The Role of Immune Cells in Liver Regeneration

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Abstract: The liver is the only organ that can regenerate and regain its original tissue-to-body weight ratio within a short period of time after tissue loss. Insufficient liver regeneration in patients after partial hepatectomy or liver transplantation with partial liver grafts often leads to post-hepatectomy liver failure or small-for-size syndrome, respectively. Enhancing liver regeneration after liver injury might improve outcomes and increase patient survival. Liver regeneration comprises hepatocyte proliferation, and hepatic progenitor cell expansion and differentiation into hepatocytes. The immune system is intensively involved in liver regeneration. The current review provides a comprehensive overview of the diverse roles played by immune cells in liver regeneration. Macrophages, neutrophils, eosinophils, basophils, mast cells, platelets, dendritic cells, type 1 innate lymphoid cells, B cells, and T cells are implicated in promoting liver regeneration, while natural killer cells and overactivated natural killer T cells are supposed to inhibit hepatocyte proliferation. We also highlight the predominant underlying mechanisms mediated by immune cells, which may contribute to the development of novel strategies for promoting liver regeneration in patients with liver diseases.

Keywords: liver regeneration; liver repair; hepatectomy; proliferation; hepatic progenitor cells; immune cells; macrophages

1. Introduction

Liver regeneration is a highly orchestrated and complex process that allows the liver to restore its original functional capacity after surgical resection or injury. Extensive research over the decades has unraveled the critical role of immune cells in driving and modulating the regenerative response within the liver microenvironment. The immune system, which comprises various cell types including macrophages, neutrophils, dendritic cells (DCs), innate lymphoid cells (ILCs), natural killer T (NKT) cells, B cells, and T cells, interacts intricately with hepatocytes and other nonparenchymal cells during liver regeneration. This current review provides a comprehensive overview of the diverse roles played by immune cells in liver regeneration. We also highlight the predominant underlying mechanisms mediated by immune cells, which may contribute to the development of novel strategies for promoting liver regeneration in patients with liver diseases.

2. Compensatory Regeneration and Progenitor Cell-Mediated Regeneration

Hepatic compensatory regeneration is characterized by hyperplasia of hepatocytes. After partial hepatectomy (PHx), only the remaining portion of the liver expands to the original liver-to-body weight ratio, and the structure of the excised parts does not grow back [1]. Compensatory regeneration is also observed in the reparative phase of acute liver injury induced by carbon tetrachloride (CCl4), moderate acetaminophen (APAP), and ischemia/reperfusion (I/R) [1–3]. In addition to compensatory regeneration, hepatic progenitor cell (HPC)-mediated regeneration occurs when the remaining hepatocytes fail to restore the original liver size due to excessive hepatocyte loss and/or hepatocellular proliferative signaling suppression, such as severe liver injury induced by a high dose of APAP or D-galactosamine (GaIN) [4,5]. HPCs exhibit hepatobiliary characteristics and represent a transitional cellular state between biliary epithelial cells (BECs) and hepatocytes. The
NOTCH and WNT signaling pathways determine the cell fate of HPCs to BECs and hepatocytes, respectively, during severe chronic liver injury or cholestatic liver injury [6,7]. The appropriate manipulation of HPC expansion and transdifferentiation toward hepatocytes would be greatly appreciated for facilitating liver regeneration.

