Liver Regeneration after Liver Transplantation

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Key Words

Liver regeneration • Transplantation • Cytokines • Growth factors • Graft size

Abstract

Background/Purpose: The liver has a remarkable capacity to regenerate after injury or resection. The aim of this review is to outline the mechanisms and factors affecting liver regeneration after liver transplantation. **Methods:** Relevant studies were reviewed using Medline, PubMed and Springer databases. **Results:** A variety of cytokines (such as interleukin-6 and tumor necrosis factor- α), growth factors (like hepatocyte growth factor and transforming growth factor- α) and cells are involved in liver regeneration. Several factors affect liver regeneration after transplantation such as ischemic injury, graft size, immunosuppression, steatosis, donor age and viral hepatitis. **Conclusion:** Liver regeneration has been studied for many years. However, further research is essential to reveal the complex processes affecting liver regeneration, which may provide novel strategies in the management of liver transplantation recipients and donors.

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Introduction

The liver is an important organ within the body that has a central role in metabolic homeostasis as it is responsible for the metabolism, synthesis, storage and redistribution of nutrients, carbohydrates, fats and vitamins [1]. The liver produces large numbers of serum proteins including albumin and acute-phase proteins, enzymes and cofactors. Importantly, it is the main detoxifying organ of the body, which removes wastes and xenobiotics by metabolic conversion and biliary excretion [2].

Hepatic regeneration following resections or injury involving less than 70% of total liver mass proceeds uneventfully until restitution of the original liver mass is complete, often within 3–6 months in an otherwise healthy human liver [3].

The amazing regenerative capacity of the liver is most clearly shown by the two-thirds partial hepatectomy (PH) model in rodents, which was pioneered by Higgins and Anderson [4] in 1931. In this model, two thirds of the liver is surgically removed, and the remaining liver enlarges until the original liver mass is restored within 5–7 days after surgery.

Regenerative activity is proportional to the regenerative stimulus until 70% of the liver has been resected or

destroyed. Thereafter, there is a marked decline (but not to baseline levels) in regenerative activity despite increased stimulation [5].

Hepatic resection is safely accomplished for malignant and benign disease because of the ability of the liver to regain its functional mass in a matter of days or weeks. Liver regeneration is a very complex process involving the activation and interaction of multiple cytokines and growth factors that regulate cell growth and proliferation. Simultaneously, liver cells continue to function while undergoing mitosis in order to reestablish the organ's mass and architecture [6].

This amazing regenerative capacity of the liver after hepatic resection has recently become even more significant with the advent of new techniques and procedures in liver transplantation [7].

Regeneration is crucial in liver transplantation. In cadaveric transplantation, hepatocyte loss occurs due to (1) ischemia/reperfusion injury which is inevitable because of the necessary preservation period from procurement to implantation, regenerative mechanisms are actively engaged after transplantation depending on the length and degree of the cold ischemic injury, and (2) the alloimmune response require replacement [8].

Regeneration is also critically required in the setting of living donor liver transplantation (LDLT) because even though the ischemic injury is minimized in LDLT, as the preservation period is very short, this technique supplies a graft, which is by definition too small, by transplanting only 50–60% of the expected liver volume in adults; this requires vigorous immediate hepatocyte proliferation. Both the recipients and the donors must rely on the rapid regeneration of a partial liver in addition to maintaining the basic metabolic functions of the liver [7].

The remarkable increase in the number of cases of liver transplantation, especially LDLT, in recent years has brought the topic of liver regeneration after liver transplantation to the forefront.

Molecular and Cellular Characteristics of Liver Regeneration

Cell division is rarely seen in hepatocytes in the normal adult liver, as these cells are in the G₀ phase of the cell cycle. However, after PH approximately 95% of hepatic cells, which are normally quiescent, rapidly reenter the cell cycle [9].

Individual hepatocytes have an amazing replicative capacity, as only a few hepatocytes are required to restore liver mass after profound liver injury [10, 11].

Early studies in rodent models show that after PH, hepatocytes begin to replicate within 24 h, with biliary epithelial cells and Kupffer cells replicating soon after [9].

All evidence to date has shown that the fully differentiated hepatocyte is the replicating cell that accounts for the rapid regeneration after PH [12].

