Review

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Inflammation and portal hypertension – The undiscovered country

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Summary

Portal hypertension has traditionally been viewed as a progressive process, involving ultrastructural changes including fibrosis, nodule formation, and vascular thrombosis, leading to increased intrahepatic resistance to flow. However, it is increasingly recognized that a significant component of this vascular resistance results from a dynamic process, regulated by complex interactions between the injured hepatocyte, the sinusoidal endothelial cell, the Kupffer cell and the hepatic stellate cell, which impact on sinusoidal calibre. Recent findings suggest these haemodynamic findings are most marked in patients with superimposed inflammation. The precise mechanisms for vascular dysfunction in cirrhosis with superimposed inflammation remain to be fully elucidated but several studies over the past decade have started to generate the hypothesis that inflammation may be a key mediator of the pathogenesis and severity of portal hypertension in this context. This review provides a comprehensive overview of the biological mechanisms for inflammation playing a key role in the severity of portal hypertension, and illustrates potential novel therapies that act by modifying these processes.

Introduction

Portal hypertension is a milestone in the progression of cirrhosis and heralds the onset of the most fatal complications of liver disease such as variceal haemorrhage (VH), hepatic encephalopathy, and ascites. The pathobiology of portal hypertension involves changes in hepatic architecture leading to increased intrahepatic resistance to flow. Furthermore, insights into the vascular biology of cirrhosis have demonstrated that a significant proportion of intrahepatic resistance is modifiable, as a consequence of sinusoidal endothelial dysfunction and the effect of contractile myofibroblasts and pericytes [1,2].

The development of robust techniques for the measurement of sinusoidal pressure, through the hepatic venous pressure gradient (HVPG), led to landmark observations into the natural history of portal hypertension, and clear associations were found between the degree of portal hypertension, and complications of cirrhosis and mortality [3,4]. However, the contextual basis of these longitudinal studies was in 'early' cirrhosis, prior to the development of complications of advanced cirrhosis such as bacterial infection and renal failure. Recent insights into the natural history of cirrhosis have led to a re-appraisal of the pathophysiological basis of portal hypertension in advanced cirrhosis. Indeed, it is increasingly recognized that the description of cirrhosis represents a diverse group of patients, with varying degrees of hepatic fibrosis and systemic manifestations [5].

These observations are complimented by the recent description of the syndrome of acute-on-chronic liver failure (ACLF), where hepatic and systemic inflammation lead to an acute deterioration of liver function, regardless of underlying stage of cirrhosis, either secondary to superimposed liver injury or due to precipitating factors such as infection [6]. The large, prospective CANONIC study defined ACLF as an acute decompensation of cirrhosis, associated with (i) single- or multi-organ failure, and (ii) high 28-day mortality (>15%) [7]. Organ failure was defined based on a modified SOFA score adapted for patients with cirrhosis (CLIF-SOFA score). Thus, ACLF is distinguished from acute decompensation of cirrhosis (AD) by the presence of organ failure, associated with a marked systemic inflammatory response, leading to a high short-term mortality. Conceptually, the development of ACLF marks a departure from the traditional

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stepwise view of progression of cirrhosis and portal hypertension. In the CANONIC study, patients with previously well-compensated cirrhosis had a significantly *higher* mortality following the development of ACLF than those with decompensated cirrhosis, marking a sharp contrast to the dogma of progressive liver disease. Moreover, patients with ACLF have been shown to have the highest portal pressures [8,9], although the CANONIC data also demonstrated that GI bleeding is not a major feature of ACLF, suggesting that the pathophysiological relevance of portal hypertension and intrahepatic resistance in ACLF may relate to decreased liver perfusion and consequently liver failure.

This aim of this review is to describe recent developments in the pathobiology of portal hypertension, in the context of these recent insights into ACLF. This article is timely, since it builds on this work defining ACLF and describing the key role of innate immunity and inflammation in the evolution of this syndrome. As such, this review seeks to delineate the role of innate inflammation on portal hypertension, and describes novel strategies and potential targets for therapy.

