Macrophages as a Cell-Based Therapy for Liver Disease

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Macrophages as a Cell-Based Therapy for Liver Disease

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Abstract

Liver failure arising from acute and chronic liver disease is an unmet clinical need that urgently requires novel therapeutic options in addition to orthotopic liver transplantation. Cell therapies offer new strategies to recover liver function through the reconstitution of healthy parenchyma and resolution of tissue pathology. Macrophages are professional phagocytes that comprise a key part of the innate immune system providing an important defense mechanism against invading pathogens. Macrophages are an inherently diverse cell type with respect to ontogeny, tissue distribution, phenotype, and function. The ability of macrophages to afford innate immunity, efficiently scavenge apoptotic/necrotic cells, and modulate local tissue microenvironment makes them an attractive cell therapy candidate for various diseases. This review aims to outline the rationale and utility of macrophages to serve as a potential cell therapy for liver disease.

Keywords

- macrophages
- cirrhosis
- acetaminophen
- liver immunology
- cell therapy

Death from liver disease continues to increase in contrast to other chronic conditions.[1,2] Cirrhosis of the liver is the result of prolonged injury to the liver arising from multiple etiologies. Cirrhosis-related deaths accounted for over a million deaths globally in 2010 with mortality rates increasing substantially in the United Kingdom.[3,4] In the United Kingdom, chronic liver disease accounts for the majority of liver transplantations, with a relatively lower incidence of transplantations to treat acute liver failure (ALF) (NHS Interim Report on Liver Transplantation, 2018). ALF is a relatively rare but life-threatening critical illness with acetaminophen (APAP) poisoning alone accounting for half of ALF cases in the United States equating to nearly 500 deaths annually.[5,6] Liver failure arising from either acute or chronic injury is limited to orthotopic liver transplantation (OLT) as the only curative option. Liver transplantation alone is inadequate with demand for grafts outweighing supply of suitable organs. Furthermore, the surgical procedure carries significant morbidity and mortality, and patients are committed to life-long immunosuppression.[7] Therefore, there is an urgent requirement for the development of alternative therapies for acute and chronic liver diseases. Despite the relative success of therapeutic interventions for specific etiologies (e.g., novel antiviral therapy for hepatitis C virus infection, alcohol abstinence for alcoholic liver disease), patients often present to medical attention late when cirrhosis and related complications have already occurred.[8] Therefore, there is a need to explore novel therapies for both acute failure and chronic liver disease to provide additional therapeutic options beside organ transplantation.

Cell Therapies for Liver Disease

Cell therapies herald a new era in medicine, offering alternative strategies to promote the functional recovery of diseased or injured tissues. Cell therapies are an attractive therapeutic approach because they promote the repair of a patient's own tissue using a fully defined (i.e., Good Manufacturing Practices [GMP]-compliant) cellular product that can be produced at scale, and delivered to patients. In the context of liver disease, several cell types have been tested in both preclinical animal models and human trials with varying degrees of success in terms of safety and efficacy (see ►**Table 1**). The first evidence of the feasibility of cellular therapy for liver disease was gained from hepatocyte transplantation for metabolic liver disease.[9–11] Two studies performing intrasplenic transplantation of allogenic hepatocytes in ALF patients with hepatic encephalopathy grade $>$ 3 showed minimal improvement in survival.[12] In the group listed for OLT, hepatocyte transplantation improved cardiovascular stability but did not significantly

* *Philip J. Starkey Lewis and Francesca Moroni have equal contribution.*

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Table 1 Cell therapy strategies that have been trialed for liver disease