3. Macrophages

Macrophages have been recognized as the most significant contributors to liver regeneration. In the liver, macrophages mainly comprise liver resident macrophages known as Kupffer cells and recruited monocyte-derived macrophages [8]. During the early phase of liver regeneration after PHx, Kupffer cells are the key players in initiating hepatocyte proliferation through the tumor necrosis factor (TNF)α and interleukin (IL)-6 [1]. Kupffer cell depletion by liposome-encapsulated clodronate (CLDN) delays liver regeneration by impairing TNFα and IL-6 expression [9–12]. Kupffer cells could also remove shear stress-induced senescent liver sinusoid endothelial cells (LSECs) to promote liver regeneration, and its depletion accumulates senescent LSECs and disrupts liver regeneration after PHx [13]. However, other groups reported that Kupffer cell depletion by gadolinium chloride or CLDN enhanced liver regeneration after PHx [14–16]. Kupffer cell depletion abolishes the induction of IL-10 to prolong the short life of TNFα mRNA [15], which could subsequently induce IL-6 production in hepatocytes [16–19]. Moreover, Kupffer cell depletion alters the components of other intrahepatic leukocytes [20], which may compensate for hepatocyte proliferation. Given that Kupffer cell depletion causes diverse consequences after PHx in different groups, directing attention towards specific gene/protein targets on Kupffer cells could present an alternative approach to boost liver regeneration. For instance, the inhibition of colony-stimulating factor 1 receptor (CSF1R) or MER proto-oncogene tyrosine kinase (MERTK) could not only reduce cytokine expression without affecting Kupffer cell viability in vitro, but also delay liver regeneration in mice after PHx [20], indicating the pro-regenerative role of CSF1R and MERTK in Kupffer cells.

Monocytes are also increased in the blood and liver in humans [21] and mice after PHx [22–24]. Impairing monocyte infiltration in mice with C-C motif chemokine receptor (CCR)2 deficiency or treatment with a CCR2 antagonist has demonstrated impeded liver regeneration after PHx [23,25,26]. However, the chemoattractant for CCR2+ monocytes, C-C motif chemokine ligand (CCL)2, is not required for liver regeneration, which has been verified in CCL2 knockout mice [26], although CCL2 expression is elevated after PHx [23,25,26]. This indicates that other chemokines for monocyte recruitment are involved in liver regeneration after PHx. The adhesion and transmigration of recruited monocytes in the liver are then enhanced by intercellular adhesion molecule 1 in LSECs, which is induced by Kupffer cell-derived TNFα. These intrahepatic monocytes subsequently serve as chaperones for endothelial sprouting by local secreting factors (WNT5a and angiopoietin [ANG]-1) and activating NOTCH1 to stabilize stalk cells, promoting vascular growth and eventually supporting liver regeneration [27].

In liver injury, Kupffer cells undergo programmed death, and the death of Kupffer cells facilitates bacterial clearance, inflammation resolution, and liver repair [8,28]. However, experimentally depleting Kupffer cells with CLDN inhibits liver regeneration in mice with a liver injury induced by CCl4 or bile duct ligation (BDL) [29,30], suggesting the importance of the process of programmed Kupffer cell death in liver regeneration. With the death of Kupffer cells, infiltrated proinflammatory lymphocyte antigen 6 family member C1 (Ly6C)high monocyte-derived macrophages become the predominant population in the macrophage pool at the early phase of liver injury [31]. Ly6C<sup>high</sup> monocyte-derived macrophages are later switched into restorative Ly6C<sup>low</sup> monocyte-derived macrophages, which indicates the initiation of the reparative phase [8]. Ly6C<sup>low</sup> monocyte-derived macrophages have been reported to express matrix metallopeptidases (MMPs) (e.g., MMP9, MMP12, MMP13, MMP14), growth factors (e.g., hepatocyte growth factor [HGF], insulin-like growth factor [IGF]), and phagocytotic genes (macrophage receptor with collagenous structure [MARCO]) to enable wound healing, dead cell clearance, and inflammation.
resolution in order to provide a pro-proliferative niche for hepatocytes [32–39]. Notably, Ly6C<sup>low</sup> monocyte-derived macrophages have also been reported to directly promote hepatocyte proliferation in vitro [40].

Alternatively activated macrophages (M2 macrophages), which have different gene signatures from restorative Ly6C<sup>low</sup> macrophages [32], have also been reported to stimulate hepatocyte proliferation and liver repair after liver injury [36]. During *Listeria monocytogenes* or *Salmonella enterica* infection, Kupffer cells undergo early rapid necroptosis and release IL-1β to induce hepatocytes to produce IL-33 which, together with basophil-derived IL-4, promotes the alternative activation of monocyte-derived macrophages, contributing to liver repair and the restoration of liver homeostasis [41]. In addition, the adoptive transferring of human or mouse bone marrow-derived M2 macrophages could exhibit highly phagocytic effects, reduce inflammation, and trigger hepatocyte proliferation in mice with APAP overdose [36].

