

## REVIEW

## Liver Regeneration in Chronic Liver Injuries: Basic and Clinical Applications Focusing on Macrophages and Natural Killer Cells

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## SUMMARY

This review summarizes the role of macrophages and NK cells in liver progenitor-like cell (LPLC)-induced liver regeneration during chronic liver injuries. Macrophages are beneficial to the proliferation and differentiation of LPLCs. NK cells can promote LPLC-induced liver regeneration, but overactivation may lead to high serum levels of IFN- $\gamma$ , thus inhibiting liver regeneration.

**BACKGROUND & AIMS:** Liver regeneration is a necessary but complex process involving multiple cell types besides hepatocytes. Mechanisms underlying liver regeneration after partial hepatectomy and acute liver injury have been well-described. However, in patients with chronic and severe liver injury, the remnant liver cannot completely restore the liver mass and function, thereby involving liver progenitor-like cells (LPLCs) and various immune cells.

**RESULTS:** Macrophages are beneficial to LPLCs proliferation and the differentiation of LPLCs to hepatocytes. Also, cells expressing natural killer (NK) cell markers have been studied in promoting both liver injury and liver regeneration. NK cells can promote LPLC-induced liver regeneration, but the excessive activation of hepatic NK cells may lead to high serum levels of interferon- $\gamma$ , thus inhibiting liver regeneration.

**CONCLUSIONS:** This review summarizes the recent research on 2 important innate immune cells, macrophages and NK cells, in LPLC-induced liver regeneration and the mechanisms of liver regeneration during chronic liver injury, as well as the latest macrophage- and NK cell-based therapies for chronic liver injury. These novel findings can further help identify new treatments for chronic liver injury, saving patients from the pain of liver transplantations. (*Cell Mol Gastroenterol Hepatol* 2022;14:971–981; <https://doi.org/10.1016/j.jcmgh.2022.05.014>)

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The liver is a unique organ because of its strong regeneration ability when it is damaged. After the injury occurs, it can often restore its original size and function in a short period of time.<sup>1</sup> Liver regeneration can be more precisely defined as compensatory hyperplasia in which the remaining liver tissue expands to meet the metabolic demands of the body. In cases of partial hepatectomy (PHx) and

acute liver injury (ALI), the damaged area can be replaced by the replication of existing hepatocytes.<sup>2–4</sup> The underlying mechanisms have been finely described.

In contrast, the regeneration in chronic liver diseases is usually involved with liver progenitor-like cells (LPLCs) and various immune cells and often accompanied by extensive necrosis and apoptotic areas,<sup>4,5</sup> thus requiring deep understanding in LPLC-induced regeneration. LPLCs, which are potentially residing in the canals of Hering, are activated in the context of severe chronic liver injury. After activation, LPLCs can proliferate into both hepatocytes and cholangiocytes, thus inducing liver regeneration.<sup>6</sup> Because liver injury that accompanies LPLC responses is often associated with inflammation and fibrosis, scientists have researched the interaction between LPLCs and immune cells. This review aims to summarize and clarify the role of 2 important subsets of innate immune cells, macrophages and natural killer (NK) cells, in LPLC-induced liver regeneration during chronic liver injury, thus providing new insights into potential new treatments for chronic and severe liver diseases.

## Liver Regeneration in Chronic Liver Diseases

With a strong ability to regenerate, the liver can restore its original size and function within a short period of time. When part of the liver parenchyma is lost or damaged, such as PHx or ALI, the remaining hepatocytes can quickly enter the cell cycle to supplement and regenerate the liver, which is called compensatory proliferation. Under these circumstances, Wang

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**Abbreviations used in this paper:** ALI, acute liver injury; BEC, biliary epithelial cell; BMDM, bone marrow-derived macrophage; FGF, fibroblast growth factor; Fn14, fibroblast growth factor-inducible 14; HGF, hepatocyte growth factor; Hh, hedgehog; HSC, hepatic stellate cell; HybHP, hybrid hepatocytes; IFN, interferon; IL, interleukin; MH, mature hepatocyte; natural killer, natural killer; NKT cells, cells expressing NK and T-cell markers; PHx, partial hepatectomy; PTEN, phosphatase and tensin homolog; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; TWEAK, tumor necrosis factor-like weak apoptosis inducing factor.