Cell type	Use	Safety	Efficacy	Strengths and limitations
Hepatocytes[25,26]	Humans (small RCTs)	High risk of thrombosis	Reduction of LDL (familial hypercholesterolemia) Reduction of need of factor VII replacement (congenital deficiency) Reduction of ALT and BIL (biliary atresia) No efficacy proven in ALF and other congenital and metabolic diseases (PFIC, OTC, and ASL deficiency)	Poor engraftment Difficult expansion Difficult cryopreservation
iPSCs[20]	Animals	High teratogenic risk	Improvement in survival (50 vs. 0% survival at day 3 in treatment vs. control) Reduction of fibrosis (50% reduction at Sirius red staining)	Teratogenic Generate organ buds
Fetal hepatocytes[22]	Animals	Safe in rats with no evidence of oncogenesis at 3 mo	Improvement in bilirubin of around 50% in Gunn's rats Improvement in survival	Isolation from aborted fetus Poor expansion
HPCs[27,35]	Animals	Little risk of thrombosis	Not proven	Poor engraftment Easy isolation Good cryopreservation No human studies
hBTSC[29,30]	Humans (2 case reports)	Safe	Improvement of MELD (from 24 to 20) Improvement of CP score (from 12 to 10) but not sustained at 1 y	Multipotent
HSCs[33,34,36,37]	Humans (RCT and pilot study)	Safe (no SAR or SUSAR in RCT)	Transient nonstatistically significant improvement in bilirubin in pilot study. No evidence of improvement in MELD in large RCT	Poor engraftment Good ex vivo expansion Good cryopreservation
FLSPCs[31,32]	Animals	Safe in rats (small size, can be used in low numbers)	Improvement in albumin	Require regenerative stimuli to proliferate Pluripotent Good cryopreservation
MSCs[46,48,49]	Humans (RCT)	Safe (no SAR or SUSAR in RCT)	Among all markers only Alb improved with statistically significance compared with controls (from 30 to 35)	Easy to expand in vitro Immunomodulatory
Macrophages[78,81]	Animals On-going phase 1/2 clinical trial	Safe (no evidence of cytokine storm in mice models)	Improvement in albumin (~5–10% in treatment group) Reduction of fibrosis (reduction of 25% of collagen I and hydroxyproline staining in treatment vs. control)	Immunomodulatory Antifibrotic

Abbreviations: ALF, acute liver failure; ALT, alanine aminotransferase; ASL, argininosuccinate lyase; CP score, Child–Pugh score; FLSPC, fetal stem/progenitor cell; hBTSC, human biliary tree stem cell; HPC, hepatocyte progenitor cell; HSC, hematopoietic stem cell; iPSC, induced pluripotent stem cell; LDL, low-density lipoprotein; MELD, Model for End-Stage Liver Disease; MSC, mesenchymal stem cell; OTC, ornithine transcarbamylase; PFIC, progressive familial intrahepatic cholestasis; RCT, randomized control trial; SAR, serious adverse reaction; SUSAR, suspected unexpected serious adverse reaction.

ameliorate liver function.[13] Therefore, hepatocyte transplantation has only shown utility to bridge to OLT. Experiments in rodents have shown hepatocytes transferred via the hepatic portal vein cause significant thrombosis and ischemia-reperfusion injury.[14] Even when intrasplenic approach is adopted, engraftment of hepatocytes is extremely limited as over 90% of transplanted cells are phagocytosed by Kupffer cells (KCs).[15]

Since adult hepatocytes have limited availability, there is a worldwide effort to produce functional hepatocytes from pluripotent cells to generate a potentially limitless source of hepatocyte-like cells (HLCs) for drug screening and medical use.[16] Recently, there have been great advances producing HLCs that recapitulate the biology of bona fide adult hepatocytes.[17] For instance, HLCs can be derived from induced pluripotent stem

