosine amino-transferase while expression of glucose 6-phosphatase and α1-antitrypsin was weakly noted. Purified LECs were thereafter intrasplenically injected into SCID mice. The immuno-histochemical analysis of transplanted mice livers performed 1 month post-transplantation showed the presence of human albumin positive cells or cell foci within the recipient mice parenchyma. Mesenchymal stem/progenitor cells were also isolated from healthy human liver and are able to proliferate *in vitro*[21,22]. Liver mesenchymal-like cells we isolated in our hands, expressed both hepatic and mesenchymal markers among which albumin, CYP3A4, vimentin and α-smooth muscle actin. *In vitro* differentiation studies demonstrated that these mesenchymal-like cells are preferentially predetermined to differentiate into hepatocyte-like cells but not into adipocytes or osteocytes[22]. Ten weeks following intrasplenic transplantation into uPA$^{+/+}$-SCID mice, recipient livers showed the presence of human hepatocytic cell nodules positive for human α-fetoprotein, pre-albumin and albumin. In SCID transplanted liver mice, human hepatocyte-like cells were mostly found near vascular structures 56 days post-transplantation. These data showing the ability of isolated adult derived liver mesenchymal-like and epithelial-like cells to proliferate and to differentiate *both in vitro* and *in vivo* lead to propose healthy adult human liver itself as an attractive source of expandable cell populations with stem/progenitor cell properties that could be used for stem cell therapy in human liver diseases.

Bone marrow stem cells

Bone marrow, with its hematopoietic and mesenchymal stem cell compartments, is the largest source for extra-hepatic stem cells. Bone marrow stem cells are able to trans-differentiate into mature hepatocytes both *in vitro* and *in vivo*[23] although the mesenchymal cell type has been shown to be the most potent component regarding hepatic differentiation[24]. Nevertheless, we recently demonstrated that while these cells acquired *in vitro* some phenotypic and functional features of mature hepatocytes, they partially preserved their initial mesenchymal phenotype[25]. In this study, we demonstrated that to obtain an advanced maturation of their hepatocyte-like phenotype *in vivo*, bone marrow mesenchymal stem cells must be *in vitro* conditioned or directly infused into the liver microenvironment[25].

Following peripheral blood stem cells or bone marrow transplantation, up to 7% of hepatocytes are of donor origin, and these cells were shown to persist up to one year after transplantation[26]. However, the data using bone marrow stem cells for liver regeneration are not definitely conclusive regarding the ability to repopulate the liver *in vivo*[27,28] and the involved trans-differentiation mechanisms (fusion or direct differentiation)[24,29]. The study conducted by Rountree et al, demonstrated that mouse bone marrow cells are not involved in hepatocytes, cholangiocytes or oval mouse cells replacement[28] whereas in the rat model, some of hepatic stem cells have been shown to arise from the bone marrow[30].

Although abundant, the documented data are not able to clearly suggest this cell source as useful for liver regeneration and seem to be dependent of the animal model.

Fetal hepatic cells

Fetal hepatocytes represent an alternative source but ethical concerns may prevent the use of this material even for

therapeutic purpose. Nevertheless, fetal hepatocytes show high proliferative capacity, are less immunogenic and more resistant to cryopreservation and ischemic injury, properties that could enhance their engraftment within the recipient liver. These cells can be isolated with a non-perfused collagenase method and are able to expand *in vitro* and to engraft within the recipient liver

Embryonic stem cells

Embryonic stem cells have been reported to differentiate into hepatocyte-like cells *in vitro* and *in vivo*[31]. The recent developments in the field of hepatocyte differentiation may bring additional information regarding the plasticity of these cells. However, before clinical application, additional studies need to explore the tendency of these cells to form teratomas and their ability to induce an immune response.

Other sources

Other progenitor/stem cells are able to differentiate into hepatocyte-like cells *in vitro* and to engraft within the recipient liver such as umbilical cord blood stem cells[32], amniotic epithelial cells[33] and placenta stem cells[34]. Human cord blood cells transplanted into mice were able to trans-differentiate into hepatocytes with presence of human centromeric DNA and albumin synthesis[35]. We recently isolated mesenchymal stem cells from Wharton's jelly and demonstrated their *in vitro* expression of several markers of hepatic lineage including albumin, α-fetoprotein, cytokeratin-19, connexin-32 and dipeptidyl peptidase IV[36]. Such expression is up-regulated after *in vitro* hepatogenic differentiation and correlated to glycogen storage and urea production. Engraftment capacity of these cells in recipient mice livers was demonstrated after 4 weeks post-transplantation using immuno-histochemistry, for the expression of human albumin, α-fetoprotein and fibronectin, while cytokeratin-19 was completely down-regulated.