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et al<sup>7</sup> reported that the self-renewing cells adjacent to the central vein of the liver lobule, Wnt-responsive Axin2<sup>+</sup> hepatocytes, could subserve homeostatic hepatocyte renewal. These specific hepatocytes express stem cell marker Tbx3, which is widely expressed in early liver hepatoblasts and is important for the initiation of hepatocyte differentiation,<sup>8,9</sup> thereby exhibiting unipotent stem cell-like characteristics. However, with a deeper investigation in Axin2<sup>+</sup> hepatocytes, Sun et al<sup>10</sup> demonstrated that the liver regeneration induced by PHx was enabled by the proliferation of hepatocytes throughout the liver, rather than by a pericentral population described before. In detail, hepatocytes throughout the liver could up-regulate Axin2 after PHx and ALI, thus contributing to the liver regeneration. This controversy may be due to the use of Axin2 heterozygous mice model, which would result in the proliferation of Axin2<sup>+</sup> hepatocytes over time. No matter which type of hepatocytes is responsible for the restoration, the liver regeneration after PHx and ALI only induces the proliferation of residual hepatocytes to restore liver mass. However, when chronic injury leads to acute loss of a large number of parenchymal tissues or exhausted hepatocyte proliferation ability, the regeneration process during chronic liver diseases was not mediated by the remaining hepatocytes. According to the severity of the chronic liver injury, 2 types of cells can be alternatively activated. Under severe or chronic injury conditions most hepatocytes become unresponsive. Font-Burgada et al<sup>11</sup> have identified a novel hepatocyte population and name hybrid hepatocytes (HybHP), which constitutively reside in portal triads of healthy liver and express low amounts of Sox9 and normal amounts of HNF4 $\alpha$ . They show higher repopulation potential than conventional hepatocytes without originating hepatocellular carcinoma.<sup>11</sup> However, if the injuries persist and the proliferation of HybHP and other hepatocytes fail to restore the liver, LPLCs with positive biliary epithelial cell (BEC) markers will be alternatively activated.<sup>5</sup> The persistent chronic liver injury will severely impair the hepatocyte proliferation ability through excess inflammation, scarring, and epithelial abnormalities.<sup>4</sup> Also, when the damage become extensive and sustained, HybHP will be killed, no longer contributing to the regeneration process.<sup>11</sup> Thus, the alternatively activated LPLCs play an essential role in severe chronic liver injury. LPLCs have bipotent capacity to differentiate into both mature hepatocytes (MHs) and BECs with a capacity for duct formation.<sup>12</sup> In addition, the *in vivo* injection of dedifferentiated LPLCs *in vitro* has been proved to repopulate chronically injured liver tissue with high efficiency.<sup>13</sup> According to the studies conducted by Shin et al,<sup>14,15</sup> Forkhead box L1 LPLCs and their descendants are required for the development of hepatocytes after chronic liver injuries, especially contributing to the liver parenchyma during the recovery phase. During the injury phase, LPLCs are responsible for the supply of newly formed hepatocytes, which can remove excessively accumulated fat.<sup>15</sup>

### **Activation and Expansion of Liver Progenitor-Like Cell-Induced Liver Regeneration**

The activation and expansion of LPLCs have been detected in patients with various liver diseases such as

alcoholic and nonalcoholic liver disease,<sup>16</sup> chronic viral hepatitis,<sup>17,18</sup> and cholestatic hepatitis.<sup>19</sup> In humans, the combination of hepatocyte loss and impaired hepatocyte proliferation is required to activate the proliferation of LPLCs, because there has been discovered an inverse correlation between the number of LPLCs and the number of hepatocytes expressing Ki67.<sup>20</sup> On the basis of the characteristic markers for LPLCs (CK19, EPCAM, Foxl1, Sox9, NCAM), the possible origins of LPLCs include BECs, hepatoblasts in fetal liver, bone marrow cells, and hepatic stellate cells (HSCs).<sup>21,22</sup>

Hedgehog (Hh) ligands are LPLCs proliferation activators. With the increase in death rate of hepatocytes, dying hepatocytes in chronic liver injuries have been proved to generate Hh ligands. Because Hh ligands are viability factors for LPLCs and myofibroblasts, they can then activate LPLC-induced liver regeneration but also induce fibrogenesis.<sup>23</sup> In addition to its direct effect on LPLCs, Hh ligands also have capacity to indirectly enhance the proliferation of LPLCs by activating HSCs into matrix-producing myofibroblasts.<sup>24</sup> Ruddell et al<sup>25</sup> have suggested that lymphotoxin beta receptor on HSCs may be involved in paracrine signaling with nearby lymphotoxin beta receptor-expressing LPLCs, which is consistent with earlier research that identified the paracrine signaling between HSCs and LPLCs.<sup>26</sup>