cells (iPSCs) or embryonic stem cells (ESCs). In vitro studies demonstrate that HLCs can be generated in a stepwise protocol from iPSCs.[18,19] iPSC-derived cells could be sourced from individual patients to allow production of autologous cells for transfer back into the same patient.[20,21] However, these have not been tested yet in clinical trials for acute or chronic liver disease. In parallel, fetal hepatocytes have been proposed as an alternative strategy to improve engraftment and function. Preclinical studies provided evidence of fetal hepatocyte metabolic function in rats.[22,23] In patients, allogenic fetal hepatocytes were transplanted intraperitoneally to seven patients with ALF but led to recovery in only three subjects with advanced encephalopathy.[24] Several limiting factors preclude hepatocyte therapy as an effective therapeutic strategy for liver disease including risk of thrombosis, the unstable ex vivo phenotype, and poor hepatocyte engraftment.[14,25,26] To mitigate these limitations, alternative cell types have been considered, for example, hepatocyte progenitor cells (HPCs). HPCs are found in adult livers in the canal of Hering and can become activated after liver injury to repopulate the organ with functional hepatocytes or biliary cells.[27] HPCs are an attractive option for cell transplantation due to their small size (5–15 μm), reduced risk of embolism, ease of cryopreservation, tolerability toward ischemia, and minimal immunogenicity.[27] However, HPCs are a relatively scarce cell type in adult liver, and engraftment represents a major obstacle for clinical use, although recent work showed coating the cells with hyaluronic acid provided a modest improvement in engraftment in mice.[28] Furthermore, the differentiation of human HPCs to mature hepatocytes has not yet been convincingly demonstrated. HPCs are present in fetal liver in high numbers and can be easily isolated and cultured. Therefore, fetal human biliary tree stem cells (hBTSCs) have been considered for HPC therapy.[29] hBTSCs have been tested in two case reports in cirrhotic patients after transplantation of 4 to 6×10^7 cells via the hepatic artery. Although this technique appeared safe, biochemical improvements were only transitory suggesting limited efficacy.[30] Another approach involves use of fetal stem/progenitor cells (FLSPCs), highly proliferative precursor cells of endodermal origin with the capacity to form hepatocytes and bile duct epithelial cells (sourced from rats at ED14). Although FLSPCs have not yet been tested in humans, they have been shown to repopulate large areas of rat parenchyma, maintain high proliferation rates, and retain differentiation potential posttransplantation even after cryopreservation.[31,32] In contrast to the poor replicative capacity of ex vivo hepatocytes, hematopoietic stem cells (HSCs) are highly proliferative with the ability to transdifferentiate into mature hepatocytes thereby representing an appealing cell therapy for liver disease.[33–35] A randomized-controlled trial of HSC-like bone marrow-derived mononuclear cell transfer to 30 cirrhotic patients on the OLT waiting list suggested transient improvement of albumin in treated subjects. However, this was not statistically significant and the overall liver function did not improve as per the Child–Pugh score.[36] Moreover, a U.K.-based clinical trial recently reported that CD-133+ HSC therapy in conjunction with granulocyte-colony stimulating factor (G-CSF) did not improve liver function in cirrhotic patients.[37] Mesenchymal stem cells (MSCs) are multipotent cells that can be readily isolated from adult bone marrow or umbilical cord tissue and can expand in vitro with capacity to differentiate into several lineages including hepatocytes.[38–40] MSCs are known to exhibit immunomodulatory functions and have been shown to reduce inflammation, reduce injury, and protect against hepatocyte apoptosis in several liver injury (acute liver injury [ALI]) models.[41–45] MSCs have also been tested in cirrhotic patients demonstrating minor improvements in liver synthetic function and Model for End-Stage Liver Disease (MELD) score, although MSCs require further evaluation in larger clinical trials with predefined primary endpoints.[46–49] Studies to date have involved small numbers of patients with short-term endpoints, thus evidence of long-term benefit and a clear understanding of the mechanism of action of MSCs is required.[50] In summary, various cell therapies hold potential to provide new strategies to treat liver disease. However, many cell therapy candidates are not yet ready to be tested in clinical trials. Engraftment represents a major obstacle in diseased liver tissue for various cell therapies designed to improve hepatic function. Further work to elucidate the mechanisms of action of each cell therapy candidate will help design early clinical trials to test safety and efficacy. Data from clinical trials are currently at an early stage and have only showed limited success at best so far.[49,51–53]