In the normal adult rat, the turnover rate is approximately one mitosis per year, but PH greatly stimulates the rate of mitosis. Liver weight doubles in 48 h reaching a normal size in 5–7 days, although regeneration continues for 15–16 days. A burst of DNA synthesis in hepatocytes begins 15–18 h after PH – as they enter the S phase of cell cycle – and reaches a peak in 24 h and then declines. A second but lower maximum is reached at about 56 h. However, the induction of DNA synthesis occurs later in the nonparenchymal cells (at about 48 h for Kupffer and biliary epithelial cells, and at about 96 h for endothelial cells). Subsequent levels of DNA synthesis in hepatocytes are lower, as complete restoration of liver mass requires an average of approximately 1.6 cycles of replication in all cells. By comparison, the peak in DNA synthesis in mice occurs later (36–40 h after PH) and varies between strains. The onset of DNA synthesis is well synchronized in hepatocytes, beginning in cells that surround the portal vein of the liver lobule and proceeding towards the central vein. Regenerating hepatocytes may divide more rapidly than their capacity to reestablish a sinusoidal pattern and thus maintain the liver plate-sinusoid plate relationship. Rapidly generating hepatocytes are swollen and hydrophilic compared with normal hepatocytes, their nuclei are enlarged with more crisply defined chromatin, and nucleoli are prominent. The functional capacity of effectively regenerating hepatocytes is diminished compared with that of normal hepatocytes. The regenerative response involves hypertrophy and hyperplasia [2, 13].

Liver regeneration after PH is carried out by proliferation of all the existing mature cellular populations composing the intact organ. These include hepatocytes, biliary epithelial cells, Kupffer cells and stellate cells. All of these cells proliferate to rebuild the hepatic tissue [14, 15].

In addition to the highly differentiated cells that can readily proliferate, there is also a secondary proliferative compartment within the liver composed of the intrahepatic stem and progenitor cells, which constitutes a functional segment of the biliary system. Oval cells are thought to be the progeny of these cells, and in models of liver in-

jury, caused by the administration of D-galactosamine, acetyl aminofluorene, dipin or the combination of carbon tetrachloride (CCl₄) administration and PH, regeneration seems to be quite dependent on the proliferation and differentiation of oval cells [16, 17]. These cells, however, are not present in PH models without injury [12, 18].

Liver repopulation and transplantation studies indicate that bone marrow stem cells might have the capacity to differentiate into hepatocytes, but it is unclear whether this transition is a rare event or the normal means by which the liver replenishes its hepatocyte pool when faced with certain types of injury. However, other studies have indicated that rare cell fusion events between bone marrow stem cells and hepatocytes could give the appearance that the resulting cells are derived from bone marrow stem cells when they are, in fact, of hepatocyte origin [2, 19, 20].

On a molecular level, studies in the past decade have elucidated cytokine pathways, transcriptional regulation and patterns of gene activation after PH in animal models. These cascades are initiated within minutes to hours after PH [7].

It has become increasingly apparent that specific cytokines [tumor necrosis factor (TNF)- α , interleukin (IL)-6], growth factors [hepatocyte growth factor (HGF), transforming growth factor (TGF)- α], and transcription factors [nuclear factor (NF)- κ B, signal transducer and activator of transcription (STAT3), AP-1 (activator protein-1), C/EBP β (CCAAT/enhancer-binding protein β) and Foxm1B] play an important role in the initiation and maintenance of liver regeneration [21].

Previous studies suggest that tri-iodothyronine (T₃) regulates liver regeneration after a 70% partial hepatectomy in the albino rat [22]. Insulin and glucagons also have a potential role in liver regeneration [23].

In animal models, in which hepatocytes are directly damaged and thereby induced to undergo necrosis, similar growth factor-mediated and cytokine-mediated pathways are activated as occurs after PH. Proliferation of hepatocytes is also involved in liver regeneration that occurs after massive hepatocyte necrosis, or apoptosis that is induced by hepatic toxins such as CCl₄ or a systemically introduced Fas ligand, but the cell cycle response is not as synchronized [24, 25].

There are also significant changes in liver architecture during liver regeneration, both after PH and liver necrosis. The size of the liver lobules is remarkably larger and the thickness of the hepatocyte plates is almost twice the size of the normal cell thickness. Previous studies suggest that slow lobular reorganization is taking place for sev-

eral weeks, and eventually liver histology becomes indistinguishable from the original [2, 26].

Initiation of Liver Regeneration

For regeneration to take place, the hepatocytes must gain proliferative capacity to enter the cell cycle and respond to other growth factors such as HGF, TGF- α , and heparin-binding epidermal growth factor, a process referred to as 'priming' [27].

A wide variety of genes are differentially expressed during the first few hours after PH (the priming phase); many of these genes are involved in the cytokine network like TNF- α and IL-6 [28–30]. The important role of TNF- α and IL-6 in triggering the regeneration was proven by the inhibition of DNA replication by anti-TNF antibodies [28], the blockage of liver regeneration in IL-6 and TNF receptor type I (TNF-R1) knockout (KO) mice [31, 32], and the correction of the defect in TNF-R1 KO mice by an IL-6 injection [32].

