32 Cell Transplantation

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Introduction

For numerous liver diseases of childhood, liver transplantation (LT) is a lifesaving procedure. However, it requires scarce organs, a highly experienced team to manage the surgical procedure, complications, and follow-up and lifelong immunosuppression for the recipient.[1] Living donor organs and split livers gave us the proof of concept that a partial organ is sufficient to restore liver metabolic functions.[2] Liver cell therapy (LCT), where cells rather than organs are transplanted in the patient, was first evaluated in acute liver failure to support liver function while awaiting spontaneous recovery or as a bridge to transplantation. Recently, LCT has been proposed as a treatment in itself to overcome LT barriers with an off the shelf, easily injectable, and reversible procedure. In addition, it has the advantage of not inducing a strong immunogenic response.[3] Initially, LCT was performed for liver-based inborn errors of metabolism (IEM) with hepatocytes isolated from livers not suitable for LT,[4] but stem cells are increasingly of interest for acquired liver diseases.[5]

Hepatocyte Transplantation

Clinical Application

Hepatocyte transplantation (HT) was translated to human medicine in the 1990s to overcome the limitations of LT—lack of donors, intensive surgery, cost, immunosuppression. In 1994, Habibullah et al. reported on intraperitoneal fetal hepatocytes administration in seven patients with acute liver failure; one child was included in the study and survived the acute decompensation.[6] The next year, Grossman et al. published the first intraportal injection of autologous hepatocytes transduced with a low-density lipoprotein (LDL) receptor in five patients (three children) with familial hypercholesterolemia; LDL cholesterol decreased in three of them.[7] The autologous procedure was developed to avoid immunosuppression and the allogenic variable.[8] Finally, in 1997, an intraportal heterologous hepatocyte infusion in a 5-year-old boy diagnosed with ornithine transcarbamylase (OTC) deficiency was reported. His ammonia and glutamine levels returned to normal values at discharge. Unfortunately, the child died 43 days post-HT of liver biopsy complications. Since the first report in 1994, HT has been evaluated in 58 children for indications ranging from biliary atresia[9] by acute liver failure (Table 32.1) to liver-based IEM (Table 32.2), with variable clinical success. At least 52% of them (30/58) received LT after HT. Currently, HT may be considered a bridge to transplantation, especially for patients with liver-based IEM who usually face a long waiting time before LT.[10]

Material Source

The first HT procedure was performed with using fetal hepatocytes, generating some ethical considerations.[6] One team used magnetic activated cell sorting to purify hepatic progenitor cells from fetal hepatocyte based on the CD326 expression.[11]

Today, the main source of cells for HT are the livers unsuitable for LT, such as reduction remnants or unused split livers, damaged livers, or livers from young donors. Steatotic livers are of lesser quality for hepatocytes.[12] Hepatocytes seem to tolerate ischemia well in comparison with cholangiocytes. Yet, their viability has been shown to be inversely

TABLE 32.1 Clinical Hepatocyte Transplantation in Pediatric Acute Liver Failure

Cause	Age	Effect, Outcome	Reference
Drug-induced	16 years 12 years 10 years	Ammonia reduction, death, 2 days post-HT Ammonia reduction, death, 7 days post-HT Ammonia reduction, death, 7 days post-HT	100
	6 months	Ammonia reduction, life support withdrawal and death, 7 days post-HT	31
	13 years	Death, 4 days post-HT	101
	14 years	Ammonia reduction and improved encephalopathy, LT 1 day post-HT	8
Idiopathic	8 years	Intraperitoneal injection of fetal hepatocytes, full recovery	6
	3 years 5 years	Ammonia reduction and improved encephalopathy in both Full recovery and immunosuppression weaned; successful bridge to LT 4 days post-HT	100
	3.5 months	No clear benefit, LT 1 day post-HT	102
Virus-induced	4 years	Ammonia reduction and improved encephalopathy, intracranial hypertension on day 2	8
	3 weeks	Ammonia reduction, death, 11 days post-HT	27