Role of Macrophages in Liver Disease

The liver contains the largest population of tissue resident macrophages in the body in the form of KCs located within the hepatic sinusoids.[54,55] In the steady state, KCs provide important hepatic innate immunity by efficiently phagocytozing gut-derived pathogens (e.g., *Escherichia coli* and bacterial products) from portal blood, thereby providing an immunological barrier between the gut and the systemic circulation.[56] KCs are also highly adapted to remove apoptotic debris (principally dead erythrocytes), particulate matter, and are involved in the clearance of several serum proteins (►**Fig. 1A** and **B**).[57,58] KCs have been shown to be implicated in the early activation of the innate immune system after a hepatotoxic event, for example, during APAP overdose. Hepatocyte necrosis releases a plethora of proinflammatory signals, including several danger-associated molecular patterns (DAMPs), chemokines, and cytokines that can activate resident macrophages via Toll-like receptor signaling resulting in the recruitment of circulating monocytes and other inflammatory cells to the liver.[59,60] However, recent studies have shown in ALI, there is a substantial loss of KCs at peak injury leading to a deficit in hepatic innate immunity.[61,62] During APAP-induced liver injury, uncontrolled inflammation resulting from massive hepatocyte necrosis coupled with KC loss results in a sepsis-like condition termed systemic inflammatory response syndrome (SIRS)—a major determinant of clinical outcome in patients with APAP-induced ALI.[63] Likewise, in chronic end-stage liver disease, when KC-mediated barrier function is diminished, patients are prone to developing bacterial and fungal infections representing a major trigger of acute-on-chronic liver failure.[64,65] Clearly, liver resident KCs are critical in maintaining important