Lipopolysaccharide (LPS), a component of the innate immune system that interacts with the LPS receptor on Kupffer cells, is important in triggering liver regeneration after PH and in other models of liver injury through stimulation of the production of inflammatory chemokines and cytokines, including IL-6 and TNF- α [33, 34]. Moreover, members of the complement cascade, C3a and C5a, interact with their receptors on Kupffer cells to stimulate IL-6 and TNF- α release [35].

Extracellular matrix remodeling after PH plays an important role in the initiation of liver regeneration by altering the balance between mitogens and mitoinhibitors by causing release (local and circulatory) and activation of HGF while releasing TGF- β ₁ extensively in the circulation, where TGF- β ₁ is bound and inactivated by α ₂-macroglobulin [26, 36].

Other events, like the expression of β -catenin [37] and the Notch-1 intracellular domain [38] in hepatocyte nuclei within 15–30 min after PH, participate in the initiation of regeneration as elimination of the expression of these proteins by RNA interference [38] decreases the regenerative response.

The precise orchestration of all the above-mentioned events is probably required to initiate the regenerative process.

Pathways of Liver Regeneration

Investigators have been able to elucidate cytokine-dependent and cytokine-independent pathways that are crucial for liver regeneration and connect many of the identified proteins to these pathways [2, 25].

Fig. 1. Pathways of regeneration. **a** Cytokine-dependent pathway. **b** Cytokine-independent pathway. VEGF = Vascular endothelial growth factor; uPA = urokinase plasminogen activator.

Cytokine-Dependent Pathway

This pathway relies on IL-6 and TNF- α , with some contribution from other cytokines such as IL-1 (fig. 1) [21]. IL-6, previously thought to be only a proinflammatory cytokine, seems to be an essential growth factor in the process of liver regeneration [39]. Mice lacking IL-6 or the TNF receptor have a blunted DNA response after PH, resulting in liver necrosis and failure. A single dose of recombinant IL-6 before resection reverses this trend [31, 32].

On the other hand, Sakamoto et al. [40] reported that liver regeneration in IL-6-deficient mice is essentially normal even though there is decreased activation of STAT3.

Experimental evidence supports the concept that IL-6 is released from Kupffer cells and followed by a transcriptional cascade which then results in hepatocyte replication [41].

IL-6 and TNF- α are believed to be released from Kupffer cells in response to an initiating stimulus, such as LPS, within the liver or from an outside environmental stimulus [21].

The binding of IL-6 to its receptor IL-6R, which is associated with two subunits of gp130, stimulates the tyrosine kinase activity of the associated Janus kinase family member, usually JAK1. Activated JAK then phosphorylates the associated gp130 and STAT3 on a tyrosine resi-

due, which results in the dimerization of STAT3. Dimerized STAT3 translocates to the nucleus and activates the transcription of target genes. Stimulation of gp130 also activates the MAPK (mitogen-activated protein kinase) signaling cascade and, although the exact mechanism remains unconfirmed at present, evidence indicates that JAK1 activates the SH2 domain containing tyrosine phosphatase SHP2 and recruits growth factor receptor-bound protein 2/son of sevenless (GRB2-SOS). GRB2-SOS activates Ras, which leads to the activation of Ras-MAPK-ERK (extracellular signal-regulated kinase). MAPK signaling is crucial for cellular proliferation. During liver regeneration, therefore, IL-6 activates two main pathways – through the gp130-IL-6R complex – the STAT3 and MAPK signaling pathways [2].

This process leads to the activation of a vast array of immediate and delayed early genes required for normal liver-specific metabolic functions as well as for regeneration and repair [7].

The conditional KO of gp130 within the liver had less impact on hepatocyte DNA synthesis after hepatectomy than would have been expected on the basis of the phenotypes of IL-6 or STAT3 KO mice. However, defects in cyclin E and cyclin A expression were observed in gp130-deleted livers after hepatectomy, which indicates that gp130 is important for normal cell cycle progression [42].

STAT3 and NF- κ B expression is enhanced 1 h after PH [43, 44]. However, elimination of either of these two signaling molecules does not abrogate the regenerative response, probably due to the existence of redundant pathways which complement for their loss (e.g. STAT1 assuming the role of STAT3) [26, 45, 46].

However, despite these findings, STAT3 and NF- κ B are critical for cells to progress from G1 to S phase and are crucial for activating the c-myc gene, a gene required for cell cycle progression. Cyclins are then upregulated, thus completing the cycle by moving the cell into S phase, and autonomous cellular replication is achieved [7].

TNF- α signaling is also required for a normal proliferative response after PH. This effect seems to be largely mediated by the ability of TNF- α to induce IL-6, as treatment with IL-6 corrects the defect in DNA synthesis that occurs in TNF-R1 KO mice that have had a hepatectomy [32]. TNF- α induces the production of IL-6 by upregulating NF- κ B, which activates the transcription of IL-6 [2].