Peripheral blood monocytes that could be generated for the recipient own blood[37] and adipose tissue[38] are also documented as possible sources of cells able to trans-differentiate into hepatocytes like cells.

Because of their homology to mesenchymal stem cells, we analyzed the ability of human fibroblasts to differentiate into hepatocyte-like cells and to engraft after *in vivo* transplantation. Accordingly, we incubated human skin fibroblasts with a specific differentiation cocktail *in vitro* and demonstrated that these cells presented hepatocyte-like morphology and acquired liver-specific markers on protein and gene expression levels. Furthermore, these fibroblast-derived hepatocyte-like cells displayed the ability to store glycogen and synthesize small amounts of urea. *In vivo*, transplantation of these fibroblasts into the liver of hepatectomized SCID-mice reveals an in situ engraftment within the recipient liver parenchyma and expression of hepatocyte markers such as albumin, α-fetoprotein and cytokeratin 18[39]. In this study, we demonstrate that despite lower liver-specific markers expression, *the in vitro* and *in vivo* differentiation profile of human skin fibroblasts was comparable to that of bone marrow mesenchymal-derived hepatocyte-like cells.

For the all above cited stem/progenitor cell types, engraftment and in situ differentiation were documented after transplantation. This was demonstrated as single cells or foci immuno-stained with human markers within the recipient liver parenchyma. Additional data regarding the *in vitro* and *in vivo* biology of these cells, their long term stability and safety, are mandatory before human therapeutic use.

HEPATOCYTE REJECTION

Limited engraftment and durability of the transplanted hepatocytes remain the major limits of LCT. Indeed, the majority of transplanted hepatocytes (70-80%) are early cleared by phagocytes and macrophages in the hepatic vascular spaces. Preventing this early cell loss requires not only the improvement of the quality of transplanted hepatocytes but also the understanding of the role of hepatocytes and other resident liver cells in the induction of immunologic events. The effect of immunosuppression drugs on hepatocyte viability and metabolic activity needs also to be investigated.

Hepatocytes express cell surface molecules of the major histocompatibility complex I, critical for the generation of cytolytic T lymphocytes as well as co-stimulation molecules involved in T-cell activation[40]. Adhesion molecules which may activate recruitment and migration of leukocytes to the liver are also expressed by mature hepatocytes[41].

As tissue factor dependent activation of coagulation was found to contribute to beta cell loss in islets transplantation, we investigated the potential pro-coagulant activity of hepatocyte preparations. We firstly demonstrated using different complementary techniques that isolated human hepatocyte population expresses tissue factor and induces an *in vitro* dependent pro-coagulant activity[42]. *In vivo*, following human hepatocyte infusions in a Crigler-Najjar patient, we also observed a delayed D-dimers increase[42]. Such phenomenon may impair hepatocyte engraftment and promote rejection which pathways in LCT may differ from those involved in solid organ or islet allografts transplantation[43].

In humans, immunosuppression protocols used for LCT are based, as for orthotopic liver transplantation, on calcineurin inhibitors (tacrolimus) and anti-T cell receptor monoclonal antibodies (anti-IL-2 receptors) with or without steroids. No detrimental effect of these drugs has been observed on isolated hepatocytes' viability and function[44].

Hepatocyte encapsulation is an alternative to protect the cells against immunologic response. Survival of encapsulated cells is prolonged despite the persisting development of immune response due to secreted materials across the physical barrier. The understanding of the specific immunogenicity of isolated hepatocytes may be helpful for the development of future suitable strategies

(generation of specific immunosuppressants or co-transplantation of hepatocytes with other tolerigenic cell populations).

CONCLUSION

Since the first description of hepatocyte transplantation in human 13 years ago, the technique is slowly progressing, hampered by several hurdles such as donor organ shortage and limited durability of engrafted cells. LCT is at least validated as a bridge to transplantation to stabilize the unstable patients while waiting for OLT. Cryopreservation remains widely necessary for scheduled transplantation, but causes significant damage to the cells and may interfere with the clinical success. Great expectations arise from stem cell technology, as a solution to organ shortage, but also because of possible higher engraftment and repopulation capacity. These cells may be considered as alternative to mature hepatocytes, or for co-transplantation with these cells aiming to improve durability. The lower immunogenic profile of mesenchymal cells as well as their immunosuppressive properties are additional criteria allowing their tolerance after transplantation.

