Liver progenitor cells and therapeutic potential of stem cells in human chronic liver diseases

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Abstract

Liver progenitor cells, thought to reside in the terminal bile ductules (canals of Hering) at the interface between portal tracts and liver lobule, proliferate during severe hepatic injury. They may contribute to hepatocyte regeneration, or even take over this role if the liver injury is severe and associated with an impairment of hepatocyte proliferation. They represent promising targets in an attempt to stimulate liver regeneration in chronic diseases. Recent studies on liver progenitor cell recruitment in response to injury in chronic viral hepatitis B, hepatitis C, alcoholic and non-alcoholic liver diseases are presented in this review, as well as clinical trials in which stem cells are administered as a therapeutic intervention to promote liver regeneration. Liver progenitor cell expansion is part of the disease process itself and may contribute to disease severity, mainly related to fibrosis. As the majority of these progenitor cells tend to acquire a biliary phenotype, their role in liver repair and improvement in liver function remains to be addressed. Present data on stem cell therapy are heterogeneous in terms of methods and endpoints ; thus, results need to be carefully examined prior to drawing a conclusion on possible benefits. (Acta gastroenterol. belg., **2013**, 76, **3-9**).

Key words : liver regeneration, liver progenitor cell, stem cell, bone marrow, CK7, chronic liver disease.

Introduction : liver regeneration

Hepatocytes are the major parenchymal cells, accounting for 80% of the organ volume, with important physiological roles. Morbidity and mortality associated with chronic liver disease are mostly related to hepatocyte loss and consequent liver insufficiency. Therefore, strategies that may promote liver regeneration should be explored.

Three mechanisms are responsible for hepatocyte regeneration (Fig. 1). First, hepatocytes themselves represent the main source for hepatocyte renewal. Mitotically quiescent under normal conditions, mature hepatocyte division is responsible for hepatic regeneration following liver injury with cell loss (1). Hepatocytic replication can be assessed reliably histologically by Ki67 immunolabelling, a cell cycle proliferative marker (detected by the MiB1 antibody), which demonstrates rare or no nuclear staining in normal liver whereas immunostaining positivity in hepatocytes is observed in cases of chronic hepatitis or cirrhosis (2).

Second, the adult liver contains liver progenitor cells (LPC), representing small cells relative to hepatocytes, with an oval shape (for this reason, these cells are also called oval cells in rodents). In case of massive necrosis or continuous injury, hepatocytes may be unable to provide full regeneration. Thus, LPC are mobilized and proliferate, increasing in number and differentiate into ductular cells and intermediate hepatocyte-like cells (Fig. 2) (3,4). The LPC niche is located within or next to Hering's canal which connects the portal bile duct and the bile canaliculus formed by apical surfaces of adjacent hepatocytes (3,4). To date, there is no direct evidence of further differentiation of those LPC into mature hepatocytes in humans. However, in mice, interesting data obtained by cell tracking experiments are available using either the expression of osteopontin or high-mobility group transcription factor Sox9 (expressed in the ductal plate and in bile duct cells) to trace LPC (5,6) or the adenoassociated virus serotype 8 (exhibiting a tropism for hepatocytes) to trace hepatocytes (7). Those cell tracking experiments have been performed in various conditions of liver injuries and data proof that LPC can differentiate into mature hepatocytes in mouse models of chronic liver injuries. However, the number of hepatocytes derived from LPC in these conditions is very low, the turnover of mature hepatocytes being responsible for the huge majority of the maintenance of the liver mass. Conflicting results exist on the contribution of LPC to new hepatocytes in the normal liver, after partial hepatectomy and in case of acute hepatic injury (5-7). By immunohistochemistry, LPC demonstrate a strong positive expression of cytokeratin (CK) 7, CK19, epithelial cell adhesion molecule (EpCam) and neuronal cell adhesion molecule (NCAM),

