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Review

Human Liver Progenitor Cells for Liver Repair

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Because of their high proliferative capacity, resistance to cryopreservation, and ability to differentiate into hepatocyte-like cells, stem and progenitor cells have recently emerged as attractive cell sources for liver cell therapy, a technique used as an alternative to orthotopic liver transplantation in the treatment of various hepatic ailments ranging from metabolic disorders to end-stage liver disease. Although stem and progenitor cells have been isolated from various tissues, obtaining them from the liver could be an advantage for the treatment of hepatic disorders. However, the techniques available to isolate these stem/progenitor cells are numerous and give rise to cell populations with different morphological and functional characteristics. In addition, there is currently no established consensus on the tests that need to be performed to ensure the quality and safety of these cells when used clinically. The purpose of this review is to describe the different types of liver stem/progenitor cells currently reported in the literature, discuss their suitability and limitations in terms of clinical applications, and examine how the culture and transplantation techniques can potentially be improved to achieve a better clinical outcome.

Key words: Stem/progenitor cells; Cell therapy; Metabolic disorders; Liver; Regenerative medicine

INTRODUCTION

Orthotopic liver transplantation (OLT) remains, to this day, the only definite treatment for acute liver failure and chronic liver diseases. It is also the treatment of choice for inborn error of metabolism disorders in which one liver enzyme is missing or defective, resulting in a loss of function. However, organ shortage has led scientists to explore the possibility of using liver cell therapy (LCT) as a bridge to OLT for patients suffering from liver failure or even as an alternative to OLT for patients with metabolic disorders looking for a less invasive, less risky, and less expensive option (78). LCT was first performed using hepatocytes and showed positive short-term results, making the procedure look very promising (13). Indeed, hepatocyte-based LCT led to clinical improvement shortly after cell transplantation in patients suffering from Crigler Najjar syndrome, factor VII deficiency, urea cycle disorders, Refsum disease, and fulminant hepatic failure (81,86,87). However, the procedure revealed important limitations. First, the efficacy of the treatment proved to have a limited durability, as the effects of the transplantation progressively decreased to disappear after 18–26 months (78). In addition, because of the practical difficulty in getting patients ready when fresh hepatocytes are available, most investigators had to rely on cryopreservation, a procedure hepatocytes are highly sensitive to (85). Finally, because hepatocytes lack the ability to proliferate, a fairly large number of cells needed to be transplanted to obtain a net clinical benefit, which was difficult to obtain due to organ shortage. Stem/progenitor cells have, therefore, emerged as an attractive alternative to hepatocytes in LCT, with a high proliferative capacity, a higher resistance to cryopreservation, and a capacity to differentiate into hepatocyte-like cells. Although stem/progenitor cells from various tissues such as bone marrow, Wharton's jelly, adipose tissue, and cord blood have been proposed, liver-derived stem/progenitor cells seem to be obvious candidates, as they emerge directly from the organ that needs to be repaired (12,80). In this article, we will try to review the different types of liver stem/progenitor cells, their sources, methods of procurement, and characteristics. We will then explore their suitability for clinical use in terms of their ability to differentiate into hepatocyte-like

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cells and repopulate the liver, as well as their safety. Then, we will describe the clinical applications potentially targeted by stem/progenitor cell-based LCT, those already under investigation, their results and limitations, to finally conclude with the possible steps to be taken to improve liver stem/progenitor cell-based cell therapy.

WHAT IS A LIVER STEM/PROGENITOR CELL?

As a general rule, a cell is considered a stem cell if it has the ability to self-renew, a high proliferative potential, and the capacity to differentiate into various specialized cell types. Although the terms "stem" and "progenitor" cells are often used interchangeably, "progenitor" cells usually designate descendants of stem cells lacking self-renewal capacity and giving rise to a much more restricted spectrum of differentiated cell types than stem cells. The terminology in terms of liver stem/progenitor cells is quite confusing, as different researchers tend to use different or overlapping labels, and it somewhat remains a matter of debate, particularly when it comes to determining if hepatoblasts are the progenitors of hepatic stem cells or their descendants. However, the work of Reid et al. favors a model that seems to be accepted by most, wherein three main types of stem/progenitor cells can be distinguished based on the different stages of liver development [for a detailed review, see the article by Turner et al. (93)]. Of these, hepatic stem cells are the most primitive. These small (about 8 μm) multipotent cells are believed to represent about 1% of the liver parenchyma regardless of the donor's age. They are characterized by the expression of epithelial and neural cell adhesion molecules [EpCAM, also known as cluster of differentiation 326 (CD326) and NCAM, also known as CD56], CD133, cytokeratin (CK) 8, CK18, and CK19 but lack intercellular adhesion molecule 1 (ICAM-1, also known as CD54), α-fetoprotein (AFP), and hematopoietic, endothelial, and mesenchymal markers. In addition, they express no or low levels of albumin (73,74). These cells require feeder cells or a matrix such as collagen type 3 or hyaluronan to be able to properly adhere to the culture vessel and proliferate ex vivo, which could make them more difficult to produce on a large scale under good manufacturing practice (GMP) conditions (50). Their ability to self-renew means that they can be kept in culture for many passages, which could allow for the production of a high number of cells. However, this could also lead to a higher risk of tumor development in vivo. Hepatoblasts are larger (about 11 μm) bipotent cells arising from hepatic stem cells, which possess the ability to differentiate along hepatocytic and cholangiocytic lineages. Phenotypically, they share some characteristics with hepatic stem cells, such as the expression of EpCAM (though at lower levels), CD133, and CK8, CK18, and CK19 and the absence of hematopoietic, endothelial, and mesenchymal markers (29,73,74,105). They do, however, express ICAM-1, as well as high levels of albumin and AFP (44,74). Although these cells are present throughout developmental stages, their number decreases significantly over time to reach less than 0.01% of the adult liver parenchyma, which would limit their availability. However, they have a high proliferative capacity when seeded in hyaluronan hydrogels with Kubota's medium, which helps maintain their early hepatoblast phenotype (93). Finally, committed progenitor cells are the largest in size (about 13 μm) and the least plastic, as they can only differentiate into either hepatocytes or biliary cells. They, therefore, typically harbor a phenotype characteristic of the lineage they belong to (such as albumin expression and lack of CK19 in the case of the hepatocytic lineage). However, some scientists have used the term hepatic progenitor cell to designate the human equivalent of the oval cell found in rodents: a bipotent cell with the ability to differentiate along both the hepatocytic and the cholangiocytic lineage, thus resembling the description of the hepatoblast, but which specifically arises following liver injury. In recent years, numerous laboratories have reported the isolation of hepatic progenitor cells, each with different characteristics (see Table 1 for a review). These variations could be explained by several points: (i) the different populations obtained probably represent various degrees of liver maturation and/or a difference in the origin of the cells [e.g., the subpopulation of progenitor cells expressing hematopoietic markers (c-kit, CD34) is believed to have derived from the bone marrow], (ii) there are tremendous differences in fluorescence-activated cell sorting (FACS) data analyses between laboratories when it comes to determining what percentage of expression truly constitutes positivity, and (iii) FACS analyses of progenitor cells are not always performed at the same timepoint during culture and the expression of specific markers could differ depending on the culture conditions and the cell passage (99). Committed progenitor cells still have a good proliferative capacity in vitro, but they cannot self-renew and eventually reach senescence. This limits the timeframe during which they can be used clinically but probably also reduces the associated risk of tumor induction.