As summarized above, manipulating infiltrated monocytes towards restorative/restorative Ly6C<sup>low</sup> or M2 macrophages would be a promising strategy for enhancing liver regeneration in hepatic infection and injury. Recently, the transplantation of mesenchymal stem cells (MSCs) has been established as a potential strategy to trigger restorative macrophages. MSC transplantation promotes IL-4-dependent M2 macrophage switching and hepatocyte proliferation, and improves outcomes in mice with acute liver injury induced by GaIN [42], as well as in mice with chronic liver injury induced by multiple doses of CCl<sub>4</sub> [43]. Another single-cell RNA sequencing (scRNASeq) study highlighted that MSC treatment promoted the transition of hepatic macrophages from an Ly6C<sup>high</sup> to an Ly6C<sup>low</sup> population in mice with acute liver injury [44]. Patients with acute-on-chronic liver failure (ACLF) show significant increases in the number of liver macrophages as well as reduced phagocytic activity. MSCs could improve the phagocytic ability of ACLF monocytes during coculturing in vitro. MSC therapy also directs monocyte-derived macrophages toward a highly phagocytic restorative macrophage-like phenotype to enhance hepatocyte proliferation in mice with ACLF [45]. Some other potential targets have been reported to be involved in macrophage phenotype switching. Blocking CCL5 by using a neutralizing antibody or agonist Met-CCL5 directly mediates the macrophages toward the M2/Ly6C<sup>low</sup> macrophage-like phenotype and subsequently contributes to hepatocyte proliferation in mitogen-activated protein kinase (MAPK)- and nuclear factor kappa B (NFκB)-dependent manners in mice with acute liver injury [46,47]. The mesencephalic astrocyte-derived neurotrophic factor or galectin-3 deficiency in infiltrated monocyte-derived macrophages maintain the restorative Ly6C<sup>low</sup> phenotype and support hepatocyte proliferation upon APAP treatment [48,49]. Furthermore, blocking E prostaglandin receptor 4 (EP4) by a selective antagonist, inhibitor, or genetic deletion in macrophages polarizes toward the anti-inflammatory Ly6C<sup>low</sup> macrophages and subsequently boosts liver repair after hepatic I/R injury [50]. Altogether, MSC transplantation and targeting the above gene/protein might be beneficial for liver regeneration and require further attention.

Notably, in the uninjured mouse model of PHx, whether M2 or Ly6C<sup>low</sup> macrophages positively regulate liver regeneration is controversial. The adoptive transferring of hypoxic mesenchymal stromal cells induces M2 macrophage polarization to promote liver regeneration after PHx through the secretion of exosomal microRNA-182-5p and modulating forkhead box (FOXO1)/ toll-like receptor 4 pathway [51]. CCL5 inhibition promotes reparative Ly6C<sup>low</sup> macrophage induction in a FOXO3a-dependent manner to support liver regeneration after PHx [52]. However, peroxisome proliferator-activated receptor α-deficient macrophages exhibit the M1 phenotype to contribute to IL-6 production in order to support hepatocyte survival and proliferation after PHx [53], which contradicts previously mentioned studies regarding the role of the restorative macrophage in liver regeneration after PHx. The components and functions of macrophages in the hepatectomized liver need to be further determined. Moreover, the application of restorative macrophages in PHx should be conducted with caution.
Most recently, a refined study [54] introduced some kinds of monocyte-derived macrophages other than the Ly6C<sub>low</sub>/M2 macrophages, and delicately described how these monocyte-derived macrophages repaired the liver during immune-mediated liver injury. Upon concanavalin A (ConA) treatment, monocyte-derived macrophages are rapidly recruited to encapsulate necrotic lesions and induce death-resistant SOX9<sup>+</sup> hepatocytes to form a barrier preventing further injury through activating JAGGED1/NOTCH2 signaling. The hypoxia and damage-associated molecular patterns in the necrotic environment induce a cluster of C1q<sup>+</sup> monocyte-derived macrophages and PDGFβ<sup>+</sup> monocyte-derived macrophages to collaboratively promote necrotic removal, hepatocyte proliferation, and liver repair [54].