HT, Hepatocyte transplantation; *LT,* liver transplantation.
(From Gramignoli R, et al. Clinical hepatocyte transplantation: practical limits and possible solutions. *Eur Surg Res.* 2015;54(3-4):162-177; Puppi J, Dhawan A. Human hepatocyte transplantation overview. *Methods Mol Biol.* 2009;48:11-16; Hansel MC, et al. The history and use of human hepatocytes for the treatment of liver diseases: the first 100 patients. *Curr Protoc Toxicol.* 2014;62:14.12.1-23; Khan Z, Strom SC. Hepatocyte transplantation in special populations: clinical use in children. *Methods Mol Biol.* 2017;1506:3-16.)

TABLE 32.2 Clinical Hepatocyte Transplantation in Pediatric Liver-Based Inborn Errors of Metabolism

Cause	Age	Effect, Outcome	Reference
Crigler-Najjar syndrome type 1	10 years	50% reduction in bilirubin, reduction in phototherapy, LT 4 years post-HT	4
	8 years	40% reduction in bilirubin, LT 20 months post-HT	106
	9 years	30% reduction in bilirubin, 35% reduction in phototherapy, LT 5 months post-HT	107
	1.5 years 3 years	>50% reduction in bilirubin, reduction in phototherapy, LT 8 months post-HT 30% reduction in bilirubin, LT 18 months post-HT	108
	3.5 years	Lowered serum bilirubin, outcome unknown	13
	8 years	35% reduction in bilirubin, 50% reduction in phototherapy, LT 11 months post-HT	109
	9 years 1 year	20% reduction in bilirubin, LT 6 months post-HT 25% reduction in bilirubin, LT 4 months post-HT	24, 110
	2 years	50% reduction in bilirubin, outcome unknown	11
	11 years	20% reduction in bilirubin, LT waiting list	27
	7 months	50% reduction in bilirubin and in phototherapy, psychomotor improvement, bilirubin stable at 1-year follow-up	25
	13 years 11 years	50% reduction in bilirubin, presence of bile glucuronides in bile, LT 19 months post-HT 50% reduction in bilirubin, presence of bile glucuronides in bile, LT 31 months post-HT	36

TABLE 32.2 Clinical Hepatocyte Transplantation in Pediatric Liver-Based Inborn Errors of Metabolism—cont'd

Cause	Age	Effect, Outcome	Reference
Alpha-1 antitrypsin deficiency	18 weeks	LT 2 days post-HT, cirrhosis on explant	101
Familial hypercholesterolemia	12 years 7 years 11 years	Ex vivo gene therapy with autologous cells. No benefit; 6% reduction in total cholesterol and LDL cholesterol 19% reduction in total cholesterol and LDL cholesterol	7
	12 years	13% reduction in total cholesterol and LDL cholesterol	111
Factor VII deficiency	3 months 35 months	70% reduction in rFVII requirement, LT 7 months post-HT 70% reduction in rFVII requirement, LT 8 months post-HT	112
	4 months	Reduction in rFVII requirement, outcome unknown	13
Progressive familial intrahepatic cholestasis type 2	32 months 16 months	No benefit (cirrhosis established):, LT 5 months post-HT No benefit (cirrhosis established), LT 14 months post-HT	108
Phenylketonuria	6 years	Reduction in phenylalanine levels and improved dietary tolerance up to 3 months post-HT (cells from "domino" GSD1b liver), PAH activity on liver biopsy at 11 months post-HT	15
Tyrosinemia type 1	59 days	Improved coagulopathy and bilirubin, LT 45 days post-HT (cirrhosis on explant)	25
Glycogen storage disease type 1a	6 years	Reduction in hypoglycemic episodes and cholesterol and triglycerides levels, no hypoglycemic admission at 1-year follow-up	25
Glycogen storage disease type 1b	18 years	Improved blood glucose, decreased epistaxis, normal G6Pase activity on liver biopsy at 8 months post-HT	113
Mild Zellweger spectrum disorder	4 years	40% reduction in pipecolic acid for 18 months, decreased cholestasis and abnormal bile acid, psychomotor improvement, outcome unknown	35
Primary hyperoxaluria type 1	15 months	Reduction un plasma oxalate, liver-kidney transplant 13 months post-HT	30
Urea cycle defects			
Ornithine transcarbamylase deficiency	5 years	Ammonia reduction and protein tolerance, death by sepsis 43 days post-HT	26
	5 years	Ammonia reduction, normal glutamine, death 45 days post-HT	28
	10 hours	Ammonia reduction and protein tolerance, LT 6 months post-HT	114
	1 day	Ammonia reduction, increased urea, protein tolerance, auxiliary partial LT 7 months post-HT and neurologically normal	14
	14 months	Ammonia reduction, increased urea, psychomotor improvement, LT 6 months post-HT	10, 115
	1 day	Ammonia reduction, increased urea, protein tolerance, auxiliary partial LT 7 months post-HT	116
	6 hours 9 days	Ammonia reduction, increased urea, normal urine orotic acid, death 4 months post-HT Ammonia reduction, protein tolerance, normal urine orotic acid, LT waitlist 6 months post-HT	117
	12 years	Ammonia reduction, increased urea, normal glutamine, septic death 30 days post-HT	25
	11 days	Ammonia reduction, neurologically normal 3 months post-HT	118
	7 months	No effect, LT 4 months post-HT	33
Argininosuccinate lyase deficiency	3.5 years	Ammonia reduction, psychomotor improvement, LT 18 months post-HT	29, 106

