

Re-Engineering the Liver with Natural Biomaterials

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Abstract

The extensive regenerative capacity of hepatocytes and the key roles of the liver in metabolic processes have generated interest in the liver as an appropriate target for cell and gene therapy. If cells were considered as natural biomaterials, then liver cell transplantation would fall within the general field of bioengineering. While unmodified hepatocytes engraft in the liver and ectopic sites, biological modifications and optimization of bioengineered systems would facilitate engraftment and survival of transplanted cells, especially in ectopic locations. Acute liver failure, chronic liver disease and metabolic deficiency states are among the conditions that can potentially be treated by cell transplantation. In acute liver failure, cell transplantation into the liver, along with the creation of an extrahepatic reservoir of cells might be required because engraftment and proliferation of transplanted cells in the liver needs time. In other situations, gradual liver repopulation alone might well be effective without additional manipulations.

Key Words: Liver, hepatocyte, transplantation, treatment

INTRODUCTION

Throughout the world, the availability of organs for transplantation has been greatly limited. As a consequence, despite progressive improvements in the success of organ transplantation, the number of patients awaiting organ transplant has continued to rise. Orthotopic liver transplantation (OLT) is no exception to this general rule. Innovative strategies such as split liver transplantation or living-related liver transplantation have not decreased the waiting lists for OLT. This chronic lack of organ supply has generated interest in cell-based approaches. At one level, the development of bioartificial liver-assist devices is being considered for supporting the survival of patients, as a bridge toward OLT or for tiding over exacerbations in the case of chronic liver failure. At another level, cell transplantation has been proposed

to develop an extrahepatic reservoir of hepatocytes or to repopulate the liver, thus promoting recovery from acute liver failure and allowing the treatment of chronic liver disease. Moreover, in several conditions, transplantation of normal hepatocytes has the potential of correcting metabolic deficiency states. Examples include kernicterus, due to congenital jaundice, encephalopathy, due to hyperammonemia, and familial hypercholesterolemia.

The liver contains many types of cells, including hepatocytes, bile duct cells and littoral cells (endothelial cells, stellate cells and Kupffer cells). Hepatocytes constitute the largest fraction of liver cells (~60%). In principle, transplantation of hepatocytes would be similar to transfusion of individual blood cells rather than whole blood transfusion. Transplantation of isolated hepatocytes is attractive for multiple reasons. Hepatocyte transplantation is technically simple because cells can be injected through vascular catheters, and does not require the removal of the native liver, which confers an element of "reversibility" to the procedure, because in contrast with OLT cells can be transplanted repeatedly and far more readily.

In the development of hepatocyte transplantation, the availability of methods to isolate cells following enzymatic digestion of the liver has been critical.^{1,2} A significant body of literature concerning hepatocyte transplantation has developed over the past three

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decades. Among the most exciting recent developments, which are moving this area toward clinical applications are the following: First, the establishment of optimal ways allowing transplanted hepatocytes to engraft, survive and function; Second, the demonstration of the efficacy of therapeutic liver re-population in animal models to establish which diseases would be suitable for hepatocyte transplantation; and Third, the identification of alternative sources of hepatocytes, which do not affect organ transplantation programs.

Engraftment, survival and function of transplanted hepatocytes: In early studies, the fate of transplanted hepatocytes was examined in ectopic locations.^{3,4} Localization of transplanted hepatocytes within the liver required an ability to distinguish between transplanted and host cells, which required further developments. It should be noted that hepatocytes are rejected promptly when mismatched, although major histocompatibility antigens are displayed on hepatocytes in a less pronounced fashion than on other cells, such as the endothelial cells in the liver. Also, the host immune response, following transplantation of whole liver, may be different from that following transplantation of allogeneic hepatocytes. Certainly, immunosuppression is effective at preventing rejection of allogeneic hepatocytes. On the other hand, transplantation of syngeneic cells, which refers to cells derived from inbred animals with a shared genetic composition, and which are similar to those obtained from identical twins, is associated with indefinite cell survival. Many of our insights into transplanted hepatocyte biology have been obtained in transplantation systems using syngeneic cells.