Although Kupffer cells and monocyte-derived macrophages show heterogeneity, they may collaborate and share some of the same signaling pathways as well. Macrophages with von Hippel–Lindau tumor suppressor deletion accelerate hepatocyte proliferation in mice with fibrosis in a hypoxia-inducible factor 1α/vascular endothelial growth factor (VEGF)-dependent manner [55]. In addition to the direct effect of VEGF on LSECs, the VEGF receptor (VEGFR)1+ and VEGFR3+ macrophages stimulated by VEGF further benefit sinusoidal reconstruction and liver repair after acute liver injury induced by I/R. The loss of VEGFR1 or VEGFR3 leads to the loss of the reparative capacity in macrophages [56,57]. Moreover, macrophages may share WNT signaling, which is also involved in liver regeneration. Wntless deletion in macrophages attenuates liver regeneration in mice after PHx [58], which is attributed to insufficient WNT2 and WNT9b expression [59]. Despite LSECs being the main source of WNTs [59], WNTs from pericentral LSECs mainly maintain the pericentral gene expression gradient in the normal liver. Upon APAP treatment, WNTs from macrophages support midzonal and periportal hepatocytes to maintain liver function. And during the regenerative phase, macrophage-derived WNTs are indispensable for the promotion of hepatocyte proliferation to repopulate the hepatocyte pools [60].

In liver regeneration involving HPCs, hepatic macrophages exert diverse roles. In mouse models induced by 2-acetylaminofluorene/PHx, 3,5-diethoxycarbonyl-1,4-dihydro collidin (DDC) diet or choline-deficient, ethionine-supplemented (CDE) diet, Kupffer cell depletion by CLDN lessens TNFα and IL-6 production and reduces HPC expansion [61–63]. In addition, Kupffer cells support the initial accumulation of monocyte-derived macrophages which, together with Kupffer cells, increase TNFα expression for HPC proliferation [63,64]. In the in vitro coculturing system, M1 macrophages directly promote HPC self-renewal through the JAGGED1/NOTCH signaling pathway [65]. NOTCH and WNT signaling could direct the specification of HPC in vivo. In DDC-induced biliary injury, NOTCH activation induces HPCs to differentiate into cholangiocytes, while in CDE-induced hepatocellular injury, macrophages engulf hepatocyte debris and subsequently release WNT3α to maintain NUMB expression in HPCs through canonical WNT signaling, contributing to the differentiation into hepatocytes by inhibiting NOTCH signaling [7].

In addition to hepatic macrophages, extrahepatic macrophages are also involved in liver regeneration. In a sterile liver injury model of touching a heated thermo probe to the hepatic surface, GATA6<sup>+</sup> peritoneal macrophages, which are prenatally developed and present in the peritoneal cavity, have been observed to migrate to the subcapsular liver within 1 h post-injury. These macrophages further switch into M2 macrophages to induce liver repair [66]. In the spleen, CD169<sup>+</sup> macrophages are maintained by B cells to contribute to liver regeneration by inducing IL-6 after PHx [67]. Diphtheria toxin (DT)-induced loss of CD169<sup>+</sup> macrophages in CD169-DT receptor mice attenuates liver regeneration after PHx [67]. However, whether B cell-maintained pro-proliferative CD169<sup>+</sup> macrophages exist not only in the spleen, but also in the liver, is not yet defined.