Several studies have demonstrated the important role of tumor necrosis factor-like weak apoptosis inducing factor (TWEAK) pathway in the initiation and expansion of LPLCs.<sup>27-30</sup> Because TWEAK has no effect on mature cells, it has a selective mitogenic effect for LPLCs, stimulating the proliferation of LPLCs through its receptor fibroblast growth factor-inducible 14 (Fn14).<sup>27</sup> In a choline-deficient ethionine-supplemented diet mouse model of chronic liver injury, LPLC numbers reduced significantly in Fn14<sup>-/-</sup> mice. The stimulation of TWEAK led to the activation of nuclear factor kappa B and dose-dependent proliferation of LPLCs, indicating that TWEAK can act directly and stimulate LPLC mitosis in an Fn14-dependent and nuclear factor kappa B-dependent fashion.<sup>28</sup> On the basis of the detected mRNA expression of TWEAK in monocyte and macrophage cell population,<sup>31,32</sup> Kupffer cells or infiltrating macrophages are believed to be the source of endogenous TWEAK. Because of the fact that TWEAK blockade or the Fn14 deficiency does not completely inhibit the proliferation of LPLCs,<sup>27</sup> other pathways appear to be involved in activating their proliferation.

Considering the associated inflammation and fibrosis in LPLC-induced liver regeneration, immune cells and fibroblastic cells surrounding LPLCs are determined important in regulating the process. Cell-to-cell interactions involve paracrine growth factors that can be grouped into several major families,<sup>33</sup> among which the fibroblast growth factor (FGF) family has been identified to be related to the epithelial morphogenesis, repair, and cyto-protection.<sup>34</sup> As a member of FGF family, FGF7 expression has been proved to induce concomitantly with LPLC response in mouse models of liver injuries, identifying FGF7 as a potential therapeutic target for liver diseases. In addition, FGF9 is expressed in hepatocytes, serving as a mediator of hepatocyte

proliferation. Liu et al<sup>35</sup> have demonstrated that LPLCs can be induced to differentiate into hepatocyte-like cells in the presence of FGF9, as well as hepatocyte growth factor (HGF), suggesting the role of FGF family not only in the initiation of LPLC but also in the differentiation of LPLC into hepatocytes.

### *Differentiation of Liver Progenitor-Like Cell-Induced Liver Regeneration*

With the bipotent capacity to differentiate into both MHs and BECs, inducing LPLCs to differentiate into hepatocytes is another important process in LPLC-induced liver regeneration. HGF was originally characterized as a potent mitogen for MHs,<sup>36</sup> with its biological effects mediated by a single tyrosine kinase receptor c-Met.<sup>37</sup> It is not surprising to find that HGF/c-Met signaling acts as an essential pathway for the differentiation of LPLCs and is required for the induced liver regeneration.<sup>38</sup>

The antagonistic interplay between Wnt and Notch signaling has been reported to crucially affect liver regeneration in fibrotic liver.<sup>39,40</sup> The balance of these 2 signaling can determine the direction of LPLC differentiation. Playing a crucial role in the regeneration of chronic liver diseases, the Wnt signaling has been studied in various mouse models.<sup>39,41-47</sup> During hepatocyte regeneration, macrophage derived Wnt3a maintains Numb expression, thereby inhibiting Notch signaling.<sup>41,42</sup> With its direct influence on LPLCs, Wnt3a secreted from macrophages can promote the differentiation toward hepatocytes.

Other pathways involved in the activation and expansion of LPLCs have also been researched, including thyroid hormone signaling,<sup>48,49</sup> HIPPO/YAP pathway,<sup>50-53</sup> and Jag1/Notch signaling.<sup>54-57</sup> The various roles of above-mentioned pathways in LPLC initiation and proliferation are summarized in [Figure 1](#).

### *Macrophage in Liver Progenitor-Like Cell-Induced Liver Regeneration*

Because inflammation has been demonstrated to initiate tissue repair by elimination of the causes of injury such as infectious agents and necrotic cells,<sup>58,59</sup> increasing number of studies have been carried out to identify the roles of immune cells in chronic liver injury-induced liver regeneration ([Figure 2](#)). In response to liver injury, liver tissues may initiate the activation of various immune cells, among which the macrophages play a predominant role. In addition to the liver-resident macrophages, also named Kupffer cells, which represent nearly 20% of the liver nonparenchymal cells, hepatic macrophages may also be derived from infiltrating blood monocytes.<sup>60,61</sup> As the immune barrier of liver tissue, Kupffer cells can influence other immune cells through complex cell-cell interactions and secretion of cytokines.<sup>62</sup>