Continued

TABLE 32.2 Clinical Hepatocyte Transplantation in Pediatric Liver-Based Inborn Errors of Metabolism—cont'd

Cause	Age	Effect, Outcome	Reference
Carbamoyl phosphate synthase I deficiency	2.5 months	Ammonia reduction and increased urea, LT 15 months post-HT	27, 117
	4 months	No effect, LT 3.5 months post-HT	33
Citrullinemia	25 months	Ammonia reduced and increased urea, outcome unknown	(Lee et al., unpublished)
	3 years	Ammonia reduction, increased urea, protein tolerance, outcome unknown	117

G6Pase, Glucose-6-phosphatase; *GSD1b,* glycogen storage disease type 1b; *HT,* hepatocyte transplantation; *LDL,* low-density lipoprotein; *LT,* liver transplantation; *rFVII,* recombinant factor VII; *PAH,* phenylalanine hydroxylase.

(From Gramignoli R, et al. Clinical hepatocyte transplantation: practical limits and possible solutions. *Eur Surg Res.* 2015;54(3-4):162-177; Pareja E, Gomez-Lechon ML, Tolosa L. Alternative cell sources to adult hepatocytes for hepatic cell therapy. *Methods Mol Biol.* 2017;1506:17-42; Puppi J, Dhawan A. Human hepatocyte transplantation overview. *Methods Mol Biol.* 2009;48:11-16; Hansel MC, et al. The history and use of human hepatocytes for the treatment of liver diseases: the first 100 patients. *Curr Protoc Toxicol.* 2014;62:14.12.1-23; Khan Z, Strom SC. Hepatocyte transplantation in special populations: clinical use in children. *Methods Mol Biol.* 2017;1506:3-16.)

correlated to ischemia time.[13] Hepatocytes can also be isolated from segment IV, with or without caudate lobe during a split-liver procedure[14] or from non-heart-beating donors.[13] As for domino liver transplantation, hepatocytes collected from an explanted liver affected by a specific IEM are suitable for HT in patients with another IEM.[15,16] A 6-year-old child with tetrahydrobiopterin unresponsive phenylketonuria received hepatocytes isolated from the native liver of a patient transplanted for glycogen storage disease type 1b. Phenylalanine levels returned to normal, and their half-life decreased significantly after the procedure. To date, no difference in clinical outcome has been reported based on the hepatocyte origin.