STEM/PROGENITOR CELL PROCUREMENT

Cell Isolation Methods

The methods of stem/progenitor cells isolation vary greatly from investigator to investigator (Table 1). Indeed, some laboratories see the "emergence" of progenitor cells following in vitro culture onto collagen-coated flasks of the parenchymal (or, less often, the nonparenchymal) liver fraction collected following collagenase digestion of the liver, a technique commonly termed "plate and wait." Hepatic stem cells have been shown to remain viable for 7 days under ischemic conditions and will most likely be the predominant population isolated under prolonged ischemic conditions (83). In addition, the plate and wait method

Table 1. Source and Properties of Human Liver Stem/Progenitor Cells

Origin	Source	Cell Procurement Method	Markers	Comments	References
Parenchymal fraction	Human fetal liver (15–25 weeks)	Isolation of $EpCAM^+$ cells	$EpCAM^+$, $CD29^+$, $CD49f^+$, $CD90^+$, $CD34^+$, HLA class I^+, $CD45^-$, $CK18^+$, $CK19^+$, Alb^+, and $AFP^{+/-}$	Successful transplantation in patient with Crigler Najar Syndrome type 1	(39,66)
	Human adult liver	Stringent cell culture condition: "plate and wait"	$CD29^+$, $CD73^+$, $CD44^+$, $CD90^+$, AFP^+, ALB^+, CK8/18$^+$, $CD34^-$, $CD45^-$, $CD117^-$, $CD133^-$, $CK19^-$, $vimentin^+$, $nestin^+$	Pluripotent cells, hepatocyte differentiation in SCID mice	(30)
	Human fetal (18–22 weeks) and postnatal liver	Isolation of $EpCAM^+$ cells	CK8/18$^+$, $CD29^+$, $CK19^+$, $CD133^+$, $CD44H^+$, $Alb^{+/-}$, $NCAM^+$, $claudin3^+$, AFP^-, $CD117^-$, ICAM-1^-, $CD45^-$, $CD90^-$, $CD34^-$, $ASMA^-$, $desmin^-$	150 doubling in vitro, mature hepatocyte after transplantation in NOD/SCID	(74)
	Human fetal liver (6–10 weeks)	Isolation of $CD117^+$/$CD34^+$	$CD45^-$, $CD90^-$, Alb^-, $CK19^-$, $CD117^+$, $CD34^+$, Lin^-	5% implantation in nude mice with injury, hepatocyte differentiation in vivo	(55)
	Human adult liver	Stringent cell culture condition: "plate and wait"	$CD90^+$, $CD73^+$, $CD29^+$, $CD44^+$, $CD13^+$, $CD105^-$, $CD45^-$, $CD34^-$, $CD133^-$, $CD117^-$, $CK8^-$, $CK19^-$, Alb^+, AFP^-, Oct-4^-, $HNF4^-$	Hepatocyte differentiation in vitro and implantation in uPA^+/SCID mice with PH	(41,52)
	Human fetal liver (17–24 weeks)	Stringent cell culture condition: "plate and wait"	Glycogen, $G6P^+$, Alb^+, AFP^+, $CK8^+$, $DPPIV^+$, $CK19^+$	Doubling time 59–70 h, 1.5% of liver repopulation in mice	(48)
	Human fetal liver (10–15 weeks)	Stringent cell culture condition: "plate and wait"	$EpCAM^+$, $CK18^+$, $CK19^+$, $CD34^+$, $CD90^+$, c-kit^+, c-met^+, Alb^-, $vimentin^+$, AFP^-, $CD44h^+$	>100 doubling in vitro, pluripotent cells, 2% liver repopulation in vivo	(11)
	Human adult liver	Isolation of Thy-1^+ cells	Thy-1^+	Properties comparisons based on the age of the donors	(58)
Nonparenchymal fraction	Human adult liver injury	Isolation of Thy-1^+ cells	$CD34^+$ (24%), CD117 (8%), CD90 (9%), CD45 (35%), CD14 (20%), CD3 (296%)	Hepatocytes differentiation in vivo	(99)
	Human adult liver	Isolation of $CD45^+$ cells	Alb^+, $CK8^+$, $CK18^+$, AAT^+, $CYP2B6^+$		(33)
	Human adult liver	Stringent cell culture condition: "plate and wait"	$CD90^-$, $CD105^-$, c-kit^-, $CD34^-$, $CD45^-$, Oct-4^-, $CK7^+$, $CK19^+$, $OV\text{-}6^+$, b2-$microglobulin^+$	20–25 doubling in vitro, hepatic differentiation in vitro	(16)
	Human liver, normal and steatotic	Density gradient (Percoll)	$OV\text{-}6^+$, Thy^+, $EpCAM^+$	Comparison of marker expression in normal and steatotic liver	(92)

EpCAM, epithelial cell adhesion molecule; CD29, cluster of differentiation 29; HLA, human leukocyte antigen; CK18, cytokeratin 18; Alb, albumin; AFP, α-fetoprotein; SCID, severe combined immunodeficient; NCAM, neural cell adhesion molecule; ICAM1, intracellular adhesion molecule 1; ASMA, α smooth muscle actin; NOD, non-obese diabetic; Lin, lineage; OCT4, octamer-binding transcription factor 4; HNF4, hepatocyte nuclear factor 4; uPA^+/SCID, homozygous urokinase plasminogen activator-severe combined immunodeficient; PH, partial hepactomy; G6P, glucose 6-phosphate; DPPIV, dipeptidyl peptidase IV; Thy-1, thymocyte 1; AAT, α-1-antitrypsin; CYP2B6, Cytochrome P450, Family 2, Subfamily B, Polypeptide 6; OV-6, oval cell-6.