Early studies showed that transplanted hepatocytes could survive in many ectopic sites (Table 1). Among these sites, the spleen and peritoneal cavity were especially noteworthy. Mito and colleagues found that hepatocytes survived in the splenic pulp.⁵ Further work reproduced these findings and suggested that the sinusoidal organization and extracellular matrix components in spleen could support the survival of transplanted hepatocytes.⁶⁻⁸ Hepatocytes were also found to survive in the peritoneal cavity, especially after attachment to extracellular matrix components or when transplanted with other types of liver cells.⁹⁻¹¹

In the spleen, transplanted hepatocytes become organized in vascular spaces and eventually proliferate

to form confluent masses.^{5,6-8} Transplanted hepatocytes demonstrate correct ultrastructure in the spleen, including development of bile canaliculi,⁵ which is a characteristic feature of mature hepatocytes. Transplanted hepatocytes exhibit synthetic, metabolic and detoxification functions in the spleen, although bile must be drained into blood followed by excretion through the host liver. It is noteworthy that transplanted cells survive and function in the spleen throughout the lives of rodents, which would be greatly beneficial in clinical application. However, one limitation of cell transplantation into the spleen concerns its relatively small capacity; despite the replacement of up to 40% of the spleen with transplanted cells after several months, the overall mass of transplanted hepatocytes constitutes no more than a small fraction of the host's liver mass.

The analysis of cell bio-distributions following injection of cells into the splenic pulp showed that 90% or more of the transplanted cells migrate onward into the portal vein and the liver sinusoids. This could potentially be associated with the shunting of cells through portosystemic collaterals into lungs, especially in the presence of portal hypertension and portosystemic shunting (see below).

In contrast with the spleen, injecting cells into the peritoneal cavity is simple and the peritoneal cavity has a large capacity. Moreover, cells transplanted into the peritoneal cavity would not be expected to migrate into vascular beds, such as, the lungs. Furthermore, the peritoneal cavity is of interest to investigators examining the feasibility of engineering adjunct bioartificial livers derived by immobilizing

Table 1. Extrahepatic Sites with Survival Of Transplanted Hepatocytes

Vascular beds
Splenic pulp
Pulmonary capillaries
Specific body cavities
Beneath renal capsule
Peritoneal cavity
Parenchymal organs
Dorsal, inguinal or mesenteric fat pad
Pancreas
Salivary gland
Skeletal muscle
Thymus
Thyroid

hepatocytes in suitable biomaterials.¹² Since hepatocytes require anchorage to extracellular matrix components for survival, transplantation of cells into the peritoneal cavity requires the provision of microcarriers or other suitable alternatives for attachment.^{9,10} Of course, microencapsulation of hepatocytes into biomaterials may offer additional advantages, such as the prevention of allograft rejection, if the entry of immunocytes, complement and antibodies were prevented. Indeed, studies have shown that transplanted hepatocytes can engraft in the peritoneal cavity promptly and even produce extracellular matrix components of their own.⁹ However, despite such manipulations, the survival of transplanted hepatocytes has been limited in the peritoneal cavity to at most a few weeks. Moreover, analysis of the function of transplanted hepatocytes shows that liver genes are expressed better in transplanted cells located in the spleen than in those of the peritoneal cavity,^{13,14} presumably due to differences in the local prevalence of intermediary substrates, trophic factors, cytokines, cell-cell interactions, and other factors.

In many animal models of disease, the transplantation of hepatocytes into the spleen or peritoneal cavity has been proven effective at replacing deficient metabolic and genetic functions.^{4,15} Despite limitations involving cell survival and gene expression, hepatocyte transplantation into the peritoneal cavity could potentially be useful for short-term applications, such as the amelioration of hepatic encephalopathy, and perhaps other metabolic disorders, as suggested by the successful amelioration of histidinemia in a mouse model.¹¹ The availability of tissue-engineered livers could be especially helpful in supporting patients with liver failure until recovery from an acute insult, liver repopulation with cell transplantation or the performance of an OLT. Although significant progress has been made in the development of effective bioartificial liver-assist devices, further work is required to optimize systems. Among the most effective devices are those that use porcine hepatocytes, though this raises concerns regarding the transmission of novel zoonotic diseases in humans.