Hepatocyte Isolation

A two-step collagenase digestion procedure was developed by Seglen to obtain hepatocytes from rat liver.[17] The procedure was adapted to the human liver by Strom et al. and since has remained the standard protocol to isolate human hepatocytes.[18] Briefly, the liver is perfused with a buffered solution containing a calcium-chelating agent to loosen the desmosomal junctions. Then, collagenase is infused into the liver via the cannulated hepatic veins.[19] After isolation, hepatocytes are usually cryopreserved to be available off the shelf. Unfortunately, this affects their viability after thawing.[20] The whole hepatocyte isolation process has to be done under a laminar flow in a clean room, with regular bacterial and fungus checking. Moreover, because HT falls under advanced therapy medicinal product laws, the US Food and Drug Administration and European Medicines Agency require compliance with the guidelines for Good Manufacturing Practice.[12]

Quality Control

Hepatocytes must be tested for bacterial contamination and *Mycoplasma*. A Trypan blue exclusion test is usually performed to quantify cell viability before and after infusion and after thawing in case of cryopreservation[21] Viability in excess of 60% is required to use for cells for clinical application. To evaluate cell engraftment potential, hepatocytes are plated on collagen-coated dishes for 24 hours, and then adherent cells are counted and the ratio to seeded cells number calculated. Recently, an assay comprised of 11 end points was developed to quantify the metabolic capacity of isolated hepatocytes, but its clinical relevance has yet to be demonstrated.[22]

Dose and Route of Administration

A 70-kg adult liver is estimated to contain 2.8×10^{11} hepatocytes, or 4×10^{9} cells/kg of body weight.[23] HT aims to replace 2% to 5% of the patient's liver mass to restore substantial metabolic liver function, which is equivalent to 8 to 20×10^{7} cells/kg. This number of cells is usually administered in multiple infusions to avoid the risk of portal thrombosis, which may be linked to the procoagulant activity of hepatocytes.[24] In patients with liver-based IEM, HT can be repeated in case of loss of effect over time.[25]

The first and only report of HT delivered through intraperitoneal infusion was for acute liver failure, because this route allows severely ill patients to benefit from HT.[6] Intrasplenic arterial infusions have also been evaluated in patients with altered liver architecture and coagulation problems.[26] Intraportal infusions are the most common way used to deliver cells to the recipient. During the infusion, the portal pressure and vital signs must be monitored regularly.[27] Indium-111-labeled hepatocytes were intraportally infused in a 5-year-old child with OTC deficiency; it was shown the cells are preferentially retained in the recipient liver.[28]

Engraftment Evaluation and Enhancement

Hepatocyte engraftment quantification has been performed on liver biopsies with the limitations of sampling, meaning that the quantification is based on a small fraction of the whole organ, limiting background extrapolation[29] and, more

accurately, on explanted livers from patients undergoing LT after HT.[30] In donor-recipient sex mismatch, engraftment can be extrapolated from sex-determining region Y gene quantification by quantitative polymerase chain reaction.[31,32] However, this technique is limited by the background signal coming from apoptotic cells and cell debris. The enzyme activity quantification on a liver sample is clinically more relevant marker of engraftment in liver-based IEM.[4] In these diseases, the effect of HT is assessed by observing the decrease of an accumulating compound (e.g., ammonia in urea cycle disorders, UCDs)[26] or the appearance of a downstream product (e.g., urea in UCD[29] or conjugated bilirubin in Crigler-Najjar syndrome type 1 [CN1][4]).

Partial hepatectomy (PH) is regularly performed for living donor liver harvesting or liver tumor resection. This induces a strong stimulus for hepatocyte replication. PH has been used in HT to stimulate proliferation of infused hepatocytes given that, in the absence of a selective advantage, donor and recipient cells proliferate at the same rate.[7] For example, preoperative portal vein occlusion (PVO) is a common surgical procedure to induce hepatic regeneration before PH for tumor resection.[12] To our knowledge, this technique has never been applied in HT. Taken together, PH and PVO could make HT a more complex procedure, and the risk-benefit ratio would need to be carefully evaluated.