However, the increase of serum TNF- α after PH has not been universally observed, and it appears to be higher in rats than in mice, and even though TNF-R1 KO mice have multiple deficits after PH, they appear to regenerate normally [47, 48]. These data suggest that TNF itself may not be required because other ligands such as lymphotoxin- α can signal through TNF-R1 [49].

Early in the process, negative feedback mechanisms are in place with the induction of various inhibitory proteins that are important for terminating liver regeneration including TGF- β , plasminogen activator inhibitor (PAI), suppressor of cytokine signaling-3 (SOCS3) and p27 and other cyclin-dependent kinase inhibitors, which downregulate STAT3 and inhibit the IL-6 signaling pathway [2, 50].

Cytokine-Independent (Growth Factor-Dependent) Pathway

The cytokine-independent pathway also promotes cellular proliferation during liver regeneration, and is mediated by growth factors or mitogens [21].

HGF and the epidermal growth factor receptor (EGFR) ligand family are important growth factors that drive cell cycle progression during liver regeneration [49].

HGF is produced in the liver and other tissues by non-parenchymal cells, particularly stellate cells, and may act on hepatocytes by a paracrine or an endocrine mechanism. The HGF precursor, pro-HGF, is rapidly activated by proteases – such as urokinase plasminogen activator and its downstream effector plasminogen – after PH or liver injury (fig. 1). Blocking urokinase plasminogen ac-

tivator delays the appearance of HGF and thereby delays liver regeneration, whereas blocking PAI accelerates the release of HGF and thereby accelerates liver regeneration [51, 52].

HGF signals through the c-Met receptor, leading to the activation of several downstream pathways including ERK 1/2, PI3K, S6 kinase and AKT [53–55].

HGF regulates various processes in the liver including mitogenesis, motogenesis and morphogenesis as well as being a direct stimulant of hepatocyte proliferation in cultured hepatocytes. HGF/c-Met signaling is important in hepatoprotection from apoptosis and in the enhancement of hepatic repair after liver injury. In addition, HGF and its receptor c-Met are important growth factors in various tissues [51, 54, 56, 57].

The genetic elimination of HGF or its receptor c-Met is associated with embryonic lethality involving abnormalities in many organs, most notably in the placenta, and, in addition, livers of the embryos are smaller than the wild-type controls [58, 59]. Moreover, Huh et al. [57] reported that hepatocyte c-Met-deficient mice had massive mortality after PH. Of all the signals participating in the very early events after PH the signaling by HGF appears to be the most irreplaceable contributor in liver regeneration [26].

The family of ligands that binds the EGFR includes EGF, TGF- α , heparin-binding EGF-like growth factor (HB-EGF) and amphiregulin (AR). EGF is a strong mitogen for hepatocytes in culture and given to intact animals causes hepatocyte proliferation [60]. EGF is continually available to the liver through the portal vein [61]. However, the concentration of EGF in the portal vein after PH has not yet been evaluated.

Catecholamines, including epinephrine and norepinephrine, rise rapidly in the plasma after PH and stimulate the production of EGF from Brunner's glands of the duodenum [62]. Norepinephrine also enhances the mitogenic effects of EGF and HGF in hepatocyte cultures and decreases the mitoinhibitory effects of TGF- β_1 [26, 63, 64].

The expression of TGF- α mRNA, which is very low in the normal liver, increases after PH and before the onset of DNA replication. The main effect of TGF- α in the liver is the stimulation of hepatocyte proliferation [65].

Transgenic mice that overexpress TGF- α display constitutive hepatocyte proliferation and eventually develop cancer [66]. On the other hand, KO mice lacking TGF- α show no defects in liver regeneration, most probably because of the overlap between various ligands of the EGF family [67].

After PH, HB-EGF is expressed earlier than HGF and TGF- α , and HB-EGF transgenic mice with liver-targeted production have enhanced liver regeneration [68]. In addition, HB-EGF KO mice have a deficient regenerative response [69], in spite of the fact that this deficiency is partially compensated by an earlier increase in TGF- α expression.

AR also appears to contribute to liver regeneration, as mice deficient in AR have a deficient liver regeneration [70].

Vascular endothelial growth factor interacts with sinusoidal endothelial cells within the liver and causes an increase in HGF production by these cells. This vascular endothelial growth factor-mediated hepatic growth is dependent on the presence of endothelial cells, but the mechanism is unknown and is partially blocked by treatment with HGF antibodies [71].

Interactions between Cytokine-Dependent and Cytokine-Independent Pathways

The cytokine-dependent and cytokine-independent pathways in liver regeneration have been linked via common downstream signal transduction molecules [e.g. ERK and JNK (Jun amino-terminal kinase)], transcription factors (e.g. AP-1 and C/EBP β) and other molecules, e.g. insulin-like growth factor-binding protein 1 (IGFBP1), that seem to be regulated by both growth factors and cytokines (fig. 1). This allows speculation about how the combination of cytokine and growth factor signals might lead to robust liver regeneration and repair after injury or resection [2, 21].