### 4. Granulocytes

Among granulocytes, neutrophils have been the most widely investigated in liver regeneration. Neutrophils are rapidly recruited into the liver in mice and humans upon hepatectomy and injury [68–70]. Depleting neutrophils using the Gr-1 antibody delays liver
regeneration after PHx [70]. Mice specifically lacking the neutrophil C-X-C motif chemokine receptor (CXCR)2, which is the receptor for chemokines to recruit neutrophils, exhibit impaired hepatocyte proliferation during the regenerative phase of hepatic I/R injury [71]. Following the injury phase, the neutrophil reactive oxygen species (ROS) production and clearance of the apoptotic neutrophils trigger Ly6C<sub>high</sub> monocyte-derived macrophages to switch to the restorative Ly6C<sub>low</sub> phenotype. The depletion of neutrophils via the anti-Ly6G antibody or genetic deficiency of NADPH oxidase 2 impedes the phenotypic conversion of macrophages and liver regeneration [29,37,72]. Moreover, CSF-1 production in aging neutrophils enables restorative macrophage maturation to contribute to liver repair in acute liver injury induced by APAP overdose [73]. To form a pro-regenerative microenvironment, recruited neutrophils also produce cathelicidin to enhance the phagocytosis of necrotic cell debris in an autocrine manner at the early reparative phase after APAP overdose [74]. Notably, neutrophils have been reported to be a source of HGF in patients with severe alcoholic hepatitis [75]. In patients undergoing PHx, surgery induces long-lasting hepatocellular apoptosis. Neutrophils effecytose these apoptotic extracellular vesicles and are subsequently activated to release HGF rather than exhibiting the classical inflammatory response, eventually supporting human liver regeneration [76]. Altogether, neutrophils play a pro-regenerative role in liver regeneration. However, a study demonstrated that the anti-Ly6G antibody did not affect HPC formation in DDC-induced liver injury in mice [62], suggesting that neutrophils are not involved in HPC-mediated liver regeneration.

Both basophils and eosinophils secrete IL-4 to promote liver regeneration, but in different scenarios. During the reparative phase after bacterial infection, basophils are recruited and stimulated by IL-33 from hepatocytes to produce IL-4 for M2 macrophage-mediated liver repair [41]. In mice with acute sterile liver injury, eosinophils accumulate in the liver and release IL-4 to directly induce hepatocyte proliferation [77]. Dynamic changes in the hepatic mast cell number and their protease phenotypes are observed in rat liver regeneration after PHx [78]. An in vitro study also demonstrated that coculturing primary hepatocytes with bone marrow-derived mast cells stimulated hepatocyte proliferation [79]. Nevertheless, whether mast cells play a role in liver regeneration in vivo needs to be further investigated.

5. Platelets

The influx of platelets into the liver occurs as early as 5 min in mice and as early as 1.5 h in humans after PHx [80–82]. Intrahepatic fibrinogen and the von Willebrand factor have been reported to mediate platelet accumulation in the liver after PHx and drive the stimulation of liver regeneration [80,83]. Thrombocytopenia induced by the anti-mouse platelet antibody or inhibiting platelet activity through clopidogrel attenuates liver regeneration after PHx and acute liver injury [80,81,84,85]. On the contrary, thrombocytosis induced by pegylated recombinant human megakaryocyte growth and development factor or platelet infusion enhances liver regeneration in rodents [81,86–89]. In a rat model of partial liver transplantation, thrombopoietin-induced platelet accumulation in the liver graft stimulated graft regeneration without aggregating I/R injury in a Kupffer cell-involving manner [90]. In patients recovering from partial liver resection, a low platelet count is an independent predictor of the delayed postoperative recovery of liver function and is correlated with an increased risk of postoperative mortality [91]. Furthermore, in recipients undergoing living donor liver transplantation, platelet transfusion is associated with improved graft regeneration, and the number of transfused platelets is positively correlated with graft regeneration [92,93]. In summary, platelets promote liver regeneration in mouse models and recipient patients after liver transplantation.