REFERENCES

1. Berry MN, Friend DS. High-yield preparation of isolated rat liver parenchymal cells: a biochemical and fine structural study. J Cell Biol 1969;43:506-20.
2. Seglen PO. Preparation of isolated rat liver cells. Methods Cell Biol. 1976;13:29-83.
3. Otte JB, De Ville DG, Alberti D, Balladur P, De Hemptinne B. The concept and technique of the split liver in clinical transplantation. Surgery. 1990;107:605-12.
4. Reding R, De GJ, V, Delbeke I, Sokal E, Jamart J, Janssen M, et al. Pediatric liver transplantation with cadaveric or living related donors: comparative results in 90 elective recipients of primary grafts. J Pediatr. 1999;134:280-6.
5. Fox IJ, Chowdhury JR, Kaufman SS, Goertzen TC, Chowdhury NR, Warkentin PI, et al. Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. N Engl J Med. 1998;338:1422-6.
6. Muraca M, Gerunda G, Neri D, Vilei MT, Granato A, Feltracco P, et al. Hepatocyte transplantation as a treatment for glycogen storage disease type 1a. Lancet. 2002;359:317-8.
7. Sokal EM, Smets F, Bourgois A, Van Maldergem L, Buts JP, Reding R, et al. Hepatocyte transplantation in a 4-year-old girl with peroxisomal biogenesis disease: technique, safety, and metabolic follow-up. Transplantation. 2003;76:735-8.
8. Dhawan A, Mitry RR, Hughes RD, Lehec S, Terry C, Bansal S, et al. Hepatocyte transplantation for inherited factor VII deficiency. Transplantation. 2004;78:1812-4.
9. Stephenne X, Najimi M, Smets F, Reding R, De Ville DG, Sokal EM. Cryopreserved liver cell transplantation controls ornithine transcarbamylase deficient patient while awaiting liver transplantation. Am J Transplant. 2005;5:2058-61.
10. Stephenne X, Najimi M, Sibille C, Nassogne MC, Smets F, Sokal EM. Sustained engraftment and tissue enzyme activity after liver cell transplantation for argininosuccinate lyase deficiency. Gastroenterology. 2006;130:1317-23.
11. Maganto P, Cienfuegos JA, Santamaria L, Eroles G, Andres S, Pardo F, et al. Transplanted frozen-thawed hepatocytes as a therapeutic approach in liver disease. Transplant Proc. 1990;22: 2017-9.
12. Jamal HZ, Weglarz TC, Sandgren EP. Cryopreserved mouse hepatocytes retain regenerative capacity *in vivo*. Gastroenterology. 2000;118:390-4.
13. Yagi T, Hardin JA, Valenzuela YM, Miyoshi H, Gores GJ, Nyberg SL. Caspase inhibition reduces apoptotic death of cryopreserved porcine hepatocytes. Hepatology. 2001;33:1432-40.
14. Stephenne X, Najimi M, Ngoc DK, Smets F, Hue L, Guigas B, et al. Cryopreservation of human hepatocytes alters the mitochondrial respiratory chain complex 1. Cell Transplant. 2007; 16:409-19.
15. Katenz E, Vondran FW, Schwartlander R, Pless G, Gong X, Cheng X, et al. Cryopreservation of primary human hepatocytes: the benefit of trehalose as an additional cryoprotective agent. Liver Transpl. 2007;13:38-45.
16. Kuleshova LL, Wang XW, Wu YN, Zhou Y, Yu H. Vitrification of encapsulated hepatocytes with reduced cooling and warming rates. Cryo Letters. 2004;25:241-54.
17. Theise ND, Saxena R, Portmann BC, Thung SN, Yee H, Chiriboga L, et al. The canals of Hering and hepatic stem cells in humans. Hepatology. 1999;30:1425-33.
18. Fausto N, Campbell JS. The role of hepatocytes and oval cells in liver regeneration and repopulation. Mech Dev. 2003;120: 117-30.
19. Khuu DN, Najimi M, Sokal EM. Epithelial cells with hepatobiliary phenotype: Is it another stem cell candidate for healthy adult human liver? World J Gastroenterol. 2007;13:1554-60.
20. Duret C, Gerbal-Chaloin S, Ramos J, Fabre JM, Jacquet E, Navarro F, et al. Isolation, characterization, and differentiation to hepatocyte-like cells of nonparenchymal epithelial cells from adult human liver. Stem Cells. 2007;25:1779-90.
21. Herrera MB, Bruno S, Buttiglieri S, Tetta C, Gatti S, Deregibus MC, et al. Isolation and characterization of a stem cell population from adult human liver. Stem Cells. 2006;24:2840-50.
22. Najimi M, Khuu DN, Lysy PA, Jazouli N, Abarca J, Sempoux C, et al. Adult-derived human liver mesenchymal-like cells as a potential progenitor reservoir of hepatocytes? Cell Transplant. 2007;16:717-28.
23. Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, et al. Bone marrow as a potential source of hepatic oval cells. Science. 1999;284:1168-70.
24. Sato Y, Araki H, Kato J, Nakamura K, Kawano Y, Kobune M, et al. Human mesenchymal stem cells xenografted directly to rat liver are differentiated into human hepatocytes without fusion. Blood. 2005;106:756-63.
25. Lysy PA, Campard D, Smets F, Malaise J, Mourad M, Najimi M, et al. Persistence of a chimerical phenotype after hepatocyte differentiation of human bone marrow mesenchymal stem cells. Hepatology. 2007;46:1574-85.
26. Korbling M, Katz RL, Khanna A, Ruifrok AC, Rondon G, Albitar M, et al. Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. N Engl J Med. 2002;346:738-46.
27. Vassilopoulos G, Wang PR, Russell DW. Transplanted bone marrow regenerates liver by cell fusion. Nature. 2003;422:901-4.
28. Rountree CB, Wang X, Ge S, Barsky L, Zhu J, Gonzales I, et al. Bone marrow fails to differentiate into liver epithelium during murine development and regeneration. Hepatology. 2007;45:1250-60.
29. Wang X, Willenbring H, Akkari Y, Torimaru Y, Foster M, Al Dhalimy M, et al. Cell fusion is the principal source of bone-marrow-derived hepatocytes. Nature. 2003;422:897-901.
30. Oh SH, Witek RP, Bae SH, Zheng D, Jung Y, Piscaglia AC, et al. Bone marrow-derived hepatic oval cells differentiate into hepatocytes in 2-acetylaminofluorene/partial hepatectomy-induced liver regeneration. Gastroenterology. 2007;132: 1077-87.
31. Heo J, Factor VM, Uren T, Takahama Y, Lee JS, Major M, et al. Hepatic precursors derived from murine embryonic stem cells contribute to regeneration of injured liver. Hepatology. 2006;44:1478-86.
32. Kakinuma S, Tanaka Y, Chinzei R, Watanabe M, Shimizu-Saito K, Hara Y, et al. Human umbilical cord blood as a source of transplantable hepatic progenitor cells. Stem Cells. 2003;21: 217-27.
33. Miki T, Lehmann T, Cai H, Stolz DB, Strom SC. Stem cell characteristics of amniotic epithelial cells. Stem Cells. 2005;23: 1549-59.
34. Chien CC, Yen BL, Lee FK, Lai TH, Chen YC, Chan SH, et al. *In vitro* differentiation of human placenta-derived multipotent cells into hepatocyte-like cells. Stem Cells. 2006;24:1759-68.