For example, both TNF- α and HGF can activate JNK and MAPK-ERK which are crucial regulators of Jun activation, and they can also induce cell proliferation and the expression of cyclin D1, which is an important checkpoint protein in hepatic growth [72, 73].

The IL-6/TNF- α and HGF pathways both upregulate the activity of the various homo- and heterodimeric AP-1 transcription factors, including the Jun-Fos heterodimer. AP-1 activity is required for the activation of a number of proteins that are involved in growth response [74], and the cooperation of AP-1 with STAT3 amplifies the expression of genes in the liver which results in an adaptive response during liver regeneration [75].

Another possible point of intersection between HGF and IL-6 signals could be the regulation of the IGFBP1 gene. IGFBP1 encodes a promitogenic and hepatoprotective protein that, in vivo, is upregulated by IL-6 as well as, potentially, by HGF as indicated by in vitro studies [76, 77].

One important linkage between cytokines and growth factors may be the activation of matrix metalloproteinases by cytokines such as TNF- α . The activity of several matrix metalloproteinases increases after PH [78], one of them is called TGF- α -converting enzyme (TACE, also known as ADAM17). TNF- α can activate TACE, which in turn cleaves the TGF- α precursor anchored in the cell membrane. The released, active TGF- α binds to the EGFR resulting in the stimulation of cell proliferation in cultured hepatocytes [79]. TACE has also been shown to cleave the precursor forms of cytokines and many EGFR ligands including TGF- α , HB-EGF and AR [80].

Termination of Liver Regeneration

The size of the liver is highly regulated and is controlled by the functional needs of the organism [81].

The most well-known antiproliferative factors within the liver are TGF- β and related family members such as activin. TGF- β is produced mainly by hepatic stellate cells and during liver regeneration hepatocytes initially become resistant to it [25, 82]. TGF- β inhibits proliferation of hepatocytes in culture [83], suppresses the production of HGF [84], the expression of urokinase and the activation of HGF [85].

With considerable evidence discounting the role of any single cytokine (e.g. TGF- β) being the terminator of regeneration, attention has been paid to other hepatocyte mitoinhibitors.

Extracellular matrix (in the form of exogenously added matrix such as collagen gels or extracts from EHS sarcoma) inhibits cell proliferation and enhances the differentiation of hepatocytes in culture [86].

The extracellular matrix signaling involves integrin-linked kinase [87]. Liver-specific ablation of integrin-linked kinase in mice results in enhanced proliferation of hepatocytes and biliary cells and hepatomegaly in the absence of PH [88]. Moreover, liver-specific ablation of integrin-linked kinase in mice results in enhanced liver regeneration after PH and the liver did not properly terminate its growth at the end of regeneration but continued to grow, reaching a size which is 58% greater than the original liver [89].

It is reasonable to speculate that the reassembly of the extracellular matrix and the sinusoidal capillary network provides matrix-driven signaling that terminates the regenerative process. This may be direct signaling through integrins or signaling induced by TGF- β (bound to the newly synthesized decorin and exerting a tonic mitoinhibitory effect). Newly synthesized matrix would also be capable of binding HGF (a protein with high affinity to

Fig. 2. Termination of regeneration is a complex process controlled by multiple factors like TGF- β , ECM, SOCS3 and IL-6. ECM = Extracellular matrix; ILK = integrin-linked kinase.

glycosaminoglycans and heparin) and preventing it from being activated by urokinase, which disappears anyway at the end of regeneration and its expression is inhibited by TGF- β . These events would bring hepatocytes back into a state of quiescence surrounded by HGF (bound to glycosaminoglycans) and TGF- β (bound to decorin). In this scenario, early mitogenic stimuli such as HGF and EGF drive both hepatocyte proliferation and enhanced expression of TGF- β . Proliferating hepatocytes become resistant to TGF- β . However, TGF- β stimulates the production of extracellular matrix and the formation of hepatic sinusoids, and also inhibits the expression of HGF and urokinase [84, 85]. New extracellular matrix synthesis by stellate cells stimulated by TGF- β restores the binding of both HGF and TGF- β_1 and reestablishes quiescence of hepatocytes in G0 (fig. 2) [26].

Activin is an apoptogen of the TGF- β family that blocks hepatocyte mitogenesis. Activin shows diminished signaling during liver regeneration when its cellular receptor level is reduced, but the receptor level is restored once liver regeneration is terminated [90].