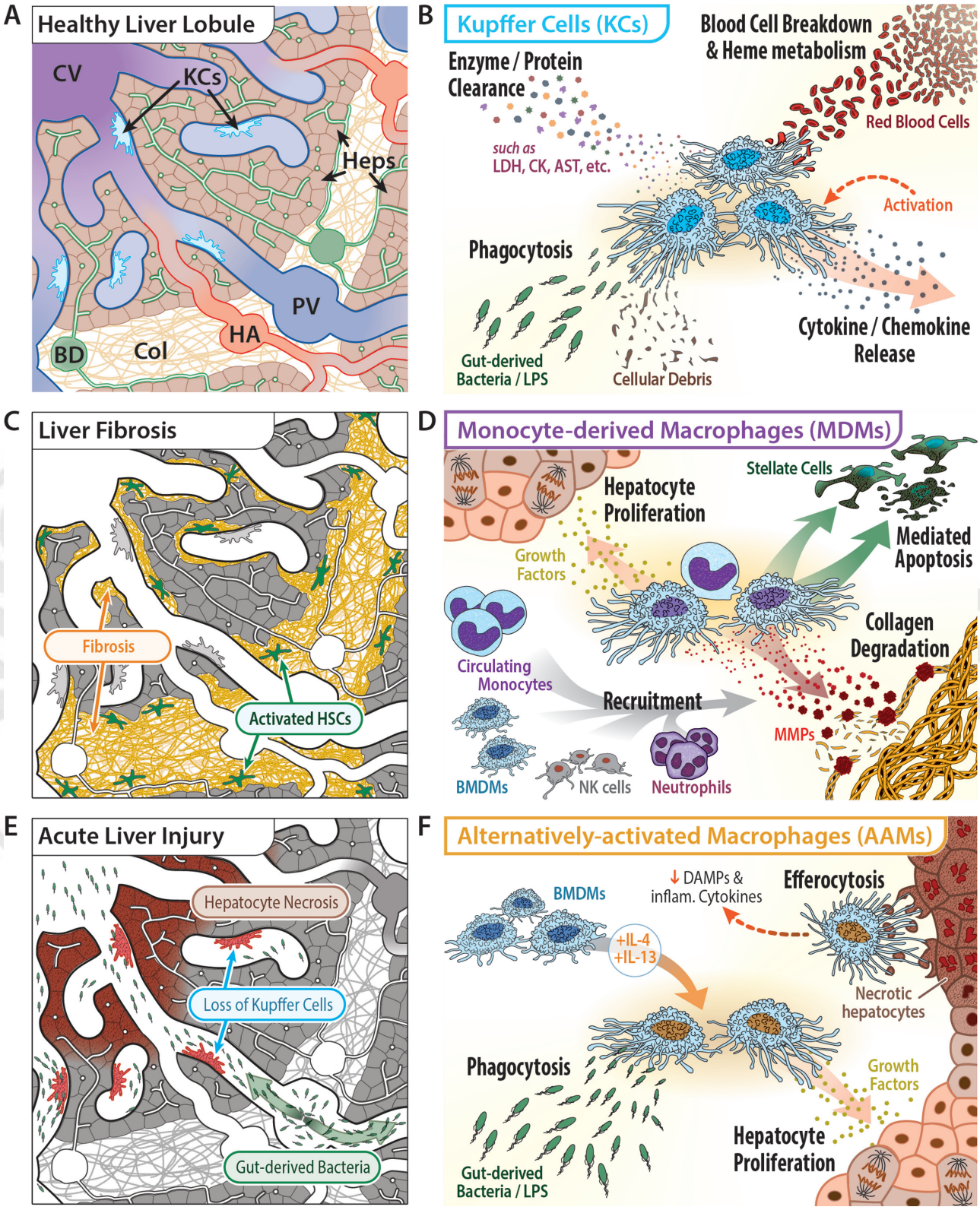

Fig. 1 [Q4](A) Kupffer cells (KCs) are liver resident macrophages located within the hepatic sinusoids that comprise part of the mononuclear phagocyte system. (B) KCs possess several important functions in the steady state including providing barrier function against gut-derived bacteria, scavenging of damaged/aged erythrocytes, and clearance of several serum proteins. (C) During chronic liver disease, hepatic myofibroblasts (activated hepatic stellate cells) deposit excessive amounts of collagen replacing hepatocytes impacting liver function. (D) Transfer of bone marrow-derived macrophages (BMDMs)/monocyte-derived macrophages (MDMs) have shown efficacy in liver fibrosis models with evidence of collagen regression, myofibroblast apoptosis, and enhanced recruitment of innate immune cells. (E) During acute liver injury, a transient loss of KCs occurs alongside massive hepatocyte necrosis causing a deficit in barrier function. (F) Supplementing the macrophage pool during acute liver injury is a potential strategy to restore hepatic barrier function. Alternatively activated macrophages (AAMs) in particular possess a high capacity for efferocytosis to resolve necrosis and in turn reduce inflammation and promote liver repair.

innate immunity and are fundamentally implicated in the pathology of liver disease.

Macrophage Therapy for Liver Fibrosis

Chronic liver disease is fundamentally distinct pathology from ALI resulting from long-term iterative injury with an inflammatory basis arising from various etiologies. Ultimately, chronic liver disease results in the deposition of large quantities of extracellular matrix (ECM). The substitution of healthy parenchyma with scar tissue (ECM proteins comprising mainly collagens) can develop into cirrhosis characterized by reduced liver function, portal hypertension, and related complications.[66] Human and mouse macrophages express several members of the matrix metalloproteinase (MMP) family of endopeptidases, including MMP8, MMP9, and MMP12.[67–71] A subset of MMPs have properties allowing them to unwind and cleave collagen helices affording them collagenolytic activity, a natural process that occurs in development and wound healing.[72] Therefore, macrophages represent a key cell type implicated in the metabolism of ECM and tissue remodeling.[73] The deranged architecture, inflammatory niche, and excessive ECM in fibrotic liver provide significant barriers for the engraftment and long-term functionality of transplanted cells. Novel strategies that target the existing hepatic scar tissue directly, using either cells or biologics, are gaining attention as an alternative to hepatocyte transplantation.[74] Novel carrier systems (e.g., liposomes[75] or mannosylated conjugated proteins[76]) have been developed to target endogenous KCs to modulate macrophage function in situ (for expert review on targeting endogenous macrophages for liver disease, refer to Tacke[77]). In parallel, transfer of exogenous macrophages (e.g., syngeneic, autologous, or allogeneic cells) represents an alternative technique to modulate the hepatic microenvironment. Intravenous injection of bone marrow-derived macrophages (BMDMs) to mice with established carbon tetrachloride (CCl_4)-induced liver fibrosis resulted in less collagen deposition, fewer hepatic myofibroblasts (activated hepatic stellate cells), and enhanced recruitment of host monocytes and neutrophils —a further source of MMP9.[78] Importantly, liver synthetic function was improved in fibrotic mice after BMDM delivery evidenced by increased serum albumin levels. In this study, macrophages were injected via the hepatic portal vein, a dosing route that may be unsuitable in cirrhotic patients due to associated coagulopathy and portal hypertension. However, a more recent study showed that transfer of classically activated macrophages (CAMs) administered to fibrotic mice via tail vein also resolved collagen efficiently.[79] Further mechanistic insight from this study revealed that recruited natural killer cells were a major source of tumor necrosis factor-related apoptosis-inducing ligand that promoted myofibroblast apoptosis. In addition, intravenous delivery of murine ESC-derived macrophages recapitulated fibrosis resolution observed with primary macrophages, although a greater number of cells were required to achieve efficacy.[80] These studies provide evidence that disease-modifying macrophages can be administered peripherally. Translational studies have since demonstrated that primary human monocyte-derived macrophages (MDMs) sourced from healthy donors have antifibrotic activity after intrasplenic cell transfer to fibrotic immunocompromised mice.[81] However, safety concerns exist using myeloid cell transfer approaches given that several groups have reported injurious responses after transplanting immature cell types in disease models, for example, bone marrow precursor cells and monocytes.[78,82,83] These findings underscore the importance of using defined protocols that yield highly enriched populations of fully mature cells qualified by a robust set of maturity markers.