could lead to the selection of dedifferentiated hepatocytes rather than true stem/progenitor cells from the native liver, as demonstrated in rat hepatocyte cultures (6). Other isolation methods include cell separation using magnetic columns (MACS) and FACS using one or more markers (see Table 1 for details). Unfortunately, it is not clear yet which markers best characterize the different progenitor populations, and investigators often have to use markers that are also expressed on other cell types. One such example is thymocyte 1 (Thy-1; CD90), which is recognized as a stem cell marker but is also expressed on a variety of other cells such as thymocytes and endothelial cells. Similarly, the marker EpCAM allows for the selection of mostly hepatic stem cells in adult livers where hepatoblast numbers are very low but will give rise to a mixed population of hepatic stem cells and hepatoblasts in younger donors and will have to be coupled to NCAM or ICAM to distinguish the two populations. Finally, variations on these protocols include the addition of a Percoll gradient before cell sorting or seeding for emergence as well as the use of different types of culture media [Dulbecco's modified Eagle's medium (DMEM) high glucose, Roswell Park Memorial Institute (RPMI) medium, Kubota's medium] and culture vessels (collagen-I-coated, fibronectin-coated, charged plastic flasks), introducing yet another opportunity to generate diverse cell populations.

Impact of Donor's Age and Disease State on the Population Isolated

Hepatic stem and progenitor cells can be isolated from various sources in terms of donor's age. Here again, there is a lack of standardization on the terminology to be used. Some laboratories use the term adult as opposed to embryonic and therefore isolate hepatic stem/progenitor cells from postnatal livers of a wide range of ages. Others also distinguish fetal, postnatal, and pediatric donors.

However, it has to be noted that access to these different sources differs from country to country and therefore influences the type of donors researchers usually work with. Indeed, teams from India are the only ones, so far, reporting the clinical use of fetal cells, while teams from Europe and China typically use neonatal or adult liver stem/progenitor cells (37,38). Overall, all three of the stem/progenitor cell types described above can be found in donors of all ages, although they are physically found in different regions of the liver and potentially in different proportions. Indeed, if the number of hepatic stem cells remains constant, the number of hepatoblasts decreases with a donor's age from about 80% of the liver parenchyma in fetal donors to reach less than 0.1% in postnatal donors (73,93). Studies are currently lacking to determine how a donor's age influences the number of hepatic progenitor cells for each of the various progenitors described. However, one recent study by Ono et al. demonstrated a tendency for the number of $CD90^+$ ($Thy\text{-}1^+$) progenitor cells to decrease with a donor's age (from 20 to 75 years old), although the result was not statistically significant, probably due to the small number of patients (58). These results suggest that researchers should be vigilant when generating hepatic progenitor cells and either try to select donor livers of the same average age or characterize the progenitors obtained from postnatal and truly adult livers to demonstrate that they are indeed the same population.

The number of stem/progenitor cells seems to fluctuate in response to liver injury or loss of liver mass as well. Weiss and collaborators have demonstrated that they can isolate a higher proportion of $Thy\text{-}1^+$ progenitor cells from regenerating livers (patients with fibrosis, cirrhosis, or nonalcoholic fatty liver disease) than from nonregenerating livers (patients with colorectal liver metastases) (99). Similarly, Tolosa et al. were able to isolate a greater number of $EpCAM^+$, oval cell 6 marker $(OV\text{-}6)^+$, and $Thy\text{-}1^+$ cells from steatotic livers than from normal livers (92). Indeed, some otherwise dormant intrahepatic progenitor cells have been described to be activated after damage in order to restore liver homeostasis (22).

ARE HEPATIC STEM/PROGENITOR CELLS SUITABLE FOR LIVER CELL THERAPY?

Hepatic stem/progenitor cells are attractive candidates for LCT, much more so than hepatocytes, because they generally exhibit a higher proliferative capacity, allowing for the generation of a large number of cells ex vivo, and are less sensitive to cryopreservation. In addition, one can expect their small size to lead to less obstruction of the portal vein and trapping by the hepatic sinusoids, translating into better transplantation efficiency (14). However, other aspects have to be evaluated before hepatic stem/progenitor cell candidates are considered suitable for LCT.

Hepatocytic Differentiation In Vitro

One of the first characteristics expected from an LCT candidate is the ability to differentiate into hepatocyte-like cells. A variety of protocols exist that can be used to assess the hepatocytic differentiation potential of stem/progenitor cells in vitro (a detailed review is available elsewhere) (79). The most common protocols employ cocktails of growth factors involved in normal liver development, such as hepatocyte growth factor (HGF), which induces proliferation, and oncostatin M (OSM), which supports cell maturation. Morphological changes offer the first clue into the degree of hepatocytic differentiation of a specific stem/progenitor cell, with cells progressively adopting a polygonal shape reminiscing of hepatocytes. These data are often complemented by gene expression studies for maturation markers such as albumin and cytochrome P450 (CYP). However, the expression of these markers is not

sufficient to prove proper hepatocytic differentiation and must be supplemented by functional tests such as albumin production, drug metabolizing P450 activity (CYP activity), glucose 6-phosphate (G6P) activity, or urea synthesis, depending on the disorder(s) targeted by the LCT (41) [for details on the tests that can be performed to demonstrate an appropriate hepatocytic differentiation in vitro, see the review by Sancho-Bru et al. (71)]. Despite tremendous efforts, most liver stem/progenitor cell differentiation protocols currently only lead to partial differentiation with the acquisition of some, but not all, of the characteristics and functions of mature hepatocytes (79). Strategies to improve these protocols and reach a more mature phenotype are therefore under investigation, such as the use of biodegradable or synthetic membranes to grow the cells onto. Piscioneri et al. have recently reported a good proliferation and differentiation of rat embryonic stem cells using chitosan membranes to mimic the liver parenchymal structure (63). The investigators demonstrated a slightly improved urea production over the collagen controls, while the proliferation rate and diazepam biotransformation remained comparable, underscoring the need for further improvement. Interestingly, Reid et al. have suggested the coculture of liver progenitor cells with mesenchymal liver cells to promote differentiation (98) and, alternatively or additionally, the use of biomatrix scaffolds comprising tissue-specific matrix components and growth factors to mimic the stem cells' environment or niche (46,96). The results are promising, but fine tuning may be required to create the proper environment for each specific stem/progenitor cell population.