Suppressors of cytokine signaling (SOCS) are important negative regulators of cytokine signaling that pre-

vent the tyrosine phosphorylation of STAT proteins. SOCS directly interact with phosphorylated JAK kinases and prevent the activation of STAT3. It has been shown that IL-6 signaling in the liver causes the rapid upregulation of SOCS3, which correlates with the subsequent downregulation of phosphorylated STAT3, thereby terminating the IL-6 signal (fig. 2) [50].

IL-6 itself could have a role in terminating the HGF signal by inducing PAI, which blocks the processing of pro-HGF into active HGF (fig. 2) [91].

Hippo kinase signaling cascade [a growth-suppressive pathway that ultimately antagonizes the transcriptional coactivator YAP (Yes-associated protein)] can also control hepatocyte proliferation [26]. Overexpression of YAP in a transgenic mouse model leads to unchecked hepatocyte proliferation, massive liver hyperplasia and hepatic carcinogenesis, while blocking the overexpression of YAP leads to a return of the liver to its normal size. It is possible, therefore, that the Hippo kinase pathway has an important role in the termination of liver regeneration and the determination of the overall liver size [92].

Fig. 3. Factors affecting liver regeneration after liver transplantation.

Factors Affecting Liver Regeneration after Transplantation

Regeneration is crucial in liver transplantation. In cadaveric transplantation, hepatocyte loss due to (1) ischemia-reperfusion injury, (2) damage that may have occurred in the donor and (3) alloimmune response, requires replacement [8].

Regeneration is also critically required in the setting of LDLT because even though the ischemic injury is minimized in LDLT, this technique supplies a graft, which by definition is too small, by transplanting only 50–60% of the expected liver volume in adults, which requires vigorous immediate hepatocyte proliferation in both the recipients and the donors [7].

Factors that have been shown to have a significant effect on liver regeneration in both experimental and clinical settings include ischemic injury, graft size, immunosuppression, steatosis, donor age and viral hepatitis (fig. 3).

Ischemic Injury

Animal studies have shown impaired regeneration when massive hepatectomy is combined with warm ischemic injury [93].

Ischemic injury, both warm and cold, is an unavoidable component of transplantation, although ischemic injury in LDLT is minimized by careful planning of the donor and recipient procedures. After prolonged cold ischemia of whole liver grafts, there is an initiation of the cell cycle pathways with an upregulation of the markers of liver regeneration. The more extensive the ischemic in-

jury, the greater the expression and activation of cytokines, transcription factors and immediate early genes and the greater the magnitude of hepatocellular replication [8].

However, the liver can only tolerate ischemic injury up to a certain point, after which the damage is too extensive and the graft is unable to maintain functional homeostasis and regenerative capabilities, which results in liver dysfunction and graft failure [94].

Theoretically, there could be an advantage in ischemic preconditioning of the graft, as it was shown to improve liver function after liver resection [95]. However, it is apparent that when rodent partial grafts are subjected to ischemic injury of more than 10 h, there is a significant effect on survival with extensive hepatic necrosis, inability to initiate or maintain the regenerative response, and decreased survival [96].

The clinical impact of cold ischemia has been demonstrated in a multicenter report of LDLT, where every hour of additional cold ischemia added a significant risk of graft loss [97].

Graft Size

The amount of liver mass transplanted has been shown to be an important variable after transplantation. Early experimental studies addressing regeneration after transplantation show that a small-for-size graft will adapt to its environment and achieve a size equal to the original native liver. It became apparent that the graft size-to-recipient ratio was critical, in that grafts that were too small had decreased survival [98].

These findings correlated with clinical experience in that small-for-size grafts regenerate to an appropriate size for the recipient. However, there was significant functional impairment of these grafts, as evidenced by cholestasis and histologic changes consistent with ischemic injury. Liver grafts with a graft weight to standard liver volume ratio of less than 40% were found to have poor graft survival and prolonged hyperbilirubinemia [99, 100].

Using animal models of partial liver graft transplantation, investigators have studied the interplay between the regenerative response and ischemic injury in the setting of 50 and 30% size grafts. The partial grafts showed a robust regenerative response if the ischemic injury was minimal. However, it became apparent that when these partial grafts were subjected to ischemic injury of moderate to prolonged time periods, there was a significant effect on survival with extensive hepatic necrosis, the inability to initiate or maintain the regenerative response, and decreased survival. This shows the diminished tolerance of small-for-size grafts for additional injury beyond transplantation itself [96, 101].

Although ischemic injury in LDLT is minimized, the amount of critical liver mass required for transplantation in living donation remains in question. Most centers have defined liver mass as graft-to-recipient body weight or as a percentage of the standard liver volume. Unfortunately, no uniform method of measuring or reporting graft volume in relation to the recipient has been established. Clinical experience with living donor and split grafts has led to an accepted lower limit of 0.8% graft-to-recipient body weight or 40% of the standard liver volume [21].

