INTRODUCTION

The term regenerative medicine arose from recent interest in tissue repair and/or regeneration by transplantation of healthy cells. Among various organs, liver is of substantial interest for cell and gene therapy (1), in particular, because this organ has extensive capacity for regenerating and repairing itself and because numerous conditions are amenable to liver-directed cell therapy (see below).

Interest has been steadily maintained over many decades in cell transplantation research, although isolation of mouse embryonic stem (ES) cells in 1981 and of human embryonic stem cells (hESC) in 1998 (2), raised extensive new hopes for major breakthroughs in cell therapy. Subsequently, isolation of person-specific induced pluripotent stem cells (iPS), first from the mouse in 2006, and then from humans in 2007 (3), added further excitement to regenerative medicine. The common belief is that stem cells are capable of self-renewal, while producing progeny that may generate an entire organism (totipotency), all lineages (pluripotency), multiple lineages (multipotency), two lineages (bipotency), or even a single lineage, through cells with extensive proliferation ability that are often designated as transit-amplifying, lineage-restricted or facultative progenitor cells. However, as demonstrated by intervening studies of stem cell plasticity, where organ-specific stem cells derived from given embryonic germlayers generated lineages arising from other germlayers and/or organs, e.g., bone marrow-derived stem cells (4,5), insights into mechanisms that could drive differentiation in stem cells to obtain mature cells were critical. However, this central issue of stem cell differentiation is unresolved at present and constitutes a major hurdle in stem cell therapy. Moreover, hESC, as well as iPS, are characterized by teratogenicity (2,3), which is another major problem for cell therapy.

Whereas stem/progenitor cells play roles in the liver (6,7), the identity of the hepatic stem cell has not been established. Also, it is unclear whether various liver cell types, which exhibit features of different germlayers, originate from a common stem cell or from different sets of stem cells. For instance, hepatocytes are thought to arise from foregut endoderm, whereas liver sinusoidal endothelial cells, the next largest liver cell compartment, as well as Kupffer cells, are thought to be of mesodermal origin, and hepatic stellate cells exhibit features of neuroectoderm. Recent studies of fetal human liver stem/progenitor cells indicate that these cells could generate multiple lineages, including hepatic, biliary and endothelial cells (8). On the other hand, mature hepatocytes exhibit stem cell-like properties with extensive capacity for proliferating and repopulating the liver, including after serial transplantation in animals across multiple generations (9). Therefore, interest in mature somatic cells that offer the capacity to repopulate the liver and to restore deficient functions is particularly appropriate. Nonetheless, supplies of donor human livers are limited, which are naturally prioritized for orthotopic liver transplantation (OLT). Among potential alternatives to adult donor tissues for cell isolation is use of fetal human tissues, especially because organ-derived fetal cells will not need induction of further differentiation in vitro and these cells may be far more readily cryopreserved compared with adult cells (8).

THE PARADIGM OF LIVER-DIRECTED CELL THERAPY

Although “stem cells” have been transplanted into numerous people worldwide with claims of various types of efficacies in many conditions, it should be obvious that these results can be properly evaluated only when appropriate data are available regarding cell targeting, engraftment, proliferation and function. Also, this should require demonstrations of disease-specific parameters to judge whether transplanted cells achieved intended outcomes. Animal studies bridge critical gaps in developing clinical protocols, as well as to resolve issues raised by clinical experiences. In the following text, some of these principles will be introduced and briefly discussed.

Based on significant amount of animal work, some 100 people have been treated with hepatocyte transplantation, including for liver failure, chronic liver disease, genetic disorders, and other conditions (10). These studies established that a large number of congenital and acquired conditions can be treated with liver cell therapy, including those where liver itself is damaged, as well as those where disease manifestations involve extrahepatic organs.
Advances in Liver-directed Cell Therapy for Hepatic and Extrahepatic Diseases

Table 1: Candidate conditions for liver-directed cell therapy

<table>
<thead>
<tr>
<th>Liver is affected by disease</th>
<th>Extrahepatic organs affected</th>
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<tr>
<td>Genetic disorders</td>
<td>Deficiency states</td>
</tr>
<tr>
<td>Crigler-Najjar Syndrome, type-I</td>
<td>Congenital hyperbilirubinemia</td>
</tr>
<tr>
<td>α-1 antitrypsin deficiency</td>
<td>Familial hypercholesterolemia</td>
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<tr>
<td>Hepatic porphyrias</td>
<td>Sporadic hypercholesterolemia</td>
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<tr>
<td>Lipidoses, e.g., Niemann-Pick disease</td>
<td>Hyperammonemia syndromes</td>
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<tr>
<td>Progressive familial intrahepatic cholestasis</td>
<td>Defects of carbohydrate metabolism</td>
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<tr>
<td>Refsum’s disease</td>
<td>Oxalosis</td>
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<tr>
<td>Tyrosinemia, type 1</td>
<td>Diabetes mellitus, type-1</td>
</tr>
<tr>
<td>Wilson disease</td>
<td>Coagulation defects</td>
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<tr>
<td>Acquired disorders</td>
<td>Immune disorders</td>
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<tr>
<td>Acute liver failure</td>
<td>Factor VII deficiency</td>
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<tr>
<td>Chronic hepatitis and cirrhosis</td>
<td>Factor IX deficiency</td>
</tr>
<tr>
<td>Fatty degeneration of liver</td>
<td>Hereditary angioedema</td>
</tr>
<tr>
<td>Liver cancer</td>
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...due to production of either mutant proteins (e.g., Crigler-Najjar syndrome, hemophilia A) or toxic proteins (e.g., oxalosis) in the liver (Table 1). Early clinical experiences substantiated the value of animal studies and established that cells can be safely transplanted in the liver, although much further work is needed to understand how engraftment and proliferation of transplanted cells could be improved for repopulating the liver. These issues are of major interest, given that most of the transplanted cells (80-90%) are rapidly destroyed in the liver and that transplanted cells do not proliferate in the healthy liver, which requires induction of damage in native cells to generate selective proliferation advantages for transplanted cells. This is currently being established by defining what mechanisms are important in the initial clearance of transplanted cells, whether this clearance may be prevented, how effective manipulations could be developed for replacing the liver to suitable extents with transplanted healthy cells, and in what ways could this impact cell therapy in specific disorders.