Mechanistically, both in vitro and in vivo studies indicate that platelets could directly promote hepatocyte proliferation by producing HGF and IGF1 [94–97]. Alternatively, as the transporter for serotonin, platelets release serotonin into the liver to promote liver regeneration through 5-hydroxytryptamine receptor 2 [84,85]. However, excessive high intraplatelet serotonin levels lead to a higher incidence of early tumor recurrence in patients
undergoing liver resection for malignant liver tumors, while excessive low intraplatelet serotonin levels lead to increased morbidity [98]. Therefore, the pharmacological intervention for platelets and platelet-derived serotonin to promote liver regeneration should be cautiously considered. Intriguingly, platelets could stimulate hepatocyte proliferation via platelet internalization by hepatocytes followed by the functional transferring of the RNA stored in anucleate platelets [99]. However, this phenomenon has only been observed in vitro [99], and further in vivo studies are warranted. Platelets have also been observed to interact with other nonparenchymal cells to promote hepatocyte proliferation indirectly. They could prime hepatic stellate cells (HSCs) to produce HGF for hepatocyte proliferation in mice [100]. Platelets adhere to LSECs after PHx and liver injury [97,100] and deploy C-X-C motif chemokine ligand 12 and VEGF to stimulate the CXCR7+ LSECs and VEGFR1+ macrophages, respectively, to induce their production of WNT2 for the purpose of facilitating liver regeneration [101]. An earlier in vitro study demonstrated that, in the presence of human platelets, human LSECs secrete IL-6 by sphingosine 1-phosphate receptor activation. Moreover, the supernatants from LSECs with platelets could significantly increase DNA synthesis in human primary hepatocytes [102]. Animal studies show that platelets promote liver regeneration by interacting with LSECs to produce IL-6 in the C-type lectin domain family 2/podoplanin- and transforming growth factor β-dependent manners [103,104]. However, another study contradicted this theory, demonstrating that supernatants from platelet/LSEC coculturing showed higher IL-6 levels, but were insufficient to increase primary hepatocyte proliferation compared to supernatants from LSECs alone [100]. Given that Kupffer cells and hepatocytes account for almost all IL-6 production after PHx [17], whether platelet/LSEC interaction-derived IL-6 plays a role in liver regeneration needs to be further determined.

6. Dendritic Cells

DCs decrease in the circulating blood in patients after major hepatectomy [21], implicating the recruitment of DCs in the liver post-hepatectomy, which has been observed in the mouse model of PHx [105]. The administration of DC poietin Fml-related receptor tyrosine kinase 3 ligand dramatically increases the number of hepatic DCs and promotes liver regeneration after PHx, probably involving estrogen-mediated immune modulation [105]. Upon hepatic I/R, monocyte-derived DCs are recruited to the boundary between damaged and undamaged areas in an EP3-dependent manner. The recruited monocyte-derived DCs release IL-13 to mediate the switching of macrophages from an Ly6C\textsuperscript{high} to an Ly6C\textsuperscript{low} pro-reparative phenotype, finally contributing to liver repair [106].