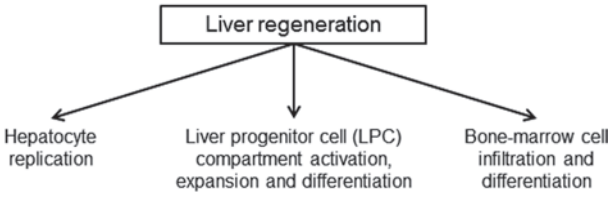

Fig. 1. — Mechanisms of liver regeneration

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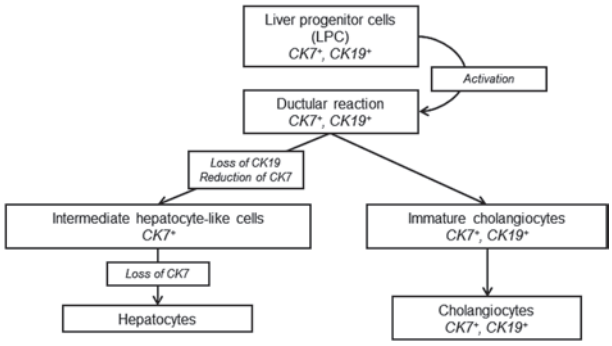

Fig. 2. — Models of LPC differentiation towards hepatocytes or cholangiocytes.

a low expression of albumin and a very low expression of alphafoetoprotein. However, this immunohistochemistry pattern is not completely specific as there may be some overlapping with non-progenitor biliary cells (4,8,9) (Fig. 2). To stain LPC, other scientists use the expression of enzymes expressed by foetal hepatocytes and oval cells such as the isoform M2 of pyruvate kinase (M2-PK) (10,11) or the π-class glutathione S-transferase (π-GST) (9,12).

The third source proposed for hepatocyte renewal is extrahepatic stem cells. Indeed, hepatocytes with a Y chromosome are found in livers from female patients who received bone marrow transplantation from a male donor (13). Furthermore, authors demonstrate, under specific in vitro conditions, the hepatic differentiation potential of CD34 positive cells from the bone marrow of human healthy donors (14). Those data suggest that adult human hepatocytes can derive from stem cells originating outside the liver, such as the bone marrow.

In this review, we describe recent studies exploring LPC compartment activation in several chronic human liver diseases including viral hepatitis B, viral hepatitis C, alcoholic and non-alcoholic liver diseases (Table 1). In addition, we report trials investigating LPC or stem cells as possible therapeutic strategies for liver regeneration (Table 2).

Hepatitis B

To date, LPC in hepatitis B have been studied in trials on chronic liver diseases with multiple causes including hepatitis B or on small groups of hepatitis B infected patients. A population of 55 patients with viral disease (with or without cirrhosis) was enrolled and 13 patients of the 55 were infected with the hepatitis B virus (15). The data suggest that inflammation may constitute a stimulus for LPC expansion. Indeed, LPC were detected in 34/55 patients and associated with severity of inflammatory cells infiltration within the liver parenchyma. Similarly, cells that stained positive for CK7, although less intense than LPC, and sharing some morphological features of hepatocytes, were closely located to the inflammatory reaction. This sign of LPC differentiation towards hepatocyte like cells in hepatitis B is also evidenced in another study with hepatitis B related cirrhosis (16) : although the majority of the cells positive for CK7 and CK19 were biliary and intermediate cell types, some CK7 positive cells with a hepatocyte morphology were also observed. Another study suggests that the hepatitis B virus itself may induce proliferation of LPC (17).

Table 1. — **Studies exploring LPC activation in human chronic liver diseases**

Author (year)	Number of cases	Liver disease (number of patients)	Staining for LPC	Ref.
Libbrecht (2000)	55	Viral hepatitis : hep B (13), hep C (38), hep B + hep C (4)	CK7, CK8, CK18, CK19	15
Wu (2009)	16	Hep B cirrhosis	CK7	17
Clouston (2005)	115	Hep C	CK7	21
Lowes (1999)	45	Hep C (15), hemochromatosis (15), alcoholic liver disease (15)	CK19, M2-PK, π-GST	11
Sancho-Bru (2012)	59	Alcoholic steatohepatitis	CK7, CK9, EpCam	24
Richardson (2007)	107	NAFLD : Simple steatosis (22), steatosis and non-specific inflammation (16), NASH (69)	CK7	31
Chiba (2011)	48	NAFLD : Brunt fibrosis stage 1 (15), stage 2 (13), stage 3 (12), stage 4 (8)	CK7, CK19	32

Hep B : hepatitis B ; hep C : hepatitis C ; NAFLD : non-alcoholic fatty liver disease ; NASH : non-alcoholic steatohepatitis ; CK : cytokeratin ; M2-PK : M2 of pyruvate kinase ; π-GST : π-class glutathione S-transferase ; EpCam : epithelial cell adhesion molecule.