Liver repopulation with transplanted cells has excited much interest, especially in view of the fact that the liver represents a natural home for hepatocytes. The identification of suitable systems for localizing transplanted hepatocytes in the liver has significantly advanced these efforts.¹⁶⁻¹⁸ These effective approaches

include the utilization of donor hepatocytes that were genetically labeled with unique transgenes. Such cells were derived from specially developed transgenic mice, which were inbred to well-established mouse strains.^{16,18} This permitted the use of genetically marked cells for both short and long-term studies of cell survival. An example of genetically marked cells is represented by mouse hepatocytes expressing hepatitis B virus surface antigen (HBsAg), which is normally absent in animals.^{16,17} Transplanted cells could now be localized in the tissues of recipient animals, including the liver, by demonstrating HBsAg mRNA by molecular hybridization. Moreover, measurement of serum HBsAg was effective in establishing the persistence of transplanted cells, as well as changes in the transplanted hepatocyte mass, in serial studies in individual animals. Subsequent to this initial demonstration, a variety of models using transgenic mice and other animals have been developed for analyzing liver repopulation.¹⁵ Among these animal models, the use of inbred F344 rats with spontaneous mutation in the dipeptidyl peptidase IV (DPPIV) gene has been highly informative.¹⁹ DPPIV is an enzyme with expression throughout the body and particularly abundant expression in the bile canalicular domains of hepatocytes. This has permitted the generation of systems where transplanted F344 rat hepatocytes, containing normal DPPIV activity, can be localized in DPPIV-F344 rats by simple histochemical methods.^{19,20}

Another approach concerned the use of cells labeled with exogenous dyes or radioisotopes, such as 111-indium or 99m-technetium, which can be localized by gamma imaging of the body with external cameras.^{17,21,22} Use of cells labeled with exogenous markers has been helpful in short-term biodistribution and cell survival analysis. As indicated above, studies showed that the injection of cells into the splenic pulp was followed by migration of transplanted cells into liver sinusoids.^{16-18,21-23} Transplanted cells are entrapped in hepatic sinusoids because of their larger size relative to the liver sinusoids. The eventual distribution of transplanted hepatocytes results in their localization in both portal vein radicles and hepatic sinusoids, with more cells situated initially in portal vein radicles.^{19,24} It is noteworthy that hepatocytes do not engraft or survive in portal vein radicles beyond the first 24–48 hours after transplantation and undergo clearance by phagocytes and macrophages.²⁴

On the other hand, transplanted hepatocytes reaching hepatic sinusoids engraft in the liver parenchyma, although here again not all transplanted hepatocytes survive. Overall, less than 20–30% of the transplanted hepatocytes that enter the liver survive in animals. It is possible that in people more transplanted hepatocytes could survive in the liver if initial cell distributions in the hepatic sinusoids were greater, and similar to animals,¹⁹ although this has to be established.

During engraftment in the liver, transplanted hepatocytes migrate from hepatic sinusoids and enter the space of Disse, which separates liver sinusoids from the liver plates.^{24,25} This process requires disruption of the sinusoidal endothelium over a period of 16–20 hours. Subsequently, transplanted cells can be demonstrated in juxtaposition to host hepatocytes. A variety of changes are encountered in the host liver immediately after cell transplantation. These include, evidence for separation of host hepatocytes in liver plates with interruption of gap junctions, extensive activation of gamma glutamyl transpeptidase expression in host hepatocytes adjacent to periportal areas, and increased hepatocyte apoptosis rates.²⁶ These changes emanate from ischemic injury to the host liver, which arise from the transplanted cells serving temporarily as emboli. Prior hepatic vasodilatation with specific drugs can prevent evidence of ischemic injury to the liver. After cells are fully integrated in the liver parenchyma during a period of several days, it is possible to demonstrate functionally intact hybrid plasma membrane structures, such as gap junctions and bile canaliculi, between adjacent host and transplanted hepatocytes.^{20,24}