7. Innate Lymphoid Cells

ILCs are lymphocytes that do not express the type of adaptive antigen receptors that are expressed on B and T cells. Currently, ILCs are classified into five subsets based on their development: natural killer (NK) cells, ILC1s, ILC2s, ILC3s, and lymphoid tissue-inducer (LTi) cells [107]. Among these cell types, NK cells are most often investigated in liver regeneration. NK cells are suggested to be increased dynamically after PHx [108], and express interferon (IFN)γ to kill regenerating hepatocytes [109]. NK depletion by the asialo GM-1 antibody could enhance liver regeneration after PHx in mice [110]. Evacuating sinusoidal NK cells by means of recombinant human CSF3 treatment facilitates liver regeneration [111]. Furthermore, the activation of NK cells by injecting polyinosinic-polycytidylic acid or murine cytomegalovirus attenuates liver regeneration, which is mediated by increased NK-derived IFNγ and TNFα [110,112]. During normal liver regeneration, NK cells increase their expression of the T cell Ig and ITIM domain (TIGIT), which negatively regulates NK-hepatocyte crosstalk to inhibit IFNγ production from NK cells. Blocking NK cell self-tolerance by deleting TIGHT increases NK-derived IFNγ expression and impedes liver regeneration [113]. However, NK cells are not likely to be involved in the liver regeneration induced by certain liver models, especially ConA-mediated liver injury and DDC-induced HPC expansion [62,114].
Most recently, an interesting study reported that environmental eustress could promote liver regeneration induced by PHx and CCl₄ through the sympathetic regulation of ILC1s to increase IL-22. T-box transcription factor 21-deficient mice, which lack ILC1s, attenuated hepatic IL-22 production and liver regeneration in mice in an enriched environment, and even in the standard environment [115], indicating the pro-regenerative role of ILC1 in liver regeneration through IL-22. Currently, the roles of ILC2, ILC3, and LTi cells in liver regeneration have not been investigated and need to be further determined. The absence of studies on ILC2, ILC3, and LTi cells can likely be attributed to the absence of the recruitment of these cells into the liver during the reparative phase.

8. Natural Killer T Cells

In mice undergoing PHx, NKT cells have been shown as the main source of IL-4 for the promotion of liver regeneration [116]. This study raises the question of which cell (NKT cells versus eosinophils [77])-derived IL-4 is more important in liver regeneration. Therefore, these studies need to be further evaluated and the findings confirmed. However, an earlier study demonstrated that, compared to NK cells, NKT cells only played a minor role in normal liver regeneration [110]. Additionally, CD1d knockout mice with NKT cell deficiency have been shown to exhibit normal liver regeneration after PHx [117]. In antibiotic-treated mice, NKT cells are overactivated by increased IL-12-expression Kupffer cells and subsequently retard liver regeneration via IFNγ [117]. Similarly, in mice infected with the hepatitis B virus, NKT cells are activated to produce IFNγ, arresting the cell cycle and inhibiting liver regeneration after PHx [118]. On the contrary, ConA-induced selective elimination of hepatic NKT cells accelerates liver regeneration after PHx in mice [119]. Therefore, targeting NKT cells might be beneficial for liver regeneration in patients specifically with hepatic NKT cell overactivation.

9. B and T Cells

Even though B and T cells are not likely involved in the HPC response [62,64], they have been reported as pro-regenerative participants in the liver. Either B, CD4⁺ T, CD8⁺ T, or γδ T cell depletion in Jh⁻/⁻, Cd4⁻/⁻ or CD4 antibody-treated, Cd8⁻/⁻ or CD8 antibody-treated, or TCRδ⁻/⁻ mice, respectively, impairs liver regeneration after PHx in mice [67,120,121]. Mechanistically, lymphoxin β (LTβ) mediated by B and T cells promotes liver regeneration [67,120], as the blocking of its receptor, LTβR, which can be expressed on hepatocytes, impedes liver regeneration after PHx [122]. However, which cell-derived LTβ is more critical for liver regeneration needs to be further investigated. γδ T cells produce IL-22 and IL-17 in a Dectin-1-dependent manner and directly induce hepatocyte mitosis in addition to promoting a regenerative phenotype in hepatic leukocytes (NK, NKT, and Kupffer cells), respectively, to support liver regeneration [121]. In chemically induced acute liver injury, CD8⁺ T cells maintain the survival of reparative monocyte-derived macrophages through the inducible T cell costimulator (ICOS)/ICOS ligand-mediated pathway [123]. Notably, regulatory T (Treg) cells, characterized as Foxp3⁺ T cells, are intensively involved in tissue regeneration in various organs, including the skin, lung, heart, central nervous system, and intestine. Tissue Treg cells hinder the early inflammatory response after tissue injury and promote the transition to a tissue milieu favoring regeneration, mostly likely through the production of IL-10 [124]. Alternatively, Treg cells produce amphiregulin, one of the ligands of the epidermal growth factor receptor, for cell proliferation, differentiation, and tissue regeneration [124–126]. Therefore, it is worthwhile to further investigate the role of Treg cells in liver regeneration.