Table 2. — **Trials on stem cells in human chronic liver diseases**

Author (year)	Number of patients	Controls ? (number)	Liver disease (number)	Protocol	Cell infusion route	Finding	Ref.
Kharaziha (2009)	8	No	Cirrhosis (8) : hep B (4), hep C (1), other (3)	BMCA + culture	Portal vein or peripheral vein	Decrease in MELD score after 24 weeks	18
Peng (2011)	158	Yes (105)	Decompensated hep B (158)	BMCA + culture	Hepatic artery	Transient improvement in MELD score	19
Terai (2006)	9	No	Cirrhosis (9) : hep C (5), hep B (3), unknown (1)	BMCA	Peripheral vein	Slight decrease in Child-Pugh score after 24 weeks	23
Yannaki (2006)	2	No	Abstinent alcoholic cirrhosis (2)	G-CSF + PBCA	Peripheral vein	Decrease in MELD score	25
Pai (2008)	9	No	Abstinent alcoholic cirrhosis (9)	G-CSF + PBCA + culture	Hepatic artery	Decrease in serum bilirubin	26
Saito (2011)	10	Yes (5)	Abstinent alcoholic cirrhosis (10)	BMCA	Peripheral vein	Improved liver function during 24 weeks	27
Spahr (2008)	24	Yes (11)	Alcoholic steatohepatitis (24)	G-CSF (5 days)	/	LPC proliferation, increase in circulating $CD34^+$ cells	28
Garg (2012)	47	Yes (23)	Acute on chronic liver failure (47) : alcoholic steatohepatitis (27), other causes (20)	G-CSF (one month)	/	Increase in $CD34^+$ liver cells, decrease in MELD score, increased 60-day survival	29

Hep B : hepatitis B ; hep C : hepatitis C ; BMCA : bone marrow cell aspiration ; G-CSF : granulocyte colony stimulating factor ; PBCA : peripheral blood cell aspiration ; LPC : liver progenitor cell.

In 16 patients with end-stage cirrhosis and positivity for hepatitis B surface antigen, the ductular reaction and CK7 staining were higher in serum HBV-DNA detectable group compared to serum HBV-DNA undetectable patients. However, the impact of viral load on LPC expansion remains to be confirmed, all cases being also characterized by severe hepatic inflammation (17).

The therapeutic impact of bone marrow stem cells for liver regeneration in hepatitis B has been studied. In a small uncontrolled trial (18), 8 patients, four of whom with hepatitis B-related cirrhosis, received autologous stem cells. Cells originated from the bone marrow and were cultured with hepatocyte growth factor and reinjected into the peripheral or portal vein after 2 months without adverse event. For the total of 8 patients enrolled in this study, the MELD score decreased after 24 weeks. However, the role of stem cell injection on this clinical amelioration remains to be addressed, as the natural history of hepatitis is variable and no control group was included. The autologous transplantation of bone marrow stem cells was further studied on 53 patients with hepatitis B-induced liver failure and compared to 105 matched patients (19). After bone marrow aspiration, mononuclear cells were isolated and cultured, then identified by flow cytometry and slowly reinjected in the hepatic artery, without complication. The transplanted group had a transient improvement of MELD score at week 3 after transplantation compared to the non-transplanted group. There was no difference in the serum ALT levels between the two groups and no significant difference in the long term follow up, and in particular no elevation in the incidence of hepatocellular carcinoma in a 192-week follow up. This is of particular interest as hepatic liver progenitor cells have also been implicated in tumorigenesis (20).