Integration of transplanted hepatocytes in the liver parenchyma is of critical significance. First, this is necessary for the physiological regulation of gene expression in transplanted hepatocytes.²⁷ Second, the presence of transplanted hepatocytes in liver parenchyma assures correct hormonal and growth factor stimulation, cell-cell and cell-extracellular matrix interactions, and survival and proliferation throughout the life of the animal.²⁸ Third, reconstitution of bile canaliculi is a requirement for the excretion of toxins into bile, which is impaired in specific disorders. Finally, entry of transplanted cells into liver plates is associated with their clearance from hepatic sinusoids, which restores normal hepatic blood flow. It has been found that cell transplantation perturbs

hepatic microcirculation with immediate attenuation of portal vein radicles, as well as impairing sinusoidal blood flow. These perturbations induce portal hypertension,^{23,24} which resolves within 2–3 hours of cell transplantation due to the early re-establishment of the hepatic microcirculation. It is noteworthy that the hepatic microcirculation suffers no significant damage following cell transplantation, despite repeated procedures,²⁹ which should be reassuring to those developing strategies for clinical hepatocyte transplantation.

The use of radiolabeled hepatocytes, as well as cell surrogates, e.g., albumin particles of an equivalent size and shape, have helped establish the significance of cell biodistributions in vascular beds,³⁰ which is important for demonstrating the safety of cell transplantation. The significance of incidental translocations of transplanted cells into lungs has also been analyzed by injecting hepatocytes intravenously. Since hepatocytes are much larger than pulmonary capillaries, intravenously injected cells are entrapped within the lungs. The potential complications included pulmonary embolism, pulmonary hypertension, cardiac arrhythmias and heart failure. Fortunately, despite injection of hepatocytes in large numbers, actual complications are rare.³⁰ This is partly due to the rapid destruction of hepatocytes in pulmonary capillaries. Recent studies have shown that transplanted cells can engraft and proliferate in the presence of acute liver injury, despite significant inflammatory activity,³¹ and this also applies to the cirrhotic liver, despite “sinusoidal capillarization” and fibrosis following deposition of extracellular matrix components.³² The safety of cell transplantation becomes relevant when liver repopulation is considered for treating individuals with acute liver failure or cirrhosis.

Analysis of the proliferative capacity of transplanted cells shows that in the normal liver, transplanted hepatocyte mass does not change significantly, throughout adult life.²⁸ After a single session of cell transplantation, 1–3% of the normal rodent liver can be repopulated. Repeated cell transplantation can increase liver repopulation further.²⁹ For therapeutic purposes, greater liver repopulation is desirable in many situations. In attempting to define ways of accomplishing this, an important concept has emerged, indicating that transplanted hepatocytes require a proliferation advantage compared with na-

tive hepatocytes for significant liver repopulation to occur. This concept has been illustrated by many animal studies. For instance, in transgenic mice expressing the urokinase-type plasminogen activator in the liver under control of the albumin promoter (alb-uPA mouse), native hepatocytes are destroyed.³³ Transplantation of normal hepatocytes, which are not injured by the transgene, leads to virtually complete replacement of the diseased liver. Similarly, transplantation of normal hepatocytes into FAH mutant mice, which constitute a model of tyrosinemia type 1 with marked liver disease, is associated with virtually complete replacement of the liver.³⁴ Selective ablation of host hepatocytes with toxins, such as carbon tetrachloride, is also associated with increased liver repopulation.³⁵ Injury of native hepatocytes with D-galactosamine, the overexpression of Mad1 transcription factor, or the use of toxic prodrugs, can also induce proliferation in transplanted cells.^{31,36,37}

Consideration has been given to enhancing the proliferative capacity of transplanted cells, for example by manipulating growth control. Mouse hepatocytes have shown extensive replication capacity in FAH mutant mice, such that a single cell can divide more than 80 times without exhausting its replication potential,³⁸ moreover, this replication capacity was not restricted to specific diploid or tetraploid subpopulations of mouse hepatocytes.³⁹ However, the results are at variance with data from rat hepatocytes, where polyploid cell fractions were less responsive to mitogenic stimulation.⁴⁰ Growth factor stimulation to induce proliferation in transplanted cells has seemed a reasonable approach, but the infusion of hepatocyte growth factor was ineffective for this purpose in rats.³⁵ Studies in animals subjected to two-thirds partial hepatectomy, which induces compensatory hepatic growth showed that transplanted hepatocytes proliferated only when cells were transplanted subsequent to partial hepatectomy,⁴¹ which induced previously unrecognized deleterious changes in the liver.