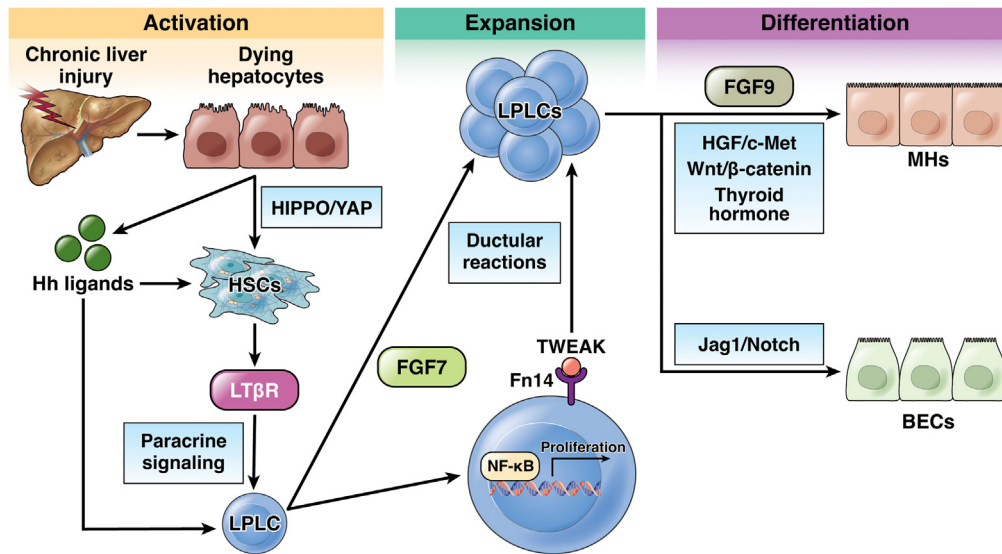
Macrophages influence both the proliferation and differentiation of LPLCs. Kupffer cells or infiltrating macrophages are believed to be the source of endogenous TWEAK.<sup>31,32</sup> A direct link has been proved between macrophage TWEAK production and paracrine signaling associated with DRs, leading to the expansion of LPLCs in

TWEAK/Fn14<sup>-/-</sup> mice model.<sup>30</sup> In addition to the TWEAK signaling, macrophages are also associated with Wnt and Notch signaling, thus playing a crucial role in the differentiation of LPLCs into hepatocytes. Wnt3a has been proved to be expressed by macrophages as a result of the phagocytosis of biological debris, implicating the influence of macrophages on environmental sensing and correction of epithelial repair from LPLCs.<sup>41,63</sup> In detail, the depletion of Kupffer cells did not influence the proliferation of LPLCs but reduced their invasive behavior.<sup>64</sup> The direct cell-cell communication between LPLCs and myofibroblasts has been shown to involve the interactions of lymphotoxin  $\beta$ ,<sup>25</sup> as well as the role of extracellular matrix deposition in LPLC expansion and differentiation.<sup>65</sup> The reduction in LPLC parenchymal invasion by the depletion of Kupffer cells has been proved to be attributed to the reduced activation of myofibroblasts and the decrease in the extracellular matrix framework, which is necessary for cell motility.<sup>64</sup>

It has been well-described that the polarization of macrophages plays an important role in liver injury and liver regeneration, with a pro-inflammatory M1 polarization and an alternative anti-inflammatory M2 polarization.<sup>66,67</sup> Similar to the opposite polarization types of macrophages, infiltrating monocytes can also be separated into 2 subtypes characterized by different Ly-6C (Gr-1) expression levels in mice, Ly-6C<sup>high</sup> monocytes, which are considered to promote tissue injury, and Ly-6C<sup>low</sup> monocytes, which can suppress inflammation and facilitate liver repair.<sup>68-70</sup> In an ALD mouse model, Ly6C<sup>high</sup> monocytes were found to develop progressively into Ly6C<sup>low</sup> monocytes upon phagocytosis of apoptotic hepatocytes in mild liver injury. However, in cases of severe injury, the phenotype switching from Ly6C<sup>high</sup> to Ly6C<sup>low</sup> monocytes might be blocked, thereby resulting in persistent hepatic inflammation and impaired liver injury.<sup>61</sup> Also, macrophage polarization has a key role in the progression of nonalcoholic fatty liver disease, driving LPLC response by Wnt3a production.<sup>42</sup> Kupffer cells also turned out to control the initial accumulation of monocyte-derived macrophages, which in turn are responsible for LPLCs proliferation.<sup>71</sup> Furthermore, Viebahn et al<sup>72</sup> identified potential synergy between LPLCs and macrophages through ligand-receptor interaction, which is based on the recruitment of macrophages to the damaged liver by LPLCs.