Liver Engraftment and Hepatocytic Differentiation In Vivo

The capacity of a specific liver/progenitor cell population to differentiate into hepatocyte-like cells in vitro offers the first demonstration of the potential usefulness of that population in treating liver disease. However, most investigators, so far, have proposed the transplantation of undifferentiated stem/progenitor cells because in vitro differentiation is a long, complicated, and costly process (particularly under GMP conditions), which may not be directly translatable to the clinic. Therefore, in vivo data must confirm the ability of these cells to engraft into the host, differentiate into functional hepatocyte-like cells following transplantation, and ameliorate symptoms. Even if animal models require higher levels of immunosuppression than patients because of the use of human cells, these models should be as close to the clinical conditions as possible to be able to predict efficiency. The degree of liver repopulation achieved seems to fluctuate depending on the animal model used. In classic models using immunodeficient mice, repopulation of the liver is about 1–5% (75). For example, Nowak et al. reported the presence of human $CD117^+/CD34^+$/lineage $(Lin)^-$ hepatic progenitor cells in the liver of D-galactosamine-treated mice 4 weeks after transplantation; however, they were only able to demonstrate a repopulation equivalent to about 5% of the host hepatocyte mass (55). Najimi et al. injected 1 million human hepatic progenitor cells into homozygous urokinase plasminogen activator-severe combined immunodeficient ($uPA^{+/+}$-SCID) mice and were able to demonstrate the presence of proliferating human cell nodules in the animals' liver 10 weeks after transplantation (52). In addition, Maerckx et al. reported engraftment of these progenitor cells and subsequent correction of the metabolic defect in a rat model of Crigler Najjar syndrome (47). Similarly, Herrera et al. used immunohistochemistry to demonstrate the presence of clusters of human liver stem cells in the liver of *N*-acetyl-*p*-aminophen-treated mice 30 days following transplantation (30). Dan et al. reported the ability of human fetal liver multipotent progenitor cells to repopulate the liver of retrorsine/CCl_4-treated recombination activating gene 2 common cytokine receptor γ chain double mutants ($Rag2^{-/-}\gamma c^{-/-}$) mice, as evidenced by the detection of human albumin in the serum of the mice 1 month posttransplantation, but they achieved less than 2% repopulation (11). These results are in accordance with reports published by Mahli et al. using human fetal liver epithelial progenitor cells (48). Finally, Schmelzer et al. showed the presence of cells expressing human albumin, CK19, an AFP in the liver of non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice within 2 days of transplantation with fetal or postnatal $EpCAM^+$ human hepatic stem cells (74). Interestingly, liver engraftment was significantly improved by CCl_4 or retrorsine treatment, suggesting the need for a stimulus. In addition, cells positive for human albumin but negative for stem cells markers were detectable within 7 days of transplantation, suggesting a rapid differentiation of the injected cells. The impact of a regenerative stimulus was confirmed by Khuu et al., who reported an increased proliferation of human liver progenitor cells in the liver of transplanted SCID mice following hepatectomy (40). Recent evidence suggests that stem cells may positively influence recovery from injury via paracrine factors that promote tissue repair. This phenomenon has been described in the stem cell treatment of several diseases requiring a surgical intervention (now in phase I clinical trial) wherein the exogenous stem cells induced tissue regeneration by activating neighboring resident stem cells, increasing angiogenesis or decreasing inflammation (9). Several stem cell types have been shown to exhibit paracrine effects, the most studied of which is the mesenchymal stem cell (MSC) (51). However, a paracrine effect would not explain the presence of metabolically active cells expressing donor proteins or enzyme activity.

More complex animal models might be needed to reach higher levels of engraftment. For example, Azuma et al. have described a triple mutant mice (FRG), fumarylacetoacetate hydrolase recombination activating gene 2 interleukin 2 receptor γ chain ($Fah^{-/-}/Rag2^{-/-}/Il2r\gamma^{-/-}$), which develops hepatocellular injury followed by death after 4–8 weeks (2). After pretreatment with a urokinase-expressing adenovirus and transplantation with human hepatocytes, they were able to observe a very high cell engraftment (30–90%) and 40% of transplantation efficiency (repopulation >1%) in these mice. Such a model might, therefore, be useful in the study of stem/progenitor cells' engraftment and their efficiency in restoring hepatic function.

Overall, these results, albeit promising, underscore the need for improvement in the engraftment and repopulation capacities of hepatic stem/progenitor cells.

Safety

From a safety standpoint, scientists must make a reasonable effort to ensure that the transplanted cells will be well tolerated, that there will be limited diffusion of the transplanted cells to organs other than the liver, and to investigate the cells' potential risk of inducing tumors.

Tumorigenicity

The tumorigenic potential of stem/progenitor cells is generally proportional to their degree of plasticity, and hepatic stem/progenitor cells are no exception. Indeed, the expression of the stem cell markers EpCAM, which is believed to play a role in cell proliferation, and CD133, which is potentially involved in stem cells' self-renewal, has been reported on hepatocellular carcinomas and is believed to carry a bad prognostic value (101,103). Similarly, the stem cell marker NCAM has been detected in various types of cancers, including hepatocellular carcinomas. These markers are, thus, currently under investigation as potential cancer stem cell markers. In addition, a study by Goyette et al. has demonstrated the expression of oval cells markers in hepatocarcinomas; however, these results are somewhat questionable considering that they are based on the study of hepatocarcinomas induced by oncogene-transfected oval cells (23). Similarly, the demonstration by Steinberg et al. that oval cells can give rise to cholangiocarcinomas was based on the study of oval cell lines previously transformed in vitro, which could bias the results (84). Clearly, more research needs to be done to determine the actual tumorigenic potential of bipotent cells. Although committed progenitors should hypothetically carry less risk for tumorigenicity, careful tumorigenic investigation is still needed for these cell populations as well. However, given the fairly young age of the technique, it is not yet clear what exact tests need to be run to ensure complete safety. In vitro tests typically include karyotyping of the cell population at various passages and fluorescence in situ hybridization (FISH) (72). However, the results of these tests are somewhat difficult to interpret. Indeed, a donor's lifestyle and environmental exposure can lead to an increased rate of cytogenetic damage (18,65). Therefore, the appearance of karyotypic abnormalities following culture does not necessarily translate into a potential for tumor formation (72). These tests can be followed by an evaluation of the cell's anchorage-independent growth and clonogenicity on soft agar, as well as an assessment of telomerase activity and cell cycle regulatory gene functionality. In vitro tests are usually complemented by in vivo demonstration of the cells' inability to induce tumor formation. Although it is clear that these experiments require immunocompromised animal models to avoid rejection of the human cells, it is not yet established which model is the most suitable to determine whether stem/progenitor cell therapy might lead to tumor formation. In addition, it is difficult to reproduce in animals, and particularly in mice, what is done in humans because of the size difference.