In conclusion, data available for hepatitis B infection suggests that LPC expansion occurs during disease progression, concurrent with liver inflammation. The majority of cells positive for cytokeratin markers demonstrates a biliary phenotype but some hepatocyte like cells are found, suggesting that LPC may contribute to hepatocyte regeneration. Data from a controlled trial on injection of bone marrow cells demonstrate a significant, although transient, improvement in liver function during follow-up compared to standard medical therapy.

Hepatitis C

Hepatitis C virus infection and its relation with progenitor cell expansion is well described in a study on 115 infected patients (21). Compared to normal liver, CK7 positive ductular reaction starting at the periphery of the portal bile ducts, as well as isolated CK7 positive cells, called hepatic progenitor cells, were observed in liver

with hepatitis C infection. The number of LPC and the extent of the ductular reaction were correlated with fibrosis stage, steatosis and inflammation. As mentioned earlier, this LPC proliferation occurred when hepatocyte replication was impaired. Indeed, higher CK7 positive cells were associated with hepatocyte replicative arrest, as evaluated by immunohistochemistry (low Ki67 and high p21 positive hepatocytes). No relationship could be observed between genotype and the intensity of the ductular reaction. The increased number of LPC detected either by staining for M2-isozyme of pyruvate kinase, CK19 or π-class glutathione S-transferase in response to progressive fibrosis has also been described in 15 infected patients (11). In this study, the isoform M2 of pyruvate kinase, expressed by fetal hepatocytes and oval cells (10), is presented as the most reliable marker for LPC as it does not stain ductal cells. Interestingly, the same increase in LPC number in relation to the fibrosis stage is described in patients with hemochromatosis or alcoholic liver disease (11), suggesting that their proliferation may be related to disease severity, rather than to the cause of liver disease. The concept that some liver cells may take their origin from outside the liver is suggested by data on autopsy and liver biopsy specimens from male patients with liver transplantation from female donors. The peak value of Y-positive hepatocytes and cholangiocytes was identified in a case of fibrosing recurrent hepatitis C (22).

In contrast with hepatitis B infection, stem cell therapy in hepatitis C has only been tested in a non-controlled and non-hepatitis C specific trial. On a total of 9 cirrhotic patients treated by autologous bone marrow cell infusion therapy, 5 patients had hepatitis C. Mononuclear cells were collected from the ilium and reinfused in a peripheral vein. Except fever, no other adverse event was noted. The patients were observed during 24 weeks, and at this time, the mean Child Pugh score was slightly decreased compared to baseline Child Pugh score. The authors suggest that bone marrow cell therapy induced hepatocyte proliferation as in some patients, a liver biopsy performed 4 weeks after the therapy showed an increase in alpha-foetoprotein (AFP) and proliferating cell nuclear antigen (PCNA) liver expression compared to the expression on the baseline liver biopsy (23).

In conclusion, LPC expansion in hepatitis C is related to fibrosis progression, steatosis, inflammation and impairment in hepatocyte replication. Data on bone marrow cell therapy is available from a small cohort of patients with various causes of cirrhosis including hepatitis C and must be interpreted with caution.

Alcoholic liver disease

Liver progenitor cells were analysed in a recent study on 59 patients with biopsy-proven alcoholic steatohepatitis (24). In these patients who had a liver biopsy performed early after hospital admission, an important LPC expansion is observed. Indeed, by microarray analysis, genes related to LPC and ductular reaction (CK7, CK19 and EpCam) are upregulated compared to healthy samples. The staining for CK7 and EpCam were correlated with the liver mRNA expression. However, by immunohistochemistry, the majority of EpCam positive cells were intermediate hepatobiliary cells and not hepatocyte like cells. In the cohort, 41% of the patients were treated with corticosteroids and the 3-month mortality was 27%. The CK7 expression, evaluated by polymerisation chain reaction (PCR), was significantly higher in patients who died after 3 months compared to those that survived. CK7 was positively correlated with MELD score, the morphometric quantification of fibrosis, but not with the degree of inflammation. This study identified LPC expansion as a marker of disease severity and poor prognosis.