### *Natural Killer Cells in Liver Progenitor-Like Cell-Induced Liver Regeneration*

Another important innate immune cell subset in liver regeneration is NK cell, which constitutes 30%–50% of the intrahepatic lymphocytes in humans, playing a vital role in suppressing hepatic bacterial and viral infections.<sup>73</sup> Although it has been proved that the activation of NK cells inhibited liver regeneration via the production of interferon (IFN)- $\gamma$  after PHx,<sup>74</sup> the role of NK cells in chronic liver injury was controversial. The activated NK cells in a CCl<sub>4</sub> mouse model turned out to inhibit the liver regeneration via the production of tumor necrosis factor  $\alpha$ ,<sup>75</sup> which also played a beneficial role in LPLCs proliferation to replace



**Figure 1. Activation, expansion, and differentiation of LPLCs.** During chronic liver injuries, dying hepatocytes generate Hh ligands, which not only directly activate LPLC-induced liver regeneration but also indirectly enhance the proliferation of LPLCs by activating HSCs into matrix-producing myofibroblasts. Also,  $LT\beta R$  on HSCs (activated via HIPPO/YAP pathway) may be involved in paracrine signaling with nearby  $LT\beta$ -expressing LPLCs. After initiation, TWEAK has a selective mitogenic effect for LPLCs, stimulating the proliferation of LPLCs through its receptor Fn14. The stimulation of TWEAK will lead to the activation of NF- $\kappa$ B and proliferation of LPLCs. FGF not only activates the proliferation of LPLCs (FGF7) but also induces the differentiation of LPLCs into hepatocytes (FGF9). HGF/c-met signaling, Wnt/ $\beta$ -catenin signaling, and thyroid hormone signaling are all proved to promote the differentiation of LPLCs into MHs, whereas Jag1/Notch signaling can induce LPLCs differentiating into BECs. BECs, biliary epithelial cells; FGF, fibroblast growth factor; Fn14, fibroblast growth factor-inducible 14; HGF, hepatic growth factor; Hh ligands, hedgehog ligands; HSCs, hepatic stellate cells; LPLCs, liver progenitor-like cells;  $LT\beta R$ , lymphotoxin  $\beta$  receptor; MHs, mature hepatocytes; NF- $\kappa$ B, nuclear factor kappa B; TWEAK, tumor necrosis factor-like weak apoptosis inducing factor; YAP, Yes-associated protein.

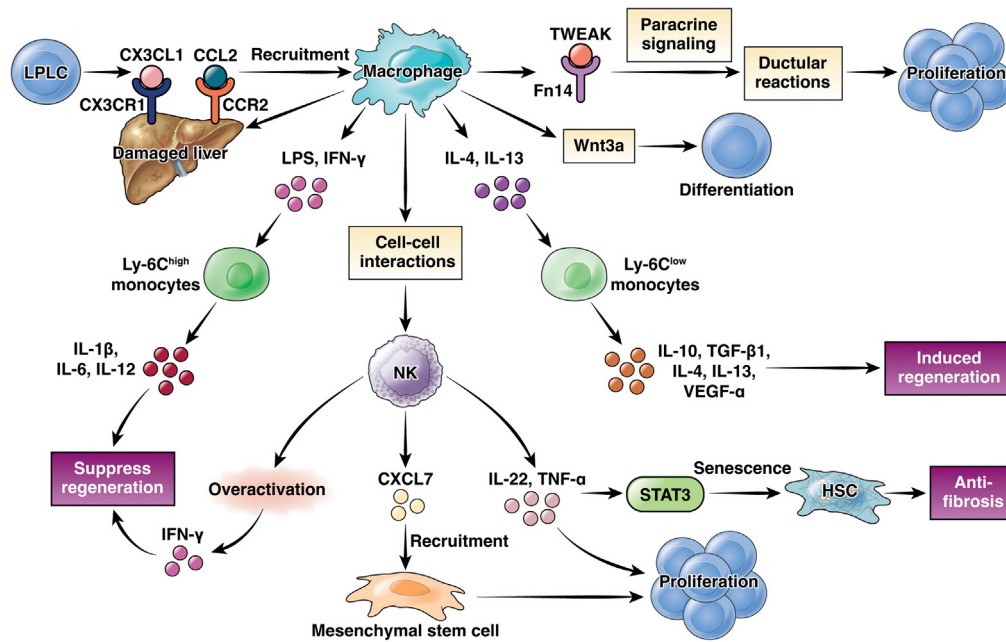
dying hepatocytes.<sup>76</sup> Also, CXCL7 secreted by NK cells could induce the recruitment of mesenchymal stem cells, thereby improving liver regeneration.<sup>77</sup> In addition, conventional NK cells have been proved to predominantly produce interleukin (IL) 22, which could promote the regeneration of epithelial cells and the production of antimicrobial peptides, effectively preventing secondary opportunistic infections during influenza infection.<sup>78</sup> Therefore, NK cells can produce cytokines that are important for liver regeneration.