**CELL ENGRAFTMENT MECHANISMS**

Work over the past 25 years established that transplanted hepatocytes engraft and function most effectively within the liver, even though cells may also engraft in ectopic sites, e.g., spleen, peritoneal cavity, etc. (11). An advantage of cell engraftment in the liver is that in the absence of allograft rejection transplanted hepatocytes can survive throughout life, at least as demonstrated in animals. It should be noteworthy that transplanted hepatocytes integrate in the liver parenchyma with reconstitution of plasma membrane structures, e.g., formation of bile canaliculi, and position-dependent gene expression. This should be extremely important for diseases requiring permanent correction, as well as for physiological behavior of cells, and for correction of conditions where toxins must be excreted into bile. However, the space in liver sinusoids is generally limited, which restricts transplantation of cells to a finite number, e.g., not more than 15% of the hepatocyte mass in the adult liver may be transplanted at any given time. Moreover, 80-90% of transplanted cells are cleared from the liver within 24 to 48 hours (Fig. 1), which permits replacement after one session of cell transplantation of only 1-3% of the hepatocyte mass. This limited cell replacement is insufficient for therapies, which led to considerations of repeated cell transplantation or of proliferation in transplanted cells (12,13). It is now well-established that transplanted hepatocytes do not proliferate in the healthy liver.

Repeated cell transplantation can progressively increase liver replacement, such that 5-8% of the hepatocyte mass was replaced after hepatocytes were transplanted thrice (13). Further increases in this extent of liver replacement by additional strategies, which are under development, will have a major impact on therapeutic outcomes in selected conditions.

Similarly, cell losses due to normal “wear and tear” must be balanced by cell replacements during liver injury, which has been verified by liver repopulation studies in animals with hepatotoxic manipulations, including drugs, chemicals, radiation, transgenes, etc. (11). Induction of transplanted cell proliferation required homeostatic mechanisms to favour transplanted cells (Fig. 2) (13). Moreover, oxidative DNA damage after liver radiation and hepatic ischemia-reperfusion was effective for liver repopulation with cells.
Inflammation in cell transplantation

The liver is a complex unit with unique morphological arrangement, including dual hepatic arterial and portal blood supply. Hepatocytes show position-dependent biological differences (5), e.g., hepatocytes in periportal areas of liver lobule undergo earlier and greater DNA synthesis during liver regeneration, whereas perivenous hepatocytes show expression of P450 genes that metabolize chemicals and drugs. While hepatocytes are the most abundant liver cell-type (~60%), other cell-types include biliary epithelial cells, hepatic stellate cells, liver sinusoidal endothelial cells (LSEC), fibroblasts, pit cells, and litorcal cells, including Kupffer cells. During liver development, paracrine signaling from primitive endothelial cells includes vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and bone morphogenetic protein (BMP), without which hepatic “differentiation” and expansion is arrested. Subsequently, complex interactions between paracrine signals, endogenous transcription factors and gene products continue during organogenesis (16). Moreover, cross-talk between liver cell types continues throughout health and disease for maintaining hepatic homeostasis. These cell-cell interactions also arise during cell transplantation, where the hepatic endothelial barrier must be disrupted for cell engraftment in the parenchyma and liver remodeling requires activation of hepatic stellate cells (13,17,18).

Recent studies showed that cell transplantation excites hepatic inflammation, including activation of neutrophils and Kupffer cells (19,20). This is deleterious for transplanted cell survival. Neutrophil and Kupffer cell responses after cell transplantation were mediated by inflammatory chemokines-cytokines, among which TNF-a played major roles. Characterization of inflammatory chemokines-cytokines after cell transplantation in syngeneic animals showed overexpression of cytokines produced in neutrophils and mononuclear cells, again incriminating those cells in the inflammatory response (20). By contrast, after allogeneic or xenogeneic cell transplants, host immune responses are more complex, with recruitment of lymphocytes, NK cells and other immune cells, although activation of cytokine-chemokine responses is recognized to be highly important, including for maturation of antigen-presenting dendritic cells. Additional inflammatory mechanisms concern activation of cyclooxygenase (Cox) pathways, which are amenable to drug interventions, including for improving cell engraftment by serving cytoprotective roles with release of VEGF and HGF (18).