As seen in experimental models, the accumulation of additional stressful stimuli, such as sepsis or renal failure, may push a relatively small graft into failure. Patients with fulminant hepatic failure and those with significant metabolic stress may require more liver volume than stable patients undergoing transplantation under elective conditions [100].

Although it has been performed successfully, many centers are not performing LDLT in acutely ill patients with fulminant failure because of the uncertainty of whether a partial graft has enough volume to support the recovery of such a recipient [81].

The administration of agents that stimulate hepatocyte proliferation by either inducing their entry into the cell cycle or maintaining them as replicative cells may help to shorten the time needed for full organ growth and improve the recovery of both donor and transplant recipient after surgery [6].

A variety of strategies have been developed to enhance liver regeneration after liver transplantation including the injection of growth factors like HGF [102] and hormones like T_3 [103–105]. However, these strategies have only been applied to rats and whether interspecies differences (rats vs. humans) will prevent the application of these approaches on humans will require further experimentation.

Immunosuppression

The clinical implications of inhibition or enhancement of the process of liver regeneration by pharmacological means becomes evident in the setting of the transplantation of grafts with significant injury from prolonged preservation and ischemia and in partial living donor grafts [21].

Within the graft environment, the host immune response must be inhibited to avoid acute allograft rejection, and inhibition of this response may also interfere with the recovery of liver grafts requiring active regeneration of hepatocytes. Glucocorticoids, routinely used in immunosuppression protocols, have been shown to markedly inhibit cell cycle progression in both PH models and in transplant models with ischemic injury [106, 107]. Cyclosporine and tacrolimus may have differential effects on regeneration in a dose-dependent fashion [108, 109]. Sirolimus (rapamycin), with its antiproliferative action, interferes with hepatocyte replication [110].

Rapid hepatocyte replication and smaller liver mass also may interfere with the metabolism and pharmacokinetics of certain drugs. Preliminary studies have shown that LDLT recipients require lower doses of tacrolimus in the early postoperative period than patients receiving whole grafts [111].

Steatosis

In the prereplicative phase of rodent liver regeneration after PH, fatty infiltration of the remnant lobes occurs. Although this phenomenon has been observed for decades, it has received renewed interest lately, as hepatic steatosis is becoming a more prevalent human disease [81].

Steatosis (fatty liver change) occurs with a number of disease processes including diabetes, acute fatty liver of pregnancy and morbid obesity. It also occurs after acute alcohol ingestion or with the use of several medications (e.g. tetracycline, fluconazole and nifedipine) [25].

Hepatic steatosis affects regeneration on several molecular levels. Steatotic livers in rats show delayed mitosis and increased mortality after PH, which may be due to

abnormal TNF and IL-6 signaling [112]. The coordinated induction of JNKs and ERKs is disrupted after PH in fat livers of ob/ob mice (a model for steatohepatitis) with enhanced ATK and inhibition of PEPCK. Cyclin D1 induction is abolished along with STAT3 and reduced ATP levels, which may arrest cell cycle progression [113].

Lipid accumulation has been associated with hepatocyte mitochondrial damage caused by free radical injury from fatty acid oxidation. Abnormalities in the induction of cytochrome P-450 may be one mechanism in the pathophysiology of these findings in fatty livers and may contribute to poor regeneration [114, 115].

Numerous animal studies have shown a marked impairment of regeneration in steatotic livers, as well as the inability to tolerate warm ischemic injury [112]. These findings correlate with the decreased survival of patients with steatotic livers following resection [116] and in clinical transplantation setting, as liver grafts with severe steatosis (>60%) have higher rates of primary nonfunction, higher transaminases and poorer graft survival, possibly as a result of the inability to initiate repair and regeneration mechanisms [117, 118].

Although deceased donor grafts with severe macrovesicular steatosis should therefore not be used, those with less than 30% macro- or microvesicular steatosis can be transplanted with immediate and long-term results similar to those of organs without fat [119]. Marcos et al. [120] showed that the function of right-lobe living donor grafts with up to 30% steatosis did not differ from fat-free grafts, and regeneration seemed not to be impaired, but many living donor programs will not use grafts with greater than 10–20% steatosis, and some centers will not accept any steatosis in living donor grafts [81].

Because donor steatosis affects both susceptibility to ischemic injury and regenerative capacity, some groups have empirically suggested increasing the necessary liver mass by 1% standard liver volume for each percentage of steatosis [7].

Steatohepatitis (inflammation of the liver in the setting of fatty infiltration) carries an even greater risk and should be considered as a contraindication for donation. In those livers, lipid accumulation is associated with hepatocyte mitochondrial damage and abnormalities in cytochrome P450 induction. This is caused by free radical injury from abnormal fatty acid oxidation and the accumulation of dicarboxylic fatty acids during intermediary metabolism. Both donors and recipients would be at risk from liver failure and hepatocyte apoptosis in this setting [81].