Irradiation of the native liver has been developed to induce a strong mitogenic signal in the liver parenchyma with a proliferative advantage of the (nonirradiated) infused hepatocytes without the potential risks of PH. Recently, Soltys et al. reported HT in two pediatric patients (aged 4 and 7 months, respectively) with UCD preconditioned with radiation therapy to the right lobe of the liver (5 and 7.5 Gy, respectively).[33] HT had no effect on metabolic control, and both patients underwent LT a few months after HT. The explanted livers were not screened for infused cells and showed no sign of radiation-induced damage. Yet, liver irradiation is not without side effects; it was shown in six adult patients treated with high-dose radiation (12-54 Gy) for biliopancreatic carcinoma, to activate stellate cells with the risk of liver fibrosis in the long term,.[34]

Immunosuppression

Immunosuppression regimens following HT have been largely inspired by LT protocols, including induction based on basiliximab at days 0 and 4 and maintenance therapy with tacrolimus to keep the serum levels at 6 to 8 ng/mL.[35] Some have added, as induction regimen, intravenous methylprednisolone followed by prednisolone daily tapered over the first 6 months post-HT.[36] Recently, a patient received antilymphocyte globulin in attempt to control. To target rejection of the infused hepatocytes and functional loss.[33] Avoid rejection of the infused hepatocytes, Grossman et al. used autologous transduced hepatocytes to treat patients with familial hypercholesterolemia, but without much clinical success.[7]

How to Overcome Hepatocyte Transplantation Barriers

Since the first report of HT nearly 25 years ago, much effort has been focused on how to translate HT into a validated clinical option. Hepatocytes are fragile cells with low proliferative capacities *in vitro*, and their engraftment is low, even with intense preconditioning methods, such as radiation therapy associated with strong immunosuppression (antilymphocyte antibody). The field of liver cell therapy is now looking for a new type of cell that can overcome the disadvantages of a fully differentiated primary cell. Mesenchymal stromal cells (MSCs) or, as recently renamed, *medicinal signaling cells,* seem to hold promise for clinical application in a near future.[37]

Stem and Progenitor Cell-Based Cell Transplantation

Stem and Progenitor Cells and Their Regenerative Potential

Stem cells are defined as unspecialized cells demonstrating self-renewal capacity, a high proliferative potential, and the ability to differentiate into multiple specialized cell types. This proliferative potential is conserved during *in vitro* cultures and, along with their fairly robust resistance to cryopreservation, offers a major advantage over hepatocytes (Table 32.3).

Embryonic stem cells (ESCs) are derived from *in vitro* culture of the inner cell mass of the developing embryo and, as such are capable of forming cells from all three germ cell layers of the body (endoderm, mesoderm, and ectoderm) both *in vitro* and *in vivo*.[38–40] In particular, ESCs are capable of differentiating into hepatocyte-like cells *in vitro* and *in vivo*, and their potential therapeutic effect in the context of liver disease has been demonstrated in animal models.[41–43] In addition, these cells have tremendous regenerative potential.[38] Therefore, they could be of interest in the treatment of metabolic diseases and acquired liver diseases.

However, their use is still limited and controversial because of ethical issues and the associated risk of tumor development.[38,44] Only a few clinical trials have been initiated so far, testing the use of ESC derivatives, all outside the field of liver therapy.[45]

MSCs are somatic cells first described in the mouse and guinea pig by Friedenstein et al. as bone marrow–derived fibroblastic cells.[46] They received their official name from Caplan in 1991, who reported the possibility of isolating, culturing, and differentiating them into bone and cartilage *in vitro*.[47] Their human counterpart was later identified by Pittenger et al. as a cell type capable of differentiating along the osteogenic, chondrogenic, and adipogenic lineages.[48] MSCs have since been reported in other tissues, such as adipose tissue, heart, cartilage, umbilical cord, Wharton jelly, and liver.[49–51] The variety of sources, along with the multiplicity of isolation methods, has led to some questions about the similarities among the different types of cells, their