If host hepatocytes are to be injured to induce proliferation in transplanted cells, this should ideally be insidious without overt liver disease. In one approach, investigators induced apoptosis in the liver, which was followed by transplantation of cells resistant to the injury.⁴² In another approach, retrorsine, a DNA-binding alkaloid, was used to inhibit mitogenic activity in host hepatocytes.⁴³ Retrorsine induces

polyploidy in the liver, meaning that cells continue to synthesize excessive amounts of DNA but are unable to divide.⁴⁴ This process is accelerated by partial hepatectomy. When normal hepatocytes are transplanted in rats treated with retrorsine and partial hepatectomy, the liver is replaced virtually completely with transplanted cells. It is noteworthy that partial hepatectomy can be substituted in this regimen by repeated administration of the thyroid hormone tri-iodothyronine (T₃),⁴⁵ which has been shown to regulate hepatic polyploidy.⁴⁶ The use of retrorsine and other toxins will be too hazardous to apply in people. However, the principle of inducing damage to native hepatocytes before cells are transplanted is being explored as a means of developing clinically effective strategies. The incorporation of radiation-induced liver injury as a priming mechanism has also been effective in repopulating the rat liver (Fig. 1).⁴⁷ Since radiation can be restricted to specific areas of the liver, widespread toxicity can be avoided, and this presents a promising lead for further development.

Hepatocyte transplantation for clinical applications: Many disorders can be potentially treated by cell transplantation (Table 2). Metabolic deficiency disorders require transplantation of normal hepatocytes expressing the deficient genetic function. One could genetically modify autologous hepatocytes obtained by resecting a portion of the liver, followed by gene transfer in culture and transplantation of corrected cells (*ex-vivo* gene therapy). *Ex vivo* gene therapy is currently hampered by difficulties in transferring genes efficiently and culturing hepatocytes without the loss of cell viability. Treatment of acquired disorders, such as chronic viral hepatitis, requires hepatocytes capable of resisting infection with viruses circulating in the host. Of course, patients with acute liver failure could be treated with healthy hepatocytes from any source.

Based upon much work using animal models, and which has been reviewed elsewhere,^{3,4,15} a significant rationale exists for hepatocyte transplantation in humans. However, hepatocyte transplantation in people has been attempted in a sporadic and nonsystematic fashion. It is necessary to conduct pilot studies initially, focusing especially upon safety issues, and such programmed development requires preliminary data before serious funding can be obtained. Initial studies