35. Ishikawa F, Drake CJ, Yang S, Fleming P, Minamiguchi H, Visconti RP, et al. Transplanted human cord blood cells give rise to hepatocytes in engrafted mice. Ann N Y Acad Sci. 2003; 996:174-85.
36. Campard D, Lysy P, Najimi M, Sokal E. Native Umbilical Cord Matrix Stem Cells express hepatic markers and differentiate into hepatocyte-like cells. Gastroenterology. 2008. In press.
37. Ruhnke M, Ungefroren H, Nussler A, Martin F, Brulport M, Schormann W, et al. Differentiation of *in vitro*-modified human peripheral blood monocytes into hepatocyte-like and pancreatic islet-like cells. Gastroenterology. 2005;128:1774-86.
38. Seo MJ, Suh SY, Bae YC, Jung JS. Differentiation of human adipose stromal cells into hepatic lineage *in vitro* and *in vivo*. Biochem Biophys Res Commun. 2005;328:258-64.
39. Lysy PA, Smets F, Sibille C, Najimi M, Sokal EM. Human skin fibroblasts: from mesodermal to hepatocyte differentiation. Hepatology. 2007;46:1574-85.
40. Chen M, Tabaczewski P, Truscott SM, Van Kaer L, Stroynowski I. Hepatocytes express abundant surface class I MHC and efficiently use transporter associated with antigen processing, tapasin, and low molecular weight polypeptide proteasome subunit components of antigen processing and presentation pathway. J Immunol. 2005;175:1047-55.
41. Morita M, Watanabe Y, Akaike T. Inflammatory cytokines upregulate intercellular adhesion molecule-1 expression on primary cultured mouse hepatocytes and T-lymphocyte adhesion. Hepatology. 1994;19:426-31.
42. Stephenne X, Vosters O, Najimi M, Beuneu C, Ngoc DK, Wijns W, et al. Tissue factor-dependent procoagulant activity of isolated human hepatocytes: Relevance to liver cell transplantation. Liver Transpl. 2007;13:599-606.
43. Lunsford KE, Horne PH, Koester MA, Eiring AM, Walker JP, Dziema HL, et al. Activation and maturation of alloreactive CD4-independent, CD8 cytolytic T cells. Am J Transplant. 2006; 6:2268-81.
44. Serrano T, Mitry RR, Terry C, Lehec SC, Dhawan A, Hughes RD. The effects of immunosuppressive agents on the function of human hepatocytes *in vitro*. Cell Transplant. 2006;15:777-83.