TABLE 32.3 Comparison of Different Cell Types Suitable for Liver Cell Therapy

Cell type	Origin	Advantages	Disadvantages
Hepatocytes	Livers unsuitable for liver transplantation (LT)	Great metabolic capacity	Poor cryopreservation resistance; no *in vitro* expansion capacity; limited donor availability
Embryonic stem cells (ESCs)	Developing embryos	Great plasticity; capacity to differentiate into hepatocyte-like cells and MSCs; great regenerative potential	Ethical issues; incomplete differentiation into hepatocyte-like cells; oncogenic risk
Mesenchymal stromal, signaling cells (MSCs)	Varied sources	Capacity to differentiate into hepatocyte-like cells; no oncogenic risk; low immunogenicity; immunomodulatory, antifibrotic and proregenerative properties; potent secretome	Incomplete differentiation into hepatocyte-like cells
Induced pluripotent stem cells (iPSs)	Adult cells reprogrammed into an embryonic state	Same plasticity as ESCs; no immunogenicity when autologous	Ethical issues; oncogenic risk, albeit lower than for ESCs; cumbersome manufacturing in case of autologous cells

phenotypes, and their functional characteristics. In an effort to clarify the subject, in 2006, the International Society for Cellular Therapy published recommendations on the minimal criteria necessary to define MSCs. These were summarized into three main points—plastic adherence, phenotypic characterization, and differentiation potential *in vitro*.[52] In addition to adhering to plastic, a characteristic often used to isolate the cells (so-called *plate and wait* method), cells have to express CD105, CD73, and CD90 (at least 95% of the population) but not express CD45, CD34, CD14, CD11c, CD79α, and HLA-DR (≤2% of the population) to be considered as MSCs. These phenotypic criteria aim to confirm mesenchymal characteristics while excluding other cell types that could be contaminating the culture.

Finally, cells have to be able to differentiate into osteoblasts, adipocytes, and chondroblasts when subjected to standard *in vitro* differentiation protocols.[52] In addition to these minimal criteria, several additional markers have been tested to further characterize these cells. Unfortunately, to this day, no unique marker has been identified for MSCs.[51] However, MSCs appear to lack most costimulatory molecules and therefore should have a low immunogenicity.[49,53] Finally, despite these common characteristics, MSCs vary in their degree of differentiation and therapeutic potential, depending on their tissue of origin.[49]

In the liver itself, several stem or progenitor cell types have been described, each with different characteristics.[5,54] Our laboratory has isolated and characterized a cell population derived by *in vitro* culture of the parenchymal fraction obtained after collagenase digestion of the human liver.[55] These cells, called *adult-derived human liver stem/progenitor cells* (ADHLSCs), display the phenotypic characteristics of MSCs but do not differentiate into osteocytes or adipocytes and only differentiate into hepatocyte-like cells. As such, they should probably be considered as progenitors rather than true stem cells.[55–57] Their hepatic predisposition can be seen as an advantage, because there is no risk of the cells differentiating into some unwanted cell type. In addition, they preferentially home to the liver, as demonstrated by the infusion of indium-labeled cells in a hemophilia patient; this is of particular importance following peripheral vein infusion, where most other MSCs tend to get trapped in the lungs.[58] Here, we have shown that although the cells were detected in the lungs soon after injection, they quickly exited the lungs to reach the liver and the ankle, showing hemarthrosis, suggesting that the cells can also home to sites of inflammation.[58]

Despite their differences, MSCs, including bone marrow, adipose tissue, liver, and umbilical cord-derived MSCs, are capable of differentiating into hepatocyte-like cells, displaying functional characteristics of hepatocytes, such as CYP3A4 activity, glycogen storage, and urea synthesis, and have, therefore, been proposed for the LCT treatment of hepatic IEM.[49,59–61] ADHLSCs have already been used clinically in the context of the first human trials under hospital exemption for the treatment of UCD, CN1, and glycogen storage disease.[62–64] In addition, they are currently in pharmaceutical clinical development. The results of a phase I prospective, open-label, multicenter, partially randomized safety study of one administration of HepaStem in children up to 17 years of age suffering from CN and UCD using three cells doses (ClinicalTrials.gov, NCT01765283) were presented,[65] and a phase II efficacy trial is ongoing in children suffering from UCD (ClinicalTrials.gov, NCT02489292).