Considering the negative effect of overactivated NK cells on liver regeneration, researchers have focused on controlling NK cells activation by inhibitory receptors.<sup>79–82</sup> The coinhibitory receptor TIGIT turned out to mediate the self-tolerance of NK cells regenerative hyperplasia by interacting with poliovirus receptor expressed on Kupffer cells, thereby limiting innate immunity against the liver regeneration.<sup>82</sup> Thus, the negative effects induced by the overactivation of NK cells can be potentially reduced by various inhibitory receptors. Considering the possible negative effects of LPLC-induced liver regeneration during chronic liver disease, such as liver fibrosis and hepatocarcinoma, NK cells have also been researched in such diseases. Recently, Saparbay et al<sup>83</sup> have demonstrated that the treatment of Everolimus could enhance the antitumor activity of immature liver-resident NK cells through the up-regulation of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), consistent with an earlier study showing that liver-resident NK cells were distinctly characterized by

expression of TRAIL,<sup>84</sup> which could attack virus-infected and tumor cells.<sup>85</sup> For liver fibrosis, NK cells could kill activated HSCs via RAE1/NKG2D- and TRAIL-dependent mechanisms, thereby controlling and ameliorating liver fibrosis,<sup>86</sup> indicating the important role of NK cells in suppressing liver fibrosis during hepatitis B virus or hepatitis C virus. Furthermore, TWEAK has been proved to play a key role in regulating goat peripheral NK cell cytotoxicity and cytokine expression levels during the progression of PPRV.<sup>87</sup> Because TWEAK signaling contributes to the initiation of LPLCs activation, it may have dual function in LPLC-induced liver regeneration.

Accounting for 10%–15% of human liver lymphocytes, NKT cells, a population of cells expressing NK and T-cell markers,<sup>88,89</sup> have also been studied in liver injury and liver regeneration. Similar to macrophages, NKT cells can also be categorized into pro-inflammatory type I NKT cells and anti-inflammatory type II NKT cells.<sup>90</sup> Type I NKT cells, also called classical or invariant NKT cells, comprise 95% of liver NKT cells and express a semi-invariant T-cell receptor, whereas type II NKT cells comprise less than 5% of liver NKT cells and express more diverse T-cell receptors.<sup>91</sup> Because most of the liver NKT cells have been proved to be invariant NKT, they were initially believed to play a negative role in liver regeneration by releasing cytokines such as IFN- $\gamma$  and IL4 or direct cytotoxicity. In hepatitis B virus transgenic mice, Dong et al<sup>92</sup> found that the impairment of liver regeneration was not only due to IFN- $\gamma$





**Figure 2. The role of macrophages and NK cells in LPLC-induced regeneration.** Macrophages can not only promote the proliferation of LPLCs through TWEAK signaling but also induce the differentiation of LPLCs into hepatocytes via Wnt3a pathway. Ly-6C<sup>high</sup> monocytes will promote tissue injury by secreting IL-1 $\beta$ , IL-6, and IL-12, whereas Ly-6C<sup>low</sup> monocytes can suppress inflammation and facilitate liver repair by secreting IL-10, TGF $\beta$ 1, IL-4, IL-13, and VEGF $\alpha$ . LPLCs can induce the recruitment of infiltrating macrophages to the damaged liver through CCL2/CCR2 and CX3CL1/CX3CR1. Macrophages can also influence other immune cells, including NK cells, through complex cell-cell interactions and secretion of cytokines. NK cells play a beneficial role in LPLCs proliferation, and CXCL7 secreted by NK cells can induce the recruitment of mesenchymal stem cells, thereby improving liver regeneration. Also, IL-22 produced by NK cells and NKT cells can increase HSCs senescence via STAT3 activation, thus alleviating liver fibrosis. CCL2, C-C motif chemokine ligand 2; CCR2, C-C motif chemokine receptor 2; CXCL7, C-X-C motif chemokine ligand 7; CX3CL1, C-X3-C motif chemokine ligand 1; CX3CR1, C-X3-C motif chemokine receptor 1; IFN- $\gamma$ , interferon  $\gamma$ ; IL, interleukin; LPS, lipopolysaccharides; NK cell, natural killer cell; STAT3, signal transducer and activator of transcription 3; TGF $\beta$ 1, transforming growth factor  $\beta$ -1; TNF $\alpha$ , tumor necrosis factor  $\alpha$ ; VEGF $\alpha$ , vascular endothelial growth factor  $\alpha$ .