Key Points

- Portal hypertension is associated with bacterial translocation (BT) and innate immune activation in cirrhosis

- Inflammation is thought to play a causal role in portal hypertension since bacterial infection increases portal pressure, and antibiotics and anti TNF-α therapy decrease portal pressure

- Mechanisms include BT leading to TLR4-mediated Kupffer cell activation and oxidative stress, with downstream effects on eNOS function and stellate cell contractility

- Novel therapies for portal hypertension acting on these processes include rifaximin, FXR agonists, statins and TLR4 antagonists

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Bacterial translocation, portal hypertension, and variceal haemorrhage

It is increasingly recognised that the unique anatomical location and vascular supply of the liver lends itself to frequent exposure to intestinal bacteria and bacterial products, particularly in the context of advanced cirrhosis and portal hypertension [10]. A broader role for gut microbiota in the development of complications of cirrhosis, such as hepatic encephalopathy and spontaneous bacterial peritonitis (SBP), has been recognised for some years. However, the immunobiology of gut bacterial translocation (BT) has only recently become a target of scrutiny, with downstream innate inflammatory responses being shown to play a role in processes such as hepatic fibrogenesis and carcinogenesis in animal models.

Bacterial translocation (BT) is defined as the passage of both viable and non-viable microbes and microbial products, such as endotoxin, from the intestinal lumen through the mucosa into mesenteric lymph nodes (MLNs) and other organs. As such, it is increasingly evident that BT is common in cirrhosis, and may

be a pathogenic event in several complications of cirrhosis. It has been shown that BT occurs in approximately 30–40% of patients with advanced cirrhosis. Indeed, positive bacterial cultures of mesenteric lymph nodes were found in 30.8% of patients with Child-Pugh C cirrhosis, compared to 8.6% of non-cirrhotics [11]. Similarly the surrogate marker of BT, lipopolysaccharide (LPS)-binding protein (LBP), was observed to be increased in 42% of cirrhotic patients [12]. In rodents, acute portal hypertension due to portal vein ligation precipitates BT [13], and in humans the degree of portal hypertension predicts the occurrence of SBP [14], suggesting that portal hypertension plays a key role in the development of BT. It has also been recognized for some years that bacterial infections are associated with a poorer prognosis from variceal haemorrhage (VH) [15]. Moreover, BT is associated with other portal hypertension-related complications, such as hepatic encephalopathy and spontaneous bacterial peritonitis (SBP) [10].

Downstream signals following BT are numerous and complex, but the immediate and dominant pathways are highly conserved innate immune signals stimulated by exposure to microbial products, or pathogen-associated molecular patterns (PAMPs) leading to activation of Toll-like receptors (TLRs) on parenchymal and non-parenchymal cells (Fig. 1). These receptors are widely expressed in the liver, but Kupffer cells (KCs) are the primary cells that respond to PAMP exposure, and adopt a pro-inflammatory phenotype through TLR-mediated signalling, producing cytokines such as TNF-α, IL-1, IL-6, and IL-12 [16]. This dysregulated pro-inflammatory cytokine response to BT is associated with severe portal hypertension in cirrhosis. Serum bacterial DNA levels, as a surrogate marker of BT, are correlated with severity of inflammation and portal hypertension in cirrhosis [17]. Moreover, in patients with SBP, elevated levels of catecholamines and TNF-α are associated with higher HVPG [18].

A causal relationship between BT-mediated inflammation and portal hypertension is further suggested in rodent models, where the administration of bacterial LPS leads to exacerbation of portal hypertension [19], whereas the use of both norfloxacin and rifaximin decrease complications of cirrhosis [20,21]. In humans, two studies demonstrate a beneficial effect of antibiotics on portal pressure, although neither is placebo-controlled [22,23]. The controlled studies of antibiotics in portal hypertension failed to show any benefit, although both showed a trend towards HVPG reduction, suggesting they were inadequately powered to demonstrate an effect [12,24]. Direct inhibition of TNF-α in patients with ACLF due to alcoholic hepatitis (AH), although not adopted due to increased overall rates of infection, has also been shown to lead to a sustained reduction in portal pressure [25].