Compared with ESCs, MSCs offer the advantage of having no oncogenic risk.[66–68] Actually, no adverse event involving tumor development linked to the use of MSCs has been reported to date (the two cases previously reported were

shown to be attributed to culture contamination with a cancer cell line), despite the large number of patients infused in many clinical trials.[51,68] However, they have the disadvantage of not fully differentiating into hepatocytes *in vitro*, probably because of suboptimal differentiation protocols and an unsuitable environment, and it is currently a matter of debate whether the true role of these cells *in vivo* is to regenerate the hepatocyte pool. In addition, it remains very challenging to detect donor MSCs *in vivo* following transplantation, despite the use of cutting edge technology, and it is therefore difficult to evaluate how well they engraft after cell transplantation.

Yamanaka et al. demonstrated the possibility of reprogramming adult cells into an embryonic state to allow them to reacquire the ability to differentiate into the three germ layers.[69–71] These cells, called *induced pluripotent stem cells* (iPSCs), harbor the plasticity of ESCs while presenting a lower, albeit still existing, oncogenic risk.[38] In addition, they can be generated from cells harvested from the patients, thereby avoiding the risk of immune rejection.[45] Like ESCs, they can differentiate into hepatocyte-like cells *in vitro*, and these iPSC-derived hepatocytes can be used for the treatment of liver disease.[72,73] However, like ESCs, the clinical development of these cells may be hindered by ethical and safety issues.[38,44]

Stem Cell-Based Therapy for Acquired Liver Diseases—Mesenchymal Stromal Cells as Medicinal Signaling Cells

Although stem cells were initially investigated in the context of liver disease for their ability to differentiate into hepatocytes *in vivo* and provide metabolic and regenerative support, it is becoming evident that they can be of great use in the treatment of acquired liver diseases. Indeed, MSCs have been shown to display tremendous immunomodulatory, antifibrotic, and proregenerative properties, influencing a wide array of cell types.[49,68,74] MSCs can stimulate the proliferation of other stem cells. In addition, they have been shown to inhibit T-cell activation and proliferation, natural killer cell cytolytic activity, and monocyte maturation into dendritic cells.[49,51,53] They can also favor the differentiation of T cells into regulatory T cells (Tregs) and reverse the phenotype of macrophages from proinflammatory M1 macrophages to antiinflammatory and proregenerative M2 macrophages.[49,75–78] Although some of these effects are believed to be mediated through cell-to-cell contact, there is accumulating evidence to support a central role for paracrine signaling. The secretome of MSCs contains a plethora of cytokines with often ambivalent potential effects, including interleukin (IL)-10, IL-1 receptor antagonists (IL-1Ras, known for their antiinflammatory properties). IL-6, despite being described as a proinflammatory cytokine, can be of use in acquired liver disease through its effect on neutrophils, nitric oxide, indoleamine 2,3-dioxygenase, and prostaglandin E2 involved in T-cell inhibition, transforming growth factor beta, which induces Treg differentiation and inhibits hepatic stellate cells (HSCs), responsible for fibrosis, and hepatocyte growth factor, which displays proregenerative and antifibrotic properties through the inhibition of HSCs.[49,75,78–80] Furthermore, MSCs are known to produce matrix metalloproteases, which help to degrade the extracellular matrix deposited by HSC during the fibrogenic process.[79] In addition, it is believed that MSCs can adapt their secretome to their environment, producing more proinflammatory cytokines during infection, and anti-inflammatory and proregenerative cytokines during injury, thereby maintaining tissue health.[51,81,82] Actually, the immunosuppressive capacity of MSCs appears to be enhanced when the cells are treated with a proinflammatory cocktail.[83] Consequently, Caplan recently suggested that the name he initially gave them be changed to *medicinal signaling cells*.[37]