Biodistribution Study

Keeping track of transplanted cells following injection into the host and among native hepatocytes is a difficult task, especially in humans where there is no difference between species. Fortunately, advances in molecular imaging have led to progress in the understanding of fundamental biological processes in living subjects (49). Among the different methods available, direct imaging appears to be the most widely used for stem cell distribution studies in humans. It is a noninvasive system that involves the labeling of stem cells with magnetic resonance imaging (MRI) or radionuclide imaging tracers ex vivo. The most commonly used agents are small paramagnetic iron oxide particles (SPIOs) for magnetic resonance imaging (MRI) and ^{111}Indium oxine for single-photon emission computed tomography (SPECT) imaging (24). The possibility of labeling cells with this radioelement has already been demonstrated in animal models injected with liver stem/progenitor cells and hepatocytes (7), as well as in patients receiving mesenchymal stem cells (20). These studies have shown that the biodistribution of donor cells greatly depends on the administration route, regardless of the cell type injected. Indeed, after intrasplenic injection, the cells migrate in large quantities from the spleen (red pulp) to the hepatic sinusoid via the portal vein system (7). This route minimizes cell aggregation in the portal vein and cell migration to the lungs. However, more cells are retained in the spleen after intrasplenic transplantation with stem/progenitor cells than with hepatocytes. In contrast, intraportal transplantation, which appears as an obvious choice for LCT, is more effective in delivering cells to

the liver but can lead to a higher number of cells migrating to the lungs. Finally, peripheric intravenous injection is the easiest and least invasive route, but it does not specifically target the liver. Indeed, Gholamrezanezhad et al. have shown that, in patients injected with MSCs via the intravenous route, a significant residual activity was first measured in the lungs within 2 h of the injection, which decreased over time from nearly 30% to 3.5% after 10 days (20). This is a serious concern, even if the cells only survive for a short period in the lung because their entry into the pulmonary vascular bed may produce embolic complications. In contrast, the signal detected in the liver was weak immediately after the injection and increased to about 15% after 10 days. Unfortunately, the signal was more important in the spleen than in the liver.

Studies performed with MSCs have shown that radiolabeling has no effect on cell viability, plasticity, and preservation of stem cell characteristics. However, metabolic activity and cell migration could be reduced, therefore moderately impairing the functional integrity of the cells (21). Engraftment of radiolabeled cells in the liver could be underestimated. Indeed, although this technique offers great sensitivity, the timing of cell distribution is limited by the decay of the label used (^{111}In has a half-life of 67 h). In addition, viable and dead cells cannot be distinguished. Other methods of cell tracking are currently under development using reporter gene imaging to generate long-term signal. Moreover, reporter proteins are only expressed by the live cells, and the signal is propagated by daughter cells. The best-known examples are the herpes simplex virus type 1 thymidine kinase (HSV1-tk) for radionuclide, luciferase (Fluc) for bioluminescence, or green fluorescent protein (GFP) for fluorescence imaging (49). Preliminary assays using these methods have already been performed in animal models with human fetal hepatocytes transplanted into NOD-SCID mice (8). However, the genetic modification of stem cells raises the concern of mutagenesis introduction, so a better knowledge will be needed before it can be used in the clinic.

Immunogenicity

Because the liver is an immunoprivileged organ and liver transplants are usually well tolerated, it is reasonable to believe that liver cells would be well tolerated as well. However, OLT patients sometimes suffer acute rejection, suggesting that the risk of cell rejection following LCT is not null and will likely vary from patient to patient. Studies on tolerance and rejection in LCT have been hindered by the lack of reliable marker for these phenomena. In addition, methods allowing one to precisely determine the number of donor cells present in the recipient liver still need to be optimized. However, the progressive loss of function seen in hepatocyte-based LCT suggests the disappearance of the donor cells, either through spontaneous cell death or subsequent to an immune reaction.

Studies by Stephenne et al. and Gustafson et al. have used the tubing loop preclinical model to demonstrate that human hepatocytes can trigger a reaction similar to the instant blood-mediated inflammatory reaction (IBMIR) seen with pancreatic islets and which involves tissue factor (TF) and the induction of the coagulation cascade (27,90). However, aside from a delay in D-dimer increase, no coagulation reaction has been demonstrated following hepatocyte transplantation in clinical settings to date, suggesting that the reaction is restricted to the small liver sinusoids (90). More recently, we have shown a similar tissue factor-mediated procoagulant activity in the adult liver progenitor cells isolated in our laboratory (89). In order to prevent any risk of inducing portal thrombosis, we routinely use a combination of unfractionated heparin and bivalirudin based on our clinical experience with hepatocyte transplantation and the amount of pediatric data available on these molecules.

Animal models of hepatocyte-based LCT have also suggested a role for macrophages/Kupffer cell-mediated inflammation in cell loss following transplantation. Indeed, these studies have shown that over 70% of transplanted hepatocytes get quickly cleared through phagocytosis and that depletion of Kupffer cells and neutrophils increases cell viability and engraftment (26,36,43). However, more studies are needed to determine whether the same is true for liver stem/progenitor cell-based LCT.

Finally, in contrast with their murine counterpart, human liver cells, in general, have been shown to be poorly immunogenic. In fact, studies from our laboratory have shown that human hepatocytes and human liver progenitor cells fail to trigger an immune response in vitro and that incubation of peripheral blood mononuclear cells (PBMCs) with allogenic hepatocytes leads to the induction of tolerogenic interleukin-10 (IL-10) producing dendritic cells and subsequent T-cell hyporesponsiveness (70). Nonetheless, Allen et al. reported the detection of $CD8^+$ alloreactive T-cells following the in vitro restimulation of recipient PBMCs with a donor-specific human leukocyte antigen (HLA) class I antigen 11 months posthepatocyte-based LCT in a patient showing signs of cellular graft loss (1). These findings suggest a potential role for the $CD8^+$ T-cell-mediated response in hepatocyte rejection. However, it has to be noted that the alloreactivity was evaluated using PBMCs or transfected B-lymphoblastoid cell lines, which, because of their immune origin, could be more immunogenic than liver cells. Furthermore, liver stem/progenitor cells are likely to be less immunogenic than mature parenchymal cells such as hepatocytes. Indeed, some MSCs, such as bone marrow (BM)-MSCs, have already been shown to modulate the function of immune cells and are currently under use in clinical settings to try and induce immunotolerance (15).

It would be reasonable to think that liver stem/progenitor cells of mesenchymal origin share these properties. In fact, we have demonstrated that the progenitor cells isolated in our laboratory can inhibit a mixed lymphocyte reaction in vitro (Sana et al., manuscript in preparation). This could be a great advantage for clinical LCT, as it would allow us to reduce or even avoid immunosuppression. However, it is not yet clear whether all liver stem/progenitor cells have immunomodulatory properties in vitro or whether these properties can be evidenced in vivo. More studies are therefore needed to characterize the immunomodulatory properties of stem/progenitor cells.