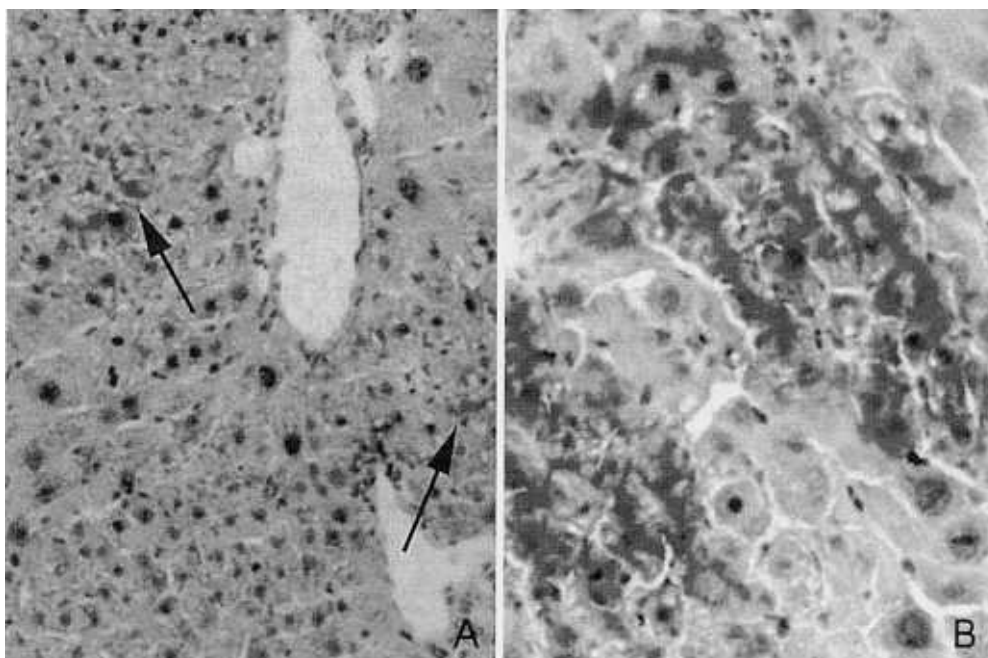


Fig. 1. Evidence for extensive liver repopulation with transplanted hepatocytes. The liver from DPPIV- F344 rats treated with radiation and two thirds partial hepatectomy before intrasplenic transplantation of DPPIV + F344 rat hepatocytes. (A) Liver from a recipient within two days of cell transplantation showing few transplanted cells (arrows). (B) Liver from a recipient two weeks later showed proliferation in transplanted cells with significantly larger transplanted cell clusters. Transplanted cells proliferate further in this situation and replace virtually the entire liver over time. Histochemical staining for DPPIV activity was utilized to localize transplanted hepatocytes, which produced red color. Hematoxylin was used to counter stain cell nuclei.

Table 2. Selected Conditions Potentially Amenable to Hepatocyte Transplantation

Liver disease present	Liver spared from disease
Congenital metabolic disease Alpha-1 antitrypsin deficiency Erythropoietic protoporphyria Lipidoses, e.g., Gaucher's disease, Niemann-pick disease Tyrosinemia, type 1 Wilson's disease	Genetically transmitted metabolic disease Congenital hyperbilirubinemia, e.g., Crigler-Najjar syndrome Familial hypercholesterolemia Hyperammonemia syndromes Defects of carbohydrate metabolism
Acquired disease Chronic hepatitis, cirrhosis Fulminant liver failure due to viral hepatitis, drug toxicity, etc.	Deficiency of circulating proteins Coagulation defects, e.g., Factor VIII or IX deficiency Hereditary angioedema

have been successful from a safety point of view. Among these early studies, Mito and colleagues determined whether hepatocyte transplantation could improve chronic liver disease.⁴⁸ They hoped that hepatocytes in the spleen would supplement liver function by constituting an additional liver mass, thereby ameliorating the consequences of chronic liver

failure. Autologous hepatocytes were isolated from patients with chronic liver disease followed by intrasplenic cell transplantation. However, since the number of viable hepatocytes that could be isolated from small cirrhotic liver segments was limited, transplanted hepatocytes represented <0.01% of the estimated host hepatocyte mass only. Survival of trans-

planted cells was demonstrated in an occasional patient with ^{99m}Tc -HIDA excretion in the spleen. However, only one of the 10 patients studied showed any improvement following cell transplantation. More patients with chronic liver disease have undergone intrasplenic cell transplantation in the United States.⁴⁹ In some patients with advanced liver failure, liver function and hepatic encephalopathy improved following hepatocyte transplantation.⁵⁰ In these patients, the presence of transplanted hepatocytes was thought to have stabilized patients, prior to eventual OLT.

In animals, hepatocyte transplantation can improve both mortality, and hepatic encephalopathy, in acute liver failure.^{51,52} When fetal human hepatocytes were injected intraperitoneally in a few patients with acute liver failure,⁵³ outcomes were believed to have improved, although whether the transplanted cells survived was not determined. More recently, Bilir and colleagues reported their experience of five patients with acute liver failure that were not candidates for OLT.⁵⁴ These patients were seriously ill with prolonged prothrombin times, hyperbilirubinemia and hepatic encephalopathy. Three of the five patients surviving for more than 48 hours after cell transplantation showed improvement in encephalopathy, ammonia levels and prothrombin times. These patients survived for 12, 28 and 52 days after cell transplantation, with localization of transplanted hepatocytes in the liver and spleen by light microscopy and fluorescent in situ hybridization with X and Y chromosome probes, although it was not reported whether transplanted cells proliferated in these patients.