The first application resulting from this potent modulatory potential has been the use of MSCs to induce tolerance during liver transplantation.[84,85] Several clinical trials are currently underway in adults, but only one trial in children from 8 weeks to 18 years of age.[38,84] The most important application, however, is the use of MSCs in immune and inflammatory disorders in various tissues, including the liver. In preclinical models, the MSCs' therapeutic potential was demonstrated in various models of acute and chronic liver diseases.[86–89] In particular, MSCs have been suggested for the treatment of liver fibrosis and cirrhosis in the context of various disorders, including nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, and primary biliary cholangitis.[90–92] There is no comprehensive study available to compare the efficiency of the various types of MSCs in acute and chronic liver diseases. However, studies comparing MSCs from two or more sources suggest a difference in potency based on the cell source and disease treated, suggesting that some MSCs may be better able to treat specific disorders.[49,93–95]

Numerous clinical trials involving MSCs in the treatment of fibroinflammatory and immune pathologies are currently underway in a wide array of organs. In the liver, most clinical trials are phase I or II trials performed in adults or children older than 15 or 16 years and targeting acute-on chronic liver failure (AoCLF), fibrosis, and cirrhosis (reviewed in Iansante et al.[38] and Alfaifi et al.[49]). Therefore, clinical trials in pediatric subjects are still lacking. However, the results from these trials may help us better understand the mechanism of actions of MSCs in liver diseases and allow us to extrapolate their safety and efficacy for use in children.

As for metabolic disorders, ESCS and iPSCs have also been suggested for the treatment of acquired liver disease but, instead of using them as hepatocyte-like cells, they are differentiated into MSCs. Once again, the main obstacles here are linked to the ethics and risk of tumor development.

Remaining Challenges and Conclusion

For the treatment of liver-based IEM, HT may be a viable alternative to LT to LT it is not available, because hepatocytes display mature functions. However, the paucity of

donors remains a problem. Hepatocytes respond poorly to cryopreservation, and transplantation of fresh hepatocytes is logistically difficult to schedule. Because of their major proliferative capacity and resistance to cryopreservation, stem cells appear to be a good alternative to HT. However, MSCs seem to be limited in their capacity to differentiate into fully matured hepatocytes *in vitro*; it is difficult to assess the level of differentiation achieved *in vivo*, and ESCs and iPSCs are plagued with ethical and safety issues. Current studies aim to optimize the differentiation of MSCs into hepatocytes and to improve the safety of ESCs and iPSCs, mostly to reduce their oncogenic risk.

Stem cells may be of better use for acquired liver diseases through their paracrine-effect on inflammation, fibrosis, and regeneration. However, even if engraftment and differentiation are not mandatory in this context, the cells have to survive long enough to exert their effect, and cell retention could therefore still be a limiting factor.

Tissue engineering strategies are currently under development to improve cell retention and potency. These seem to increase when cells are grown in three-dimensional structures. Therefore researchers are evaluating the therapeutic potential of organoids made of one or more cell types such as hepatocytes, MSCs, and endothelial cells. In addition, scaffolds can be used to seed the cells, such as decellularized bone or liver scaffolds, where the remaining extracellular matrix offers support for the cells to grow.[96] Alternatively, cells can be suspended in hydrogels and "printed" into a tissue-like structure using a bioprinter.[97] Although these techniques have shown some potential, they are still far from being able to reconstitute a whole organ, and more research remains to be performed before they can be of use in the clinic. Finally, cell retention can be improved by growing cells as sheets using a thermosensitive polymer.[98] This technique has already been used clinically in a wide range of applications in organs other than the liver.[99]

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