CLINICAL APPLICATIONS

Regulatory Requirements for the Clinical Use of Stem/Progenitor Cells

In Europe, stem/progenitor cells are considered advanced therapy medicinal products (ATMPs) and, as such, must follow a number of rules, which differ from those applying to traditional medication. Regulations on advanced therapies have been published to harmonize the rules across the European Union and facilitate the development of such therapies while ensuring the highest possible level of safety for the patients. The purpose of this review is not to detail each and every rule applying to ATMPs, and we therefore encourage readers with a special interest for this topic to refer to Regulation No. 1394/2007 for more information. Briefly, these regulations have given rise to a Committee on Advanced Therapies tasked with assessing ATMPs and supporting their development. In addition, the regulations require that ATMPs originate from organs obtained through voluntary, unpaid donation (with informed consent of the donor or next of kin) and that they are manufactured in compliance with the principles of GMP. Tissues must be obtained via a tissue bank accredited by the Ministry of Health and subjected to the rules established in the European directive on tissues and cells (EUTCD:2004/23/EC; 2006/17/EC; 2006/86/EC). They also require that the manufacturer set up a system to ensure the traceability of the ATMPs and the patients treated as well as a system to follow-up on the efficacy of the therapy and any potential adverse reaction. Developers can seek scientific advice from the European Medicines Agency (EMA) for preclinical and clinical development plans and for production. Clinical teams working within hospitals have an exemption allowing them to use these products for exploratory purposes. In any case, every effort must be made to ensure the safety of the patient.

Indications for Stem/Progenitor Cell-Based LCT

Numerous diseases have already benefited from hepatocyte-based LCT treatment, including metabolic disorders, fulminant hepatic failure, chronic liver diseases, and decompensated end-stage cirrhosis. The best results have been obtained with inborn errors of liver metabolism, such as Refsum disease, urea cycle disorders, and Crigler Najjar syndrome (53,78). The proof of concept has been established that infused cells can engraft and function in the recipient liver, but the durability of their function is limited to a period of a few months. Hepatocyte procurement is also limited by organ shortage. Liver stem/progenitor cell-derived hepatocytes are the next generation of cells for LCT development. Inborn errors of metabolism remain fairly rare, typically affecting 1/900 of live births, but are difficult to manage both for physicians and patients (78). They result from the absence or deficiency of one key enzyme in an otherwise healthy liver, leading to the improper functioning of one specific metabolic pathway. Although these disorders are not immediately fatal, metabolite accumulation can lead to mental retardation, progressive organ deterioration, and, in the long run, life-threatening events (78). Some argue that, in these patients, the healthy transplanted liver stem/progenitor cells would be at an advantage compared to the deficient resident cells. Others argue that, on the contrary, transplanted cells would have difficulties engrafting due to the lack of regeneration stimulus. However, a study by Khan et al. has reported the successful use of fetal hepatic progenitors for the treatment of hyperbilirubinemia resulting from Crigler-Najjar syndrome type 1 (38). Biliary atresia and end-stage decompensated liver cirrhosis are the most common cause of chronic liver disease in children and adults, respectively. These disorders can become life threatening, especially if the patient experiences an acute episode aggravating the symptoms. Additional reports by Khan et al. show encouraging results in the treatment of these diseases with fetal hepatic progenitors (37,39). However, recent evidence, suggesting that the efficacy of the treatment may be somewhat limited by the presence of cirrhosis, has prompted researchers to explore possible ways of improving the method (see the paragraph on improved delivery methods below). Finally, hepatocyte-based LCT has been proposed as a way of bridging patients suffering from fulminant hepatic failure to OLT. However, hepatocyte-based LCT has proven more difficult in the case of fulminant hepatic failure due to a higher risk of portal thrombosis (78). The smaller size of hepatic stem/progenitor cells could be an advantage in this case, but the amount of time needed for these cells to differentiate in vivo may make them unsuitable for the treatment of any acute disorder. A better alternative might be the cotransplantation of the stem/progenitor cells with hepatocytes: in that way, hepatocytes, which can bring the early hepatic support, would hopefully give the patient enough hepatic function to survive until the stem/progenitor cells take over.

Cell Therapy Protocols Currently Used in the Clinic

Although hepatocyte-based LCT has been performed for a little over a decade, the transplantation of stem/progenitor cells into patients is a relatively new technique, and a lot of standardization remains to be done. There is currently no consensus on the amount of cells that needs to be injected, the injection route, or frequency. However, the information already available for hepatocyte transplantation can provide a basis investigators can build upon (32,64). Generally, the cell dose used in hepatocyte-based LCT varies from 10^9 to 10^{10} cells per patient in order to replace 5–10% of the liver mass (78). The number of stem/progenitor cells required to achieve the same effect may be different owing to their higher proliferation capacity. For example, Khan et al. have reported positive results after transplanting 12–15 million fetal hepatic progenitor cells in young children (1 and 2 years old) and 80 million cells in adults (37–39). In any case, investigators may have to rely on large-scale culture conditions to obtain a sufficient number of stem/progenitor cells to inject. It is not yet clear whether immunosuppression is necessary in stem/progenitor cell-based LCT. Therefore, investigators should probably use the immunosuppression regimen traditionally given to patients receiving OLT or hepatocyte-based LCT (methylprednisolone, tacromilus, and a tapering oral dose of prednisolone) until they can demonstrate that it is not necessary.

HOW CAN WE IMPROVE STEM/PROGENITOR CELL-BASED LCT?

The success of cell therapy depends on the capacity of the injected cells to engraft into a new environment and on their capacity to proliferate, which means that they need a selective growth advantage over the host population responding to regeneration signals. Engraftment can be influenced by a variety of factors including cell quality and dose, immunosuppression, route of administration, and differentiation state (28,34). It has not yet been established how much engraftment is necessary to obtain a positive outcome. Transplantation of only 1–5% of the liver mass may be sufficient to correct the single biochemical enzymatic defect responsible for a metabolic disorder, but fulminant and chronic liver failure may require replacement of over 10% of the functional liver (59). However, the metabolic liver lacks any signaling to allow exogenous cells to implant, so in any case, the efficiency of cell transplantation needs to be improved for greater efficiency.

Preclinical Studies

The most important parameters to consider before cells can even be prepared for injection are the cell quality and the medium formulation. For practical purposes, many clinical applications rely on cryopreserved cells. Even if stem/progenitor cells are more resistant to freezing than hepatocytes, the quality of the cells after defrosting is a critical point for their capacity to engraft and can vary from donor to donor and from population to population. Several methods have already been described with other cell types to try to improve cell recovery and quality following cryopreservation and could therefore potentially be used for hepatic stem/progenitor cells. These include adding sucrose to the freezing medium, using a three-step cooling protocol with induced ice nucleation, or, as Saliem et al. have also recently reported, using a xeno-free cryoprotectant solution containing anhydrous dextrose in addition to the usual dimethyl sulfoxide (62,69,76,88).

Other parameters to be optimized concern the cells' engraftment into the host and their functionality. Most strategies currently under investigation for the improvement of LCT concern hepatocytes, but some of these could easily be adapted to stem/progenitor cells (64). The mechanism of hepatocyte engraftment into the liver offers critical steps that could potentially be improved following the injection (Table 2) (25):

(i) Immediately after infusion, cells first arrive into the hepatic sinusoid, where the majority is trapped based on their size (26). This leads to a transient portal hypertension associated with hepatic microcirculatory perturbations. One could imagine that the smaller size of progenitors would limit this phenomenon of aggregation; however, the risk still exists. To limit the narrowing and congestion of the sinusoid in the context of hepatocyte transplantation into animal models, Slehria et al. used vasodilators (phentolamine and nitroglycerin). This increased deposition of transplanted cells in the hepatic sinusoids resulted in improved cell engraftment and microcirculatory restoration (77). Other vasodilators, such as serotonin, have been described to improve regeneration and microcirculation in mice and could easily be used in the clinic (91).

(ii) Then, the onset of ischemia and maybe the presence of dead cells lead to another important event: the activation of Kupffer cells. These resident liver macrophages and neutrophils recognize the transplanted cells as "non-self," participating in the early clearance of more than 70% of these cells within 24–48 h (36). They release cytokines and chemokines and contribute to the recruitment of lymphocytes and the development of an inflammatory response that interferes with cell engraftment (43). This early cell loss could potentially be limited by controlling liver inflammation. Depletion of Kupffer cells and neutrophils by irradiation (100) or by gadolinum chloride (GdCl3) (36) to control liver inflammation

Table 2. Method of Cell Transplantation Improvement

Critical Step	Intervention	Effect	References
Vasoconstriction	Vasodilators (nitroglycerin, phentolamine)	Increased deposition of transplanted cells into the sinusoid and restoration of microcirculation	(77)
Inflammation	Inhibitor of coagulation	Effect on the innate immunity	(27)
	Celecoxib and naproxen	Interference in cyclooxygenase pathway, stimulation stellate cells	(17)
Kupffer cells	Gadolinum chloride	Depletion of Kupffer cells	(36)
	Irradiation	Inhibition of the phagocytic function of Kupffer cells	(100)
Disruption of sinusoids	Irradiation	Induce apoptosis of hepatic sinusoidal endothelial cells	(100)
	Cyclophosphamide	Improve disruption of the sinusoid and cells engraftment by accelerating entry and integration into the parenchyma	(48)
	Monocrotaline	Improve disruption of the sinusoid	(35)
Integration into the endothelium	Integrin extracellular matrix (collagen, fibronectin-like polymer)	Better adherence of transplanted cells, acceleration of liver repopulation	(45)
	Irradiation	Inhibition of the proliferation of native hepatocytes	(42)
	Retrorsine	Blockage of hepatocyte proliferation	(34)
Stimulus of regeneration	Coculture with hepatic stellate cells	Production of HGF and extracellular matrix	(60,97)
	Portal vein embolization	Stimulation of the regenerative response and improvement of cell transplantation	(10)

HGF, hepatocyte growth factor.

has been shown to improve cell engraftment and result in acceleration of liver repopulation. Another approach involves blocking the activation of the cyclooxygenase pathway contributing to the inflammatory response following cell transplantation and thereby interfering with cell engraftment. In this context, administration of naproxen and celecoxib inhibits prostaglandin-endoperoxid synthases and induces stellate cells to express the cytoprotective genes vascular endothelial growth factor (VEGF) and HGF. This, in turn, contributes to the regulation of hepatic remodeling and improves cell engraftment in animal models (17).

(iii) Finally, the remaining transplanted cell fraction enters the liver parenchyma after a few hours. Cell integration requires their translocation from the sinusoid to the space of Disse by penetration of the endothelial barrier. This mechanism occurs by vascular permeability of the hepatic endothelium, thanks to VEGF and transforming growth factor (TGF)-α released by surrounding cells, followed by endothelial disruption. The transplanted cells must then thread their way between the resident hepatocytes and reconstitute their plasma membrane to integrate the host liver parenchyma within 3–7 days (26).

Even if most of the transplanted cells are cleared by macrophages, cells can still be found in most vascular beds of ectopic sites in the body. The use of biomaterials could facilitate engraftment and help provide an effective cell therapy. Indeed, the team of Lola Reid has recently shown that the use of biomaterials such as hyaluronan improves engraftment under quiescent conditions, that is, more cells are localized in the liver and remain there longer (94). Encapsulation in polyelectrolyte fibrous scaffold could also be a solution to avoid cell dispersion as it has been done with MSCs and umbilical cord blood (UCB) (102,104). Although these results are very encouraging, the long-term effects of such a matrix transplanted in vivo have to be well characterized before further transposition to the clinic.

Several researchers have tried forcing the sinusoid disruption using various agents such as cyclophosphamide (48) or monocrotaline (35). These treatments led to a better entry and integration of transplanted cells in the animal liver parenchyma. Cell attachment to the hepatic endothelium has also been improved by intraportal infusion of collagen or fibronectin-like polymer. This promotes cell adherence to the endothelium by rapid activation of vinculin-containing focal adhesion complexes, resulting in a superior cell engraftment in treated animals (45). Several teams have proposed giving a selective growth advantage

to donor cells by blocking native hepatocyte proliferation. This creates more space into the parenchyma and avoids the overflow of cells seen in the liver after an abrupt process of regeneration (54). This inhibition can be mediated by retrorsine, a cell cycle inhibitor (34).

Finally, integration into the parenchyma requires the secretion of several factors following cell activation. Some scientists have recreated this microenvironment by a system of coculture. Wang et al. have shown that using a feeder layer (cells producing HGF) and/or the secreted extracellular matrix, cells from UCB were able to acquire morphological features, specific genes, and functions of hepatocytes within 7 days (97). In this context, activation of hepatic stellate cells occurs naturally in vivo and has been shown to positively modulate cell engraftment after hepatocyte transplantation in rats (5). Cotransplantation of stem/progenitor cells with hepatic stellate cells could amplify this phenomenon and improve cell engraftment, as it was observed with MSCs or hepatocytes in 3D culture (60,67). Other types of cotransplantation could be considered as Lola Reid's team has shown that mesenchymal companion cells had cooperative interactions with the stem/progenitor cells in vitro (74). It could also be interesting to coculture stem cells with endothelial cells. Indeed, Pedroso et al. demonstrated that the cotransplantation of $CD34^+$ cells (UCB) with $CD34^+$-derived endothelial cells in a chronic diabetic animal model improved the wound healing by decreasing the inflammatory reaction and increasing the neovascularization (61). However, one has to carefully select the companion cell type, evaluating its potential side effects and, particularly, the associated risk of cancerogenesis.