Some trials are available on regenerative medicine in alcoholic liver disease. Two patients with decompensated alcoholic cirrhosis were treated with an intravenous infusion boost of peripheral CD34 positive stem cells obtained by several leukaphereses, after stimulation by granulocyte colony-stimulating factor (G-CSF) (25). An improvement in MELD score was noted in these two patients after the two treatments (G-CSF and infusion). Nine other patients with alcohol related cirrhosis were treated with infusion of hematopoietic stem cells collected by leukapheresis after G-CSF mobilization, and cultured for amplification before readministration. Those treatments were associated with a decrease in serum bilirubin during the 3-months follow-up (26). However, the biological amelioration observed during the follow up in these two studies can not be attributed to the sole cellular therapy, such improvement being possible in alcoholic liver disease following abstinence. Ten subjects with advanced abstinent alcoholic cirrhosis were enrolled in a recent controlled study. The five subjects who received autologous bone marrow (from the ilium) infusion (via the cubital vein) had a significantly higher serum albumin, prothrombin time and total protein during the 24 week-follow up than controls (27). It is of note that the daily administration of G-CSF for 5 days, without bone marrow cell injection, is able to induce the endogenous LPC proliferation, as proved by the increased number of double positive Ki67-CK7 positive cells at day 7 in a randomized clinical trial on 24 patients with alcoholic steatohepatitis (28). However, this was not associated with an improved liver function over a 4-week observation period. Another large randomized clinical trial studying the effects of G-CSF focuses on patients with acute on chronic liver failure (23 patients in the G-CSF group and 24 patients in the placebo group). The aetiology of the acute event and the underlying chronic disease was alcohol in more than 50% of the cases. The administration of G-CSF subcutaneously over a period of one month (daily doses for the first 5 days and then every third day) was associated with a significant increase in CD34 positive cells in the liver after one month, a

significant and progressive improvement of MELD score and a better 60-day survival compared to placebo (29).

In conclusion, LPC expansion is an important feature of decompensated alcoholic liver disease including alcoholic steatohepatitis and is correlated with fibrosis and associated with a poor liver function. The administration of bone marrow cells in abstinent alcoholic liver cirrhosis is associated with amelioration of liver function in a small controlled trial. Interestingly, repeated administration of G-CSF alone is associated with an improved 2-month survival. Such treatment has shown to be able to stimulate LPC proliferation and increase liver CD34 cell population. However, the expression of CD34 within the liver cannot be solely attributable to bone marrow originating cells, as liver sinusoidal endothelial cells for example also express CD34 in chronic liver diseases (30).

Non-alcoholic fatty liver disease

There are two large trials on LPC localisation and number in non-alcoholic fatty liver disease. The first trial concerns 107 patients with non-alcoholic fatty liver disease (NAFLD) ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) (31). Interestingly, the number of LPC was increased in simple steatosis compared to normal liver. Furthermore, the ductular reaction was correlated with the stage of fibrosis and the grade of portal inflammation. The ductular reaction was proved to be related to the extent of hepatocyte replicative arrest, assessed by the hepatocyte nuclei staining for p21. This liver regeneration blockade was associated with insulin resistance. The LPC compartment has also been studied in a second large trial on 48 patients with NAFLD (32). Located in the fibrotic portal tract and fibrous septa, CK19-positive cells were increased in number in relation with fibrosis progression as assessed by Brunt fibrosis staging and Sirius red staining, and in relation with portal inflammation and hepatocellular ballooning. In this study, there was no relation between LPC and steatosis or lobular inflammation. However, the progression of fibrosis was associated with LPC senescence as shown by the increased p21 expression in the nuclei of CK19-positive cells and with the appearance of CK7 positive CK19 negative cells located in the hepatic parenchyma. In addition, the expression of macrophage chemoattractant protein 1 (MCP-1) was increased in the ductular reaction of advanced fibrotic steatotic livers compared to normal livers. Interpreted as a pro-fibrogenic factor attracting hepatic stellate cells (32), the role of MCP-1 in the modulation of the microenvironment and the possible macrophage attraction remains to be evaluated, in the light of recent experimental data on macrophage precursor therapy improving liver regeneration (33). Conversely, these two studies do not provide any evidence of hepatocyte-like cells. Furthermore, they suggest that LPC could have pro-fibrogenic effects. To date, there is no trial on regenerative medicine using bone marrow cells in NAFLD/NASH.