10. Conclusions and Future Directions

Liver regeneration is an orchestrated and complicated process that involves diverse immune cells (Figure 1). While considerable progress has been made in understanding the underlying mechanisms of the immune system in liver regeneration, numerous questions remain unanswered. Firstly, although many studies have examined the regenerative
phase, the role of immune cells in the termination of liver regeneration requires further investigation. Secondly, with the advancements in scRNAseq, it would be highly valuable to explore the heterogeneity of immune cells and elucidate their specific functions in liver regeneration. Thirdly, employing spatial technologies including spatial transcriptomics could offer insights into the interaction between immune cells, liver zonation, and liver regeneration. Furthermore, while previous studies have predominantly focused on liver regeneration induced by hepatectomy and acute liver injury, there is a need for additional research on liver regeneration/repair in chronic liver diseases, such as cirrhosis and fatty liver diseases. Lastly, understanding the discrepancies in the process of liver regeneration between animal models and human cases would greatly facilitate the translation of novel treatments for liver diseases in humans.

Figure 1. The roles and underlying mechanisms of immune cells in liver regeneration. Different immune cells play diverse roles in hepatocyte proliferation, and hepatic progenitor cell expansion and differentiation into hepatocytes after liver resection and injury. Abbreviations: ANG: angiopoietin; C1q: complement component 1q; CSF: colony stimulating factor; CXCL: C-X-C motif chemokine ligand; HGF: hepatocyte growth factor; ICOS: inducible T-cell costimulator; IFN: interferon; IGF: insulin-like growth factor; IL: interleukin; ILC: innate lymphoid cell; LT: lymphotoxin; MMP: matrix metalloproteinase; NK: natural killer; NKT: natural killer T; PDGF: platelet-derived growth factor; ROS: reactive oxygen species; TNF: tumor necrosis factor; VEGF: vascular endothelial growth factor. Created with Biorender.com, accessed on 26 July 2023.
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Abbreviations

ACLF: acute-on-chronic liver failure; ANG: angiopoietin; APAP: acetaminophen; BDL: bile duct ligation; BEC: biliary epithelial cell; CCL4: carbon tetrachloride; CCL: C-C motif chemokine ligand; CCR: C-C motif chemokine receptor; CDE: choline-deficient, ethionine-supplemented; CLDN: liposome-encapsulated clodronate; ConA: concanavalin A; CSF1R: colony stimulating factor 1 receptor; CXCR: C-X-C motif chemokine receptor; DC: dendritic cell; DCC: 3,5-diethoxycarbonyl-1,4-dihydrocollolidin; DT: diphtheria toxin; EP4: E prostanoid receptor 4; FOX: forkhead box; GaIN: D-galactosamine; HGF: hepatocyte growth factor; HPC: hepatic progenitor cell; HSC: hepatic stellate cell; I/R: ischemia/reperfusion; ICOS: inducible T cell costimulator; IGF: insulin-like growth factor; IL: interleukin; ILC: innate lymphoid cell; INF: interferon; LSEC: liver sinusoid endothelial cell; LTβ: lymphoxin β; Ly6C: lymphocyte antigen 6 family member C1; M2 macrophages: alternatively activated macrophages; MAPK: mitogen-activated protein kinase; MARCO: macrophage receptor with collagenous structure; MERTK: MER proto-oncogene, tyrosine kinase; MMP: matrix metalloproteinase; MSC: mesenchymal stem cell; NFκB: nuclear factor kappa B; NK: natural killer; NKT: natural killer T; PHx: partial hepatectomy; ROS: reactive oxygen species; scRNAseq: single-cell RNA sequencing; TNF: tumor necrosis factor; TIGIT: T cell Ig and ITIM domain; Treg: regulatory T; VEGF: vascular endothelial growth factor; VEGFR: VEGF receptor.

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