In contrast to the emergency setting of ALF, the relatively slow progression of compensated liver cirrhosis (median survival $>$ 12 years[8]) provides a therapeutic window available over a longer timeframe assuming complications can be managed and disease-inducing factors controlled (e.g., antiviral medication, cessation of alcohol consumption). Therefore, this timeframe allows the collection of a patient's own monocytes for macrophage differentiation (typically 7 days) before infusion back into the patient, that is, autologous cell therapy. Moore et al demonstrated that MDMs sourced from cirrhotic patients are phenotypically similar to healthy donor-derived macrophages in terms of MMP expression and surface marker composition.[81] Furthermore, intrasplenic transplantation of healthy human MDMs to an immunocompromised mouse model of liver fibrosis elicited regression of collagen, and reduction of liver injury markers. This work provided the platform to build a GMP-compatible pipeline to generate clinical-grade human MDMs for potential therapeutic applications.[84] A differentiation protocol now exists with clear release criteria for functionally mature human macrophages (25F9^{hi}, CD206^{hi}, CCR2^{lo}) using a defined serum-free, antibiotic-free method. Safety and efficacy studies of autologous macrophage therapy are now underway in a Phase I/II first-in-human clinical trials for the treatment of liver cirrhosis. Exogenous macrophage delivery has shown promise in preclinical models to elicit collagen regression and stimulate hepatic function (►**Fig. 1C** and **D**).

Macrophage Efferocytosis and Acute Liver Injury

Macrophages are exquisitely adapted to recognize and remove dead or dying cells from the system. Macrophages, including KCs, express a repertoire of cell surface receptors including Mer,[85] phosphatidylserine receptors,[86] lectins,[87] and scavenger receptors[88] that recognize motifs on dying cells to initiate and facilitate their internalization and degradation. Macrophage-mediated removal of dead cells, known as efferocytosis coined from the Latin term "efferre": "to bury," is thought to be a prerequisite for the resolution of inflammation by clearing the inflammatory source to allow the restitution of injured tissue.[89,90] During liver injury (e.g., during APAP overdose), there is a sudden and massive chemical insult to the liver, causing widespread hepatocyte necrosis that occurs rapidly after drug ingestion.[91] In the clinic, severe toxicity can be mitigated via the timely infusion of N-acetylcysteine (NAC; a sulfhydryl donor that boosts hepatocyte antioxidant capacity to prevent hepatocyte