Conclusion and prospects for future research

In summary, we report here general considerations on LPC characterisation and on the therapeutic use of circulating or bone-marrow derived stem cells in human chronic liver diseases. Although trials available have raised some questions, many crucial points remain to be addressed, thereby constituting prospects for future research.

A correlation between immunostaining for LPC and other histological and clinical data is described in liver diseases of various aetiologies. In chronic liver diseases, hepatocyte replicative arrest and fibrosis development have been associated with the expansion of LPC. Liver progenitor cells may differentiate into biliary like cells or hepatocyte like cells. According to data issued from various liver diseases, the majority of LPC demonstrates immunostaining pattern of a biliary phenotype. A number of CK7 positive cells with the morphological characteristics of hepatocytes have been reported in hepatitis B, hepatitis C and alcoholic liver disease but not in NAFLD. Nevertheless, in humans, the exact role and the participation of these activated and proliferative LPC to human liver regeneration and liver function remain unknown. LPC proliferation being associated with disease severity and hepatocyte proliferative capacity arrest (34), one may ask the naïve question of the release of eventual worsening (profibrogenic, other ?) factors from LPC.

There is a great interest for stem cell therapy in the medical community, and in particular among hepatologists. Indeed, liver transplantation is facing problems including organ shortage, side-effect and costs of immunosuppressive therapy. Many recent reports are published exploring the impact of circulating or bone-marrow stem cell mobilisation and/or infusion in chronic liver diseases. However, a great heterogeneity exists in terms of patient selection (single disease aetiology versus multiple causes), methods of cells collection (from the blood or from the bone marrow, use of eventual mobilization by G-CSF), cell infusion technique (number of cells used for transplantation, administration either peripherally or directly into the hepatic circulation, through the artery or the vein) and follow up (Fig. 3). In addition, the majority of clinical studies on stem cell therapy in liver diseases are small and uncontrolled. Data from a controlled trial in hepatitis B demonstrated a significant but transient benefit in terms of liver function, but further studies are needed in other liver diseases. Such investigations are of great importance as the natural history of cirrhosis is variable, and the amelioration over time for some patients is possibly due, for example, to previous medication (35), to standard of care management or to abstinence from alcohol.

Contrary to the experimental use of bone-marrow or circulating stem cells, LPC infusion, evaluated in animal experiments (36), has not been tested in humans, due to difficulties for isolation, culture and administration (37). However, interesting reports proof that the administration

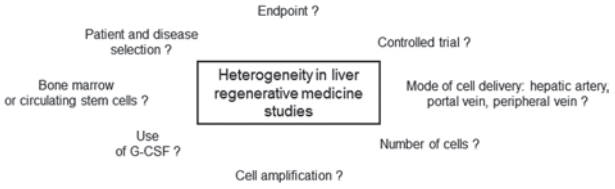

Fig. 3. — Various methods in stem cell therapy in liver diseases

of G-CSF in humans is able to stimulate LPC proliferation (28) and, if repeated, to ameliorate the liver function in the setting of acute on chronic liver failure (29). Awaiting results of large controlled trials on the use of bone-marrow stem cells, progress can be made in the comprehension or the discovering of signals that will favour or block hepatocyte replication and/or hepatocyte differentiation of LPC. Indeed, robust evidence exists proving the presence of LPC expansion in various liver diseases. The reason why this LPC presence is associated with poor prognosis and does not lead to full restoration of liver function needs further studies, as well as the precise mechanisms potentially involved in liver regeneration and repair. Finally, experimental data exploring the role of LPC interaction with liver matrix deposition or other hepatic cell types such as hepatic macrophages introduce the concept of the role of the microenvironment in the differentiation of LPC (33,38-40) and may provide opportunities for further development in regenerative medicine.

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