However, experimental data have shown that normal fat metabolism plays an important role in the regenerative phase after liver resection and helps rather than hinders regeneration. Peroxisome proliferator-activated receptor- α (PPAR- α), which regulates hepatic lipid turnover in rodents, appears to be necessary for normal liver regeneration. Mice in which the normal accumulation of fat was prevented had a profound deficit in hepatocyte proliferation, suggesting that fat accumulation may serve as a fuelling source for the energy needed by the regeneration process [121, 122].

Donor Age

Liver age is a significant factor in hepatocellular regeneration. Older livers do not regenerate as quickly as younger ones and show delayed regeneration after acute injury. Rodent models have shown a reduced and delayed DNA synthesizing capacity [123], and a clinical study showed a greater graft/standard liver volume in the young donor livers compared with middle-aged and older donor grafts. The older livers also had a higher prothrombin time in the early postoperative period [124].

The graft survival of older living donor grafts is inferior to younger grafts, and whether this is because of a decreased ability to regenerate alongside the other stresses of transplantation is yet to be shown [125].

In the transplantation setting, older grafts have poorer long-term survival when combined with longer cold ischemic times [7].

It must be emphasized that age may affect the regeneration and recovery of the living donor as well as the recipient. Many liver programs limit the upper age limit of the donor, and although no definite age has been specified, 55–60 years old is a generally accepted upper age limit [81].

Viral Hepatitis

The processes of viral replication, alloimmune response, metabolic function and cancer recurrence have all been correlated with cellular replication [81].

Because the majority of liver transplant patients have hepatitis C, it is important to know if this vigorous regenerative response in the partial graft has a significant effect on the kinetics of viral replication in hepatitis C virus (HCV)-positive individuals. Early results suggest that recipients of living-donor liver transplants may have an earlier and more severe recurrence of HCV compared with recipients of whole cadaveric liver grafts [126, 127]. However, this has not been confirmed in other studies [128–130].

Because the primary target cell for HCV replication *in vivo* is the hepatocyte, events that lead to hepatocyte proliferation may enhance HCV replication [131].

Investigators have noted that HCV RNA replication is enhanced in proliferating cells, suggesting that viral replication is regulated by cell cycle-dependent factors [132, 133].

Other Factors

There is clinical and experimental evidence that several other factors may influence the regenerative response after transplantation.

Increased portal venous flow has been implicated in a more rapid regeneration. However, increased portal pressure, as encountered in small-for-size grafts when the full portal vein flow has to traverse through a much-reduced liver size, the pressure building up in the portal vein effectively shuts down the flow through the portal arterioles and the liver becomes dearterialized leading to graft failure [134].

Poor hepatic venous drainage has been shown to inhibit regeneration and segments with poor venous drainage become atrophied with time. Female gender may have a positive effect on the regeneration of partial grafts in murine models, but no human study has confirmed this finding [81].

Donor Liver Regeneration

With the recent advent of cadaveric split and LDLT, the opportunity has arisen to study liver regeneration in humans more closely [135].

The most common question asked by potential donors is, 'When will my liver grow back and will it have normal function?'

Several reports have shown that the remnant liver of the donor grows more slowly than the transplanted portion and may not regenerate to the full volume that it had before the operation, and that ultimately it reaches approximately only 85–90% of the mass of the donor's original liver even after 1 year [135–138]. This is contrary to what was previously believed and different from rodent models [7]. However, most of the donors achieved normal liver synthetic function within 1 postoperative week and without complications [137, 138].

Rapid recipient liver regeneration may be related to high liver blood flow after LDLT because of the persistence of a hyperdynamic state, immunosuppressant administration (cyclosporine and tacrolimus stimulate liver

regeneration) or humoral factors in the recipient (HGF, IL-6 and TNF- α) [138].

Due to the fact that liver function is completely restored in the donor by 1 month after transplantation, it is possible that there is a lack of growth stimulatory signals as additional mass is not required to sustain normal hepatic function. On the other hand, it is likely that although it is capable of functioning adequately under normal conditions, the incompletely regenerated donor liver may have a deficit in its reserve capacity [135].

Conclusions

Liver transplantation has opened an exciting opportunity to study liver regeneration in humans. Several factors affect liver regeneration after transplantation such as ischemic injury, graft size, immunosuppression, steatosis, donor age and viral hepatitis. A combination of radiological imaging, liver biopsies and plasma measurements could provide significant data which would lead to a better understanding of the factors and pathways that control liver regeneration after liver transplantation; this in turn could be used to enhance liver regeneration after transplantation and to solve clinical problems such as liver failure in cases of small-for-size grafts.

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Disclosure Statement

The authors declare no conflict of interest.

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