Therefore, BT-mediated inflammation is suggested to be an important mediator of portal hypertension in advanced cirrhosis. Several markers of systemic inflammation are elevated in advanced cirrhosis, and correlate with portal hypertension and mortality, including serum CRP and IL-6 levels [26,27]. The mechanisms whereby an enhanced pro-inflammatory cytokine response to BT in cirrhosis may potentiate vascular dysfunction and intrahepatic resistance in cirrhosis are discussed below.

Mechanisms of intrahepatic resistance in hepatic inflammation

The hallmark of cirrhosis is nodular fibrosis and scarring, leading to architectural distortion of sinusoidal blood flow, however the
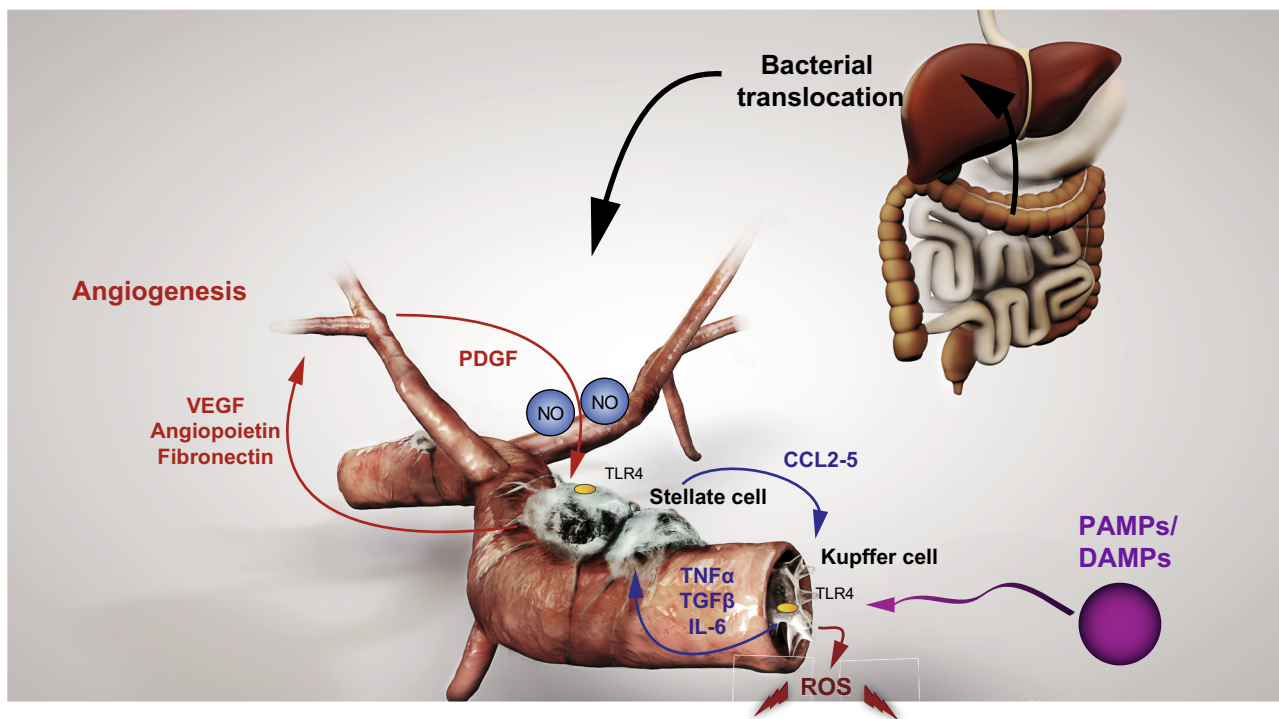
Fig. 1. The role of bacterial translocation (BT) in the pathobiology of portal hypertension. Gut-derived bacterial products (PAMPs) stimulate the hepatic innate immune system through toll-like receptor (TLR) 4 signaling, predominantly on hepatic stellate cells (HSCs) and Kupffer cells (KCs). TLR-4 mediated stimulation of HSCs leads to HSC activation and a fibrogenic, contractile phenotype, as well as KC activation through paracrine chemokine secretion (CCL2-CCL5). In turn, KCs produce TGF-β, stimulating fibrogenesis, and the pro-inflammatory cytokines TNF-α and IL-6, propagating hepatic inflammation. KCs also produce reactive oxygen species (ROS), leading to the generation of other reactive nitrogen species and local tissue damage. HSCs also interact with sinusoidal endothelial cells (SECs) in the sinusoidal niche. The SEC tonically produces nitric oxide (NO), which maintains the HSC in a quiescent phenotype. A reduction in SEC-derived NO production contributes to HSC activation and consequent fibrosis/HSC contractility. The activated HSC produces local mediators (VEGF, angiopoietin-1), which stimulate angiogenesis in the SEC and other local cells, which in turn propagates portal hypertension.