Establishing correlations between transplanted cell proliferation and improved outcomes is important because animals with acute liver failure have been shown to improve after the injection of cell fragments or conditioned medium from hepatocytes.⁵⁵⁻⁵⁷ Recent studies in animals have begun to establish that transplanted hepatocytes can engraft and proliferate in acute liver failure.^{31,36,37} Also, studies have demonstrated that mortality can be improved in animals following limited replacement of the liver with transplanted cells.^{36,37} However, more analysis is necessary to define mechanisms of liver repopulation in animals with acute liver failure.

Treatment of genetic disease represents another major category. Several patients with familial hyper-

cholesterolemia (FH) were treated with *ex-vivo* hepatic gene therapy.^{58,59} Patients with FH are at great risk of coronary artery disease due to mutation in the low density lipoprotein receptor (LDLR) gene. Using approaches defined in the Watanabe heritable hyperlipidemic (WHHL) rabbit, which is an authentic model of FH,^{60,61} *ex-vivo* gene therapy was attempted in FH patients.^{58,59} This produced a significant lowering of serum cholesterol levels but no cures, presumably because gene transfer and the magnitude of liver repopulation were limited. In more recent studies, allogeneic human hepatocytes were transplanted into the liver of a child with ornithine transcarbamylase deficiency and hyperammonemia.⁶² Hepatocyte transplantation improved the serum ammonia levels only transiently. Similarly, in a patient with Crigler-Najar syndrome type 1 with severe hyperbilirubinemia, hepatocyte transplantation improved bilirubin levels,⁶³ but the response was eventually lost.

These results re-emphasize the need for insights into strategies for liver repopulation. When the liver can be repopulated extensively, such as, by using retrorsine and partial hepatectomy or other manipulations prior to cell transplantation, it is possible to correct genetic disease completely. Examples include the Nagase analbuminemic rat, where the liver is normal but animals exhibit extremely low serum albumin levels, and the Long-Evans cinnamon rat, which exhibit extensive copper accumulation in the liver due to mutations in the *atp7b* gene, which is similar to Wilson's disease.^{64,65}

Alternative sourcing of cells

Several approaches are being tested to expand the supply of human hepatocytes. One approach concerns conditionally immortalized cells, which refers to the introduction of genes that can drive proliferation in cells. Among the oncogenes that can perturb cell growth control, the simian virus (SV) 40 T antigen has been utilized for enhancing proliferation in hepatocytes.⁶⁶ Expression of SV40 T antigen can be regulated by modulating the temperature of cells, as well as by using other transcriptional regulatory mechanisms. In this way, hepatocytes can be induced to proliferate extensively. SV40 T-containing hepatocyte lines have been generated, although problems

with oncogenetically transformed cells concern the potential for tumorigenesis. Therefore, attempts have been made to first transform human hepatocytes with the SV40 T antigen and to then remove the oncogene.⁶⁷ This approach has been successful and permitted expansion of human hepatocytes, which remained functionally intact, and were capable of improving survival in a rat model of acute liver failure.

Isolation of progenitor cells from the human liver constitutes another approach. Although a liver stem cell has not yet been identified, progress has been made in the understanding of relationships among cell lineages. For instance, stem cells from the bone marrow seem capable of originating hepatocytes.^{68,69} Progenitor cells from the pancreas are also capable of differentiating into hepatocytes.⁷⁰ The presence of progenitor cells in the rodent liver has been well established.⁷¹ Activation of progenitor cells in the adult organ requires induction, however, the fetal liver contains large numbers of progenitor cells, which can be isolated and show differentiation capacity in intact animals.⁷²⁻⁷⁴ These findings indicated that progenitor cells can be isolated from the fetal human liver.⁷⁵ Early studies with isolated human progenitor liver cells indicate that these cells can proliferate extensively in culture and differentiate into mature hepatocytes following transplantation into immunodeficient animals. In future, it may be possible to further expand the number of cells in culture, as suggested by culture conditions under which adult human hepatocytes can be maintained for months.⁷⁶ The use of xenogeneic cells, especially from pigs bred under specific-pathogen free conditions, has captured some interest.⁷⁷ If transgenic pigs expressing human complement and other genes designed to limit host immune responses become available, it is possible that limitless numbers of hepatocytes could be produced, which will greatly facilitate clinical applications.

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