death). However, in patients who present late (i.e., later than 10 hours of APAP ingestion), NAC has much reduced efficacy,[92] and APAP-induced liver injury (APAP-ALI) can result in more than 50% hepatocyte necrosis providing a massive source of inflammatory mediators and DAMPs.[59,93,94] In addition to hepatocyte necrosis, recent evidence showed there is a transient but significant loss in viable KCs at peak injury, which results in a diminished barrier function and reduced phagocytic capacity in the liver.[61,62] The gut lumen contains a huge source of bacteria and bacterial products, which can translocate to the liver parenchyma via the hepatic portal vein. KCs provide important innate immunity by recognizing and engulfing bacteria to maintain homeostasis.[55] A recent study described a further population of MDMs that exist in the liver capsule to provide innate immunity against peritoneal pathogens.[95] APAP-ALI is known to drastically reduce numbers of hepatic KCs, and therefore impair the performance of the mononuclear phagocyte system in the liver.[62] For example, the clearance of circulating microaggregated albumin is compromised in patients with APAP-ALF.[61] Indeed, the lack of innate immunity predisposes ALF patients to risk of developing serious bacterial and/or fungal infections.[96] In a study involving 50 ALF patients, 28 out of 30 patients that died had a detectable bacterial infection and all the deaths that occurred after 7 days of hospital admission were attributed to microbial infection.[97] Furthermore, a separate study reported fungal infections (candida and aspergillus) in 32% of ALF patients, in which fungal infection led to a major cause of death in 7 of the 16 infected patients.[98] Indeed, antimicrobial drugs have been trialed as a prophylactic treatment to ALF-associated infections but have resulted in only marginal benefit.[98,99] These reports suggest that impaired barrier function resulting from ALI risks the development of serious systemic infections that are a major determinant of clinical outcome.

The combination of massive hepatocellular necrosis and diminished clearance functions promotes uncontrolled inflammation. In APAP-induced ALF patients, this is recognized as SIRS and represents a key determinant of clinical outcome risking multiorgan failure and death.[63,100] Several experimental strategies to modulate hepatic immune function during ALI are now gaining attention.[101] Tissue macrophage populations are controlled, in part, through CSF 1 receptor (CSF1R) stimulation, which promotes the survival, proliferation, and differentiation of cells in the macrophage lineage.[102] Mice treated with a modified CSF1 fusion protein (CSF1-Fc) demonstrated enhanced hepatic clearance capacity by increasing numbers of both resident and infiltrating macrophages in the liver.[103] Importantly, CSF1-Fc treatment also increased macrophage accumulation at the necrotic regions and reduced serum alanine aminotransferase activity in mice with APAP-ALI. The hepatoprotective role of macrophages has been demonstrated by several groups. Chemical ablation of KCs in mice exhibited aggravated hepatic vascular permeability in liver sinusoidal endothelial cells (LSECs) after APAP-ALI.[104] Furthermore, mice lacking KCs and infiltrating MDMs show sustained necrosis and elevated serum transaminases after APAP-ALI, suggesting macrophages are required for necrosis resorption.[105–107] Timely removal of necrotic tissue is required for appropriate wound healing. Macrophages also possess paracrine functionality and are a major source of anti-inflammatory cytokines and growth factors. KCs can secrete interleukin (IL)-10 upon activation, a potent immunosuppressive cytokine that has been shown to be hepatoprotective during APAP-ALI.[107–109] Infiltrating macrophages in particular have also been shown to express high levels of Vegf, a proangiogenic cytokine associated with neovascularization in chronic injury models.[110] Hepatic macrophages isolated from APAP-injured mouse liver stimulated LSEC proliferation and migration in vitro suggesting paracrine proangiogenic function.[105] Macrophages are also known to express several WNT ligands,[111,112] which are known to stimulate β-catenin signaling in hepatocytes during liver regeneration.[113] CSF1R-mediated hepatic macrophage accumulation induced a modest but significant increase in hepatocyte proliferation after partial hepatectomy suggesting macrophages may promote parenchymal cell division, although specific factors underpinning this have not been identified.[103] Macrophages can adopt a variety of phenotypes in response to their microenvironment. Alternatively activated macrophages (AAMs) have been shown to exhibit a greater capacity for efferocytosis in vitro (discussed further below).[114] Boosting the hepatic macrophage pool to restore hepatic innate immunity, promote efferocytosis of necrotic cells, suppress inflammation, and stimulate hepatocellular proliferation may be an attractive strategy for the treatment of ALI (►**Fig. 1E** and **F**).