overproduction by activated hepatic NKT cells but might also be relevant to hepatocyte itself, which was more sensitive to exogenous stimulus. For direct hepatotoxicity, NKT cells could kill hepatocytes by releasing FasL.<sup>93</sup> However, in chronic liver injury, which will lead to liver fibrosis associated with the accumulation of collagen in the liver,<sup>94</sup> the impact of NKT cells on LPLC-induced liver regeneration seemed to be controversial. Similar to NK cells, NKT cells can inhibit HSC activation by killing activated HSCs, thus inhibiting liver fibrosis. However, the protective effect was only discovered in the earlier stages and not in the later stages of liver fibrosis.<sup>95</sup> Also, IL22 produced by NK and NKT cells has been finely demonstrated to improve liver regeneration by increasing HSCs senescence via STAT3 activation, as well as promoting LPLCs.<sup>96</sup> In addition, the chemokine receptor CXCR6, which is predominantly expressed on NKT cells, could protect liver from inflammation and fibrosis in NEMO<sup>LPC-KO</sup> mice, in which increased hepatocyte apoptosis and compensatory regeneration caused steatosis, inflammation, and fibrosis.<sup>97</sup> In NKT cell-deficient mice, the impairment of Cyclin B1 and p21 expression could induce reduced liver regeneration.<sup>98</sup> Thus, NKT cells are necessary and even beneficial for LPLCs-induced liver regeneration during chronic liver injury.

## Therapies Improve Liver Regeneration Based on Innate Immune Cells for Chronic Liver Disease

With a diversity of complications and the result of liver fibrosis and hepatocarcinoma, the treatment to chronic liver diseases associated with LPLC-induced liver regeneration is an urgent but challenging problem. So far, most of the studies have been carried out on cell therapies based on macrophages. For example, bone marrow cell therapy has shown promising protective results in liver regeneration.<sup>30,99–103</sup> A single infusion of unfractionated bone marrow cells has been proved to result in a direct activation of DRs, as well as the changes in liver structure and function, including LPLCs expansion.<sup>30</sup> More specifically, bone marrow-derived macrophage (BMDM) delivery could lead to early chemokine up-regulation, with hepatic recruitment of endogenous macrophages and neutrophils, thereby attenuating liver fibrosis in chronic liver disease.<sup>99</sup> In addition, the expression of TWEAK, mitogen that is selective for LPCs but not mature hepatocytes,<sup>27</sup> was increased in bone marrow macrophage-treated mice,<sup>99</sup> further improving LPLCs proliferation. Considering the opposite roles of the 2 subsets of macrophages in liver inflammation

and regeneration, another macrophage-based treatment for chronic liver injury can be rendering macrophages toward a more restorative phenotype or directly using polarized macrophages for cytotherapy.<sup>103,104</sup> Baeck et al<sup>104</sup> examined the role of CCL2 inhibitors, which could pharmacologically inhibit Ly6C<sup>low</sup> macrophage infiltration, with the liver fibrosis regression appeared in both models, indicating that suppressing Ly6C<sup>low</sup> macrophage infiltration could be a selective strategy for liver regeneration during chronic liver injury.

It has been proved that the M1 polarized BMDMs exhibited better therapeutic effects than non-polarized M0 BMDMs, which was attributed to their ability to attenuate liver fibrosis and improve liver regeneration by recruiting monocyte-derived macrophages and NK cells, thus promoting collagen degradation, HSC apoptosis, and LPLC proliferation.<sup>103</sup> Also, M1 macrophages could further polarize the recruited endogenous macrophages into a Ly6C<sup>low</sup> restorative phenotype,<sup>103</sup> leading to an improved regeneration in chronic liver diseases. Recently, a new treatment targeting S1PR2, which has emerged to be pro-inflammatory and pro-fibrotic by affecting the infiltration and M1 polarization of bone marrow macrophages,<sup>105-108</sup> was designed and carried out, showing that the inhibition of macrophage S1PR2 could retard liver inflammation and fibrogenesis via down-regulating NLRP3 inflammasome.<sup>109</sup> Therefore, the different polarization phenotypes of macrophages played a crucial role in liver regeneration and provided various methods for the treatment of chronic liver diseases.