key role of intrahepatic vascular tone in regulating sinusoidal pressure is well established. Molecular mechanisms of this increase in vascular tone include an imbalance of vasodilator and vasoconstrictor compounds, dysfunction of sinusoidal endothelium, and activation of contractile elements in vascular smooth muscle, portal myofibroblasts, and hepatic stellate cells (HSCs). Nitric oxide (NO) has been demonstrated to be a key regulator of intrahepatic vascular tone, and NO production from endothelial nitric oxide synthase (eNOS) in the sinusoidal endothelial cell (SEC) is decreased in cirrhosis [28,29]. However, eNOS protein levels remain unchanged, suggesting that NO production is reduced due to either post-translational modification of eNOS enzyme, such as decreased eNOS phosphorylation, or altered levels of endogenous eNOS cofactors/inhibitors. Several of these have been described in cirrhosis, including elevated levels of eNOS inhibitors asymmetric dimethylarginine (ADMA) and caveolin-1, and decreased levels of the eNOS co-factor tetrahydrobiopterin [30–32] (Fig. 2).

Following its generation in SECs, NO modulates vascular tone through a vasodilator effect on adjacent vascular smooth muscle. However, intrahepatic vascular tone is also regulated by HSCs, which adopt a myofibroblastic phenotype upon activation [2]. These activated HSCs have extensive coverage of the sinusoidal network through cellular extensions and can modulate intrahepatic resistance through contractility. Activated HSCs are

responsive to endogenous vasoconstrictors (e.g., endothelins, norepinephrine, angiotensin II, leukotrienes, thromboxane A2) leading to increased contractility and intrahepatic resistance [33–36]. The intrahepatic vasculature displays increased sensitivity to these vasoconstrictors in cirrhosis. Additionally, the activated HSCs play a key role in angiogenesis, leading to intrahepatic shunting and vascular collateral formation [37,38].

Hepatic innate immune signaling has been suggested to contribute to portal hypertension through effects on fibrosis, and on intrahepatic vascular tone. The role of PAMPs in the progression of fibrosis, in particular through TLR4 signaling, has been extensively studied. TLR4 is expressed on both parenchymal and non-parenchymal cell types in the liver, and its signaling is involved in liver injury induced by viral hepatitis, alcoholic and non-alcoholic steatohepatitis, and cholestatic and drug-induced liver diseases [16]. Several animal studies support the importance of TLR4 in liver fibrosis. Knockout mice that are deficient in TLR4, or in other signaling molecules of the TLR4 pathway such as CD14, LBP, MyD88, and TRIF, have less liver fibrosis induced by bile duct ligation (BDL) or carbon tetrachloride (CCl_4) than wild type [21,39,40]. Selective decontamination of gut flora also suppresses the increase in plasma LPS and attenuates liver fibrosis in these rodent models [39].

Although the TLR4 signaling pathway is involved in fibrosis, the elegant experiments by Seki *et al.* demonstrate that this is a