Relevance of Macrophage Phenotype and Function

Macrophages are an inherently plastic cell type capable of acquiring a spectrum of phenotypes in response to stimuli from the microenvironment. Traditionally, this phenotypic axis was defined simplistically as "M1" macrophages (classical-activation with enhanced bactericidal properties), versus "M2" macrophages (alternative-activation with enhanced tissue remodeling properties), which has provided a useful framework despite calls for a more nuanced nomenclature.[115] Functional analysis of different macrophage phenotypes can be achieved by polarizing cells in vitro using defined factors (e.g., lipopolysaccharide and interferon γ to produce CAMs, or IL-4/-13 to produce AAMs). As discussed earlier, CAMs outperformed standard BMDMs in terms of their role in collagen regression.[79] Polarized macrophages may offer greater efficacy or improved safety profiles since a polarized cell population is phenotypically more uniform. One safety concern that exists with macrophage therapy is the risk of transplanted macrophages acquiring a potentially deleterious phenotype in response to microenvironmental cues in a diseased organ. Polarizing macrophages ex vivo using high concentrations of recombinant cytokines prior to transplant may reduce this risk since there is some evidence that polarized macrophages can retain their phenotype epigenetically. [116] Safety studies that test macrophage phenotype and persistence in relevant disease models are warranted. In the setting of ALI, efficient efferocytosis of necrotic material is required to suppress

inflammation. Numerous groups have reported that AAMs have enhanced phagocytic function versus standard BMDMs or CAMs.[80,117,118] Therefore, transferring AAMs or by promoting hepatic macrophage phagocytosis may represent a therapeutic strategy in the setting of ALI.

Highly defined macrophages with desired characteristics may indeed provide more precise therapy. Tissue-resident macrophages are known to display unique gene expression profiles with considerable diversity among macrophage populations.[119] While macrophages share a common set of functions, tissue-specific functions do exist, for example, osteoclasts perform efficient bone resorption in contrast to microglia, which support neuronal circuit development.[120,121] In the liver, KCs are highly specialized at removing damaged erythrocytes from the circulation.[122] KCs express several genes involved with lipid and iron metabolism including several scavenger receptors, which are enriched in KCs versus other macrophage populations.[123] Tissue-resident macrophages, including KCs, develop from embryonic precursors with the capacity to proliferate and self-renew.[124,125] During APAP-ALI, approximately half of KCs are lost at peak injury but recover through proliferation over several days.[62] During this time, circulating inflammatory monocytes infiltrate the liver and differentiate into short-lived MDMs. Blood monocytopenia resulting from massive influx of circulating monocytes into the liver has been associated with poor prognosis in patients.[126] It has been shown experimentally that MDMs can repopulate the liver and acquire self-renewal properties, but only under specific conditions.[123] ESC-derived macrophages may resemble tissue-resident macrophages more closely with lower expression levels of *Myb* (a HSC transcription factor) compared with BMDMs.[80] The source of exogenous macrophages may have implications on the phenotype, function, and persistence of these cells in tissues after administration.

In summary, evidence suggests that macrophages play a key role in the initiation and resolution phases of both acute and chronic liver disease. The barrier function provided by KCs is essential to prevent bacteremia and systemic inflammation. Supplementing hepatic macrophage populations using exogenous cell transfer or by cytokine-induced endogenous macrophage expansion are clinically relevant strategies that have the potential to augment hepatic innate immunity during liver disease. Methods to generate clinical-grade primary human macrophages have recently been described allowing these cells to be evaluated in prospective clinical trials.

Main Concepts and Learning Points

- Kupffer cells are liver resident macrophages that possess several important functions in liver tissue, including providing barrier function against gut-derived pathogens.
- Macrophages play distinct roles in the initiation and resolution phases of liver injury, therefore are intrinsically implicated in liver disease pathophysiology.
- Patients with both acute and chronic liver disease have a perturbed phagocytic system thereby being at risk of developing serious bacterial/fungal infections.
- Strategies that restore hepatic innate immunity during liver disease through direct cell transfer or cytokine-induced macrophage replacement are gaining attention.
- Primary human macrophages can now be manufactured to meet GMP standards and clinical trials to test safety and efficacy in liver disease are underway.

Conflict of Interest[Q3]

Dr. Starkey Lewis has a patent PCT/GB2017/052769 pending. Dr. Forbes has a patent PCT/GB2017/052769 pending, and a patent UK application ref 1804255.6 pending.

Q3

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