Macrophages and NK cells are 2 subsets indispensable for liver regeneration. Therefore, scientists have researched the interplay between them in the hope of better outcomes for chronic liver diseases. Ma et al<sup>110</sup> demonstrated that the deletion of phosphatase and tensin homolog (PTEN), originally identified as a tumor-suppressor protein, could activate the M2 polarization of Kupffer cells, leading to the less activated phenotype of NK cells, probably through direct cell-cell contact or decreased secretion levels of IL12 and IL15. Furthermore, PTEN-deficient Kupffer cells secreted more growth factors required for liver regeneration,<sup>110</sup> identifying PTEN as a potential target for liver regeneration. For NK cells, they have been previously proved to be beneficial for attenuating inflammation and preventing adverse cardiac remodeling via the cell crosstalk with allogeneic human cardiac-derived progenitor cells,<sup>111</sup> implying their potential roles in LPLC-induced liver regeneration. In a Fah<sup>-/-</sup> liver failure mouse model, the activated NK cells showed a vital role in bone marrow-derived hepatocyte generation, facilitating the fusion of myelomonocyte and hepatocyte in an IFN- $\gamma$ -dependent manner.<sup>112</sup> With a low efficiency of bone marrow-derived hepatocyte generation, bone marrow transplantation has been used in severe and chronic liver diseases but showed limited efficacy. Clarifying the beneficial role of innate immune cells such as NK cells could greatly improve the outcomes of bone marrow transplantation and bring exciting news to patients with severe liver failure.

In addition, NK cells also showed direct roles in preventing liver fibrosis in a thioacetamide-induced liver fibrosis mouse model through its activation by Fasudil, a Ras homology family member A kinase inhibitor.<sup>113</sup> The activated NK cells could induce HSC apoptosis and clear senescent-activated HSCs, thereby killing HSCs and producing IFN- $\gamma$  to prevent liver fibrosis,<sup>113</sup> identifying the critical role of IFN- $\gamma$  in NK cell-related therapy for liver regeneration during chronic liver injury. Interestingly, NK cells could regulate the M1 and M2 polarization of macrophages according to the immune response phase and disease stage during liver injury-induced inflammation.<sup>114</sup> Therefore, synergies between macrophages and NK cells could be further investigated to emphasize the role of innate immune cells in LPLC-induced liver regeneration.

The studies on NKT-based treatment for chronic liver injury were limited to date because of its less importance compared with macrophages and NK cells in liver regeneration. Because NKT cells were considered pathogenic in liver diseases,<sup>115-118</sup> the selective elimination of hepatic NKT cells by concanavalin A showed accelerated liver regeneration after PHx.<sup>115</sup> Correspondingly, the accumulation of NKT cells depending on chemokine receptor CXCR6 resulted in exacerbated inflammation and promoted liver fibrosis.<sup>116</sup> Therefore, targeting CXCR6 might have the potential to treat liver fibrosis. Also, a study on commensal bacteria in liver regeneration demonstrated that this bacteria subset could promote liver regeneration by maintaining Kupffer cell tolerance and preventing the overactivation of NKT cells,<sup>119</sup> which could explain why the overuse of antibiotics induced impaired liver function and liver regeneration. With the in-depth understanding of NKT cells, Martin-Murphy et al<sup>120</sup> proved that NKT cells could protect mice from acetaminophen-induced liver injury probably by producing IL4. It remains unknown whether NKT cells can be developed to promote liver regeneration by taking advantage of 2 subpopulations with opposite effects like macrophages. Thus, further exploration is needed to investigate the therapeutic roles of NK cells and NKT cells in liver regeneration during chronic liver injury.

## Conclusions

In summary, macrophages can not only promote the proliferation of LPLCs through TWEAK signaling but also induce the differentiation of LPLCs into hepatocytes via Wnt3a pathway. The macrophage-based cytotherapy has been proved to be beneficial for liver regeneration. In addition, treatments polarizing macrophages to a more restorative phenotype can also help repair the liver. For NK cells, they play a beneficial role in viral hepatitis because of their ability to suppress liver fibrosis, which is the major negative effect of LPLC-induced liver regeneration. However, the overaction of NK cells may lead to the overproduction of IFN- $\gamma$ , thus leading to impaired liver regeneration. Thus, treatments based on NK cells mainly focus on the control of NK cells activation to take advantage of their beneficial roles. NKT cells, another subset of innate immune cells, can also be categorized into pro-inflammatory

type I and restorative type II NKT cells, but they have been less researched in liver regeneration compared with macrophages and NK cells. Considering the important role of LPLCs in liver regeneration during chronic liver injury, further studies based on these 3 kinds of innate immune cells are required because of their close relationship with LPLCs.

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