Improving Hepatic Engraftment in Patients

Unfortunately, strategies used in animal models are not always transferable to the clinic because of the toxicity or potential carcinogenicity of molecules such as retrorsine or monocrotaline. Among the new methods to improve liver repopulation, several approaches have been adapted for clinical use.

Hepatic irradiation appears to be one of the best solutions available, acting at several levels through the disruption of the endothelial sinusoids and the suppression of phagocytic activity of Kupffer cells (100) as well as the temporary inhibition of native hepatocytes' proliferation to improve cell engraftment (42). However, the release of regenerative cytokines after radiotherapy of the liver does not have the same impact in all species. Irradiation, therefore, requires special consideration before clinical use. Nonetheless, Krause et al. have successfully tested a new protocol that combines a low and fractionated irradiation dose (5×5 Gy instead of a single dose of 25 Gy) after partial hepatectomy to improve hepatocyte engraftment in a rat model (42).

Surgical practices can also be used in patients to induce a stimulus of regeneration. Partial portal vein embolization is routinely used in surgery and has been investigated in a nonhuman primate model for cell engraftment (10). Dagher et al. demonstrated that reversible embolization is sufficient to improve autologous hepatocyte engraftment in monkeys, with a liver repopulation exceeding 7% (10). This procedure has been translated to the clinic where patients underwent portal vein embolization followed by stem cell administration (19). Liver resection is another common procedure that has been considered as a pretreatment in the context of cell transplantation. However, more studies are necessary to evaluate the timing of injection and the percentage of hepatectomy needed to obtain a stimulus of regeneration advantageous for cell transplantation. Indeed, the use of massive hepatectomy as a stimulus of regeneration is still controversial (54).

Improving the Delivery Methods: Extrahepatic Sites and Bioartificial Liver

As stated above, recent evidence obtained from rat models suggests that liver cirrhosis, the consequence of most chronic liver disorders, may prevent the maturation of stem/progenitor cells and hamper the efficiency of LCT, underscoring the need for improved therapeutic modalities (68). To this end, laboratories are currently exploring new delivery methods such as the transplantation of cells into extrahepatic sites or the use of new biological or artificial liver matrices. Although these methods are currently being developed with hepatocytes, one could reasonably expect these technologies to be adaptable to liver stem/progenitor cells. For example, several laboratories have suggested the transplantation of hepatocytes at extrahepatic sites. Ohashi et al. have demonstrated the successful transplantation of murine hepatocytes under the kidney capsule or in the subcutaneous space, as well as their ability to proliferate in response to a regeneration stimulus (56). They have even shown the capacity of hepatocytes transplanted under the kidney capsule to partially correct a factor VIII deficiency in mice. However, it has to be noted that in order to give the hepatocytes a proper environment for survival, the cells had to be injected with extracellular matrix components (for transplantation under the kidney capsule) or following an angiogenic treatment allowing for the proper vascularization of the injection site (subcutaneous space). Similarly, Hoppo et al. have recently reported the capacity of hepatocytes directly injected into the peritoneal cavity of tyrosinemic mice to colonize the lymph nodes and rescue the animals from lethal hepatic failure (31). However, these experiments have, so far, been limited to animal models, and proof of their efficacy for clinical treatment is still lacking. Other approaches involve providing the cells a support system mimicking the natural liver microenvironment through the use of a

biological or artificial extracellular matrix. Various types of matrices, such as matrigel, alginate, agarose, and collagen gels, are currently being tested with more or less success [see Soto-Gutierrez et al. for a detailed review (82)]. As research in this field advances, the obstacles hampering the use of such matrices are becoming more and more apparent, the most prominent being the difficulty to create a 3D structure comparable in size and proportion to an actual liver. An interesting study by Ohashi et al. has demonstrated the successful transplantation and persistence of stacked sheets of hepatocytes into the subcutaneous space of mice (57). However, this system may not be entirely suitable for all types of clinical applications, owing to its limited plasma exchange capacity. Scientists are therefore turning their attention to a new strategy wherein livers that are unacceptable for transplantation would be decellularized by perfusion and reseeded with healthy functional cells (4,95). A detailed description of the technique can be found in the review by Badylak et al. (3). Uygun et al. have demonstrated the feasibility of the method in the rat and reported good hepatocyte viability after 24 h (95). Further studies need not be performed to confirm the potential usefulness of the technique in the context of stem/progenitor cell-based LCT.

CONCLUSIONS AND PERSPECTIVES

Hepatic stem/progenitor cell research has brought exciting new possibilities to the fields of hepatology and liver cell transplantation. Indeed, stem cells have shown multiple advantages over hepatocytes for LCT, including a high proliferative capacity and resistance to cryopreservation. In addition, their origin makes hepatic stem/progenitor cells well suited for LCT, even if they are not as readily available as stem/progenitor cells from other tissues: they are able to differentiate into hepatocyte-like cells and acquire mature hepatic functions with less risk of anarchic proliferation than more immature or multipotent cells. However, the lack of standardization in the terminology and methods of cell isolation and culture makes it difficult to have an exact idea of the state of advancement of the technique and to compare the various hepatic stem/progenitor cell types currently proposed for LCT. Clearly, more research is needed to define the tests necessary to ensure not only cell quality in terms of viability and hepatocytic differentiation potential but also their safety. In this context, investigators must reach a consensus on the tests' readouts and their analysis to better standardize the cells' characterization. Finally, researchers must work toward improving cell engraftment and differentiation following transplantation. Several strategies are currently under investigation and show promising results, such as the in vitro culture of stem/progenitor cells in the presence of factors and cell populations belonging to their own niche or the transplantation of the cells in 3D structures such as bioartificial livers. LCT using hepatic stem/progenitor cells may not be suitable for all types of liver-based disorders. In particular, disorders affecting the majority of the liver mass such as fulminant hepatic failure may be difficult to treat using this strategy due to the delay necessary for the cells to mature and become functional. However, LCT using both hepatocytes and hepatic stem/progenitor cells may allow us to bypass the problem by providing mature cells to support liver function long enough for the stem/progenitor cells to differentiate and take over.

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