In chronic liver disease, e.g., Wilson disease, liver injury typically leads to depletion of cellular anti-oxidant defense mechanisms with greater susceptibility for cell death, especially when additional insults are encountered. Exposure to noxious toxins and inflammation is associated with release of a variety of chemokines and cytokines, which further activates deleterious intracellular signaling. These mechanisms could potentially alter inflammatory and other responses leading to changes in cell engraftment and/or proliferation in chronic liver injury. Therefore, improvement in cell engraftment under these situations will also be important, particularly because the kinetics of liver repopulation is impaired in the presence of chronic liver injury of certain types.

CELL THERAPY IN SPECIFIC DISORDERS

Many cell transplantation studies addressed correction of disease in animal models. For instance, hepatocyte transplantation ameliorated hyperbilirubinemia in the Gunn rat, which models Crigler-Najjar syndrome, type-I, hypoalbuminemia in the Nagase analbuminemic rat, hypercholesterolemia in Watanabe heritable hyperlipidemic (WHHL) rabbit model of familial hypercholesterolemia (FH), copper toxicosis in the LEC rat model of Wilson’s disease, tyrosinemia in the FAH mouse model of hereditary tyrosinemia, type-I, biliary disease in the mdr2-/ mouse with cholestasis, and other animal models. These animal models are serving as the basis for the development of clinical protocols of hepatocyte transplantation. Experience with hepatocyte transplantation indicates that each condition poses unique requirements regarding the extent of liver repopulation, replacement of protein secretory function versus metabolic function, etc. Therefore, for clinical applications of cell therapy, it is appropriate to consider the capacity of transplanted cells to engraft, proliferate and restore deficient functions under both syngeneic and allogeneic settings.

Although the strategy of ex-vivo gene therapy in WHHL rabbits was applied to patients with FH (21), success was limited, due to inefficient gene transfer in cells, engraftment of only some transplanted cells, and limited liver replacement with transplanted cells. The efficacy of cell therapy in the LEC rat model of Wilson’s disease can be demonstrated by restoration of hepatic ATP7b expression, clearance of liver copper and reversal of hepatic damage. This permitted a series of studies to demonstrate how
liver could be replaced with healthy transplanted hepatocytes in LEC rats. The major findings were that hepatic radiation promoted transplanted cell proliferation with phenotypic reversal of Wilson’s disease parameters, including restoration of normal liver histology, although several months were necessary for improvement (15).

While patients with acute liver failure and chronic liver disease represent major candidate groups for hepatocyte transplantation (1, 10), it has been unclear whether cells should be transplanted in the liver, in an extraparenchymal location, e.g., the peritoneal cavity, spleen, etc., or simultaneously in the liver and an extraparenchymal location. For combined cell and gene therapy, enhancing engraftment of transplanted cells will again be critical. Although ex-vivo gene therapy is more invasive than in vivo gene therapy, it will potentially be safer because inadvertent gene transfer in undesirable cells, e.g., germline cells, or antigen-presenting cells, can be avoided. Moreover, inhibition of inflammatory responses mediated by cytokines-chemokines in this setting should be helpful for improving cell/gene therapy results. Many gene transfer vectors have been tested over the past two decades. Gene therapy with adenovirus vector to express hATP7B in LEC rats produced increased copper excretion in bile; however, toxicity of adenoviral vectors is now well recognized. Retroviral vectors require active cell replication for efficient gene transfer and may produce insertional mutagenesis. Other suitable vectors include those based on adenov-associated virus, SV40 virus, and lentivirus, which infect non-dividing cells, integrate into the genome for permanent transgene expression, and accommodate relatively large genes.

Another major paradigm of liver cell therapy is provided by recent cell transplantation studies in hemophilia A (22, 23). This condition arises from mutations in factor VIII gene located on the X chromosome. Since prevention of bleeding requires frequent administration of hFVIII protein, which is extremely expensive and may be defeated by inhibitors, permanent solutions in the form of cell and gene therapy are very attractive for hemophilia A. Surprisingly, it had been unknown as to what cell type produced and released FVIII, even though the liver was found to be a major contributor as shown by rapid correction of hemophilia A after OLT (24). It had been assumed that FVIII was produced in hepatocytes. To establish what specific liver cell type produced FVIII, we performed a series of cell transplantation studies in hemophilia A mice. This demonstrated that LSEC and not hepatocytes were the major source of FVIII since transplantation of healthy mature LSEC restored FVIII activity in hemophilia A mice, correlating with the extent of liver repopulation with transplanted LSEC.

CONCLUSIONS

The potential of organ-specific cell therapy is extensive in case of the liver, since the number of conditions amenable to liver-directed cell and gene therapy is substantial. Insights into mechanisms of transplanted cell engraftment, proliferation and function are necessary for developing clinical protocols. These insights will eventually permit cell therapy with candidate stem cells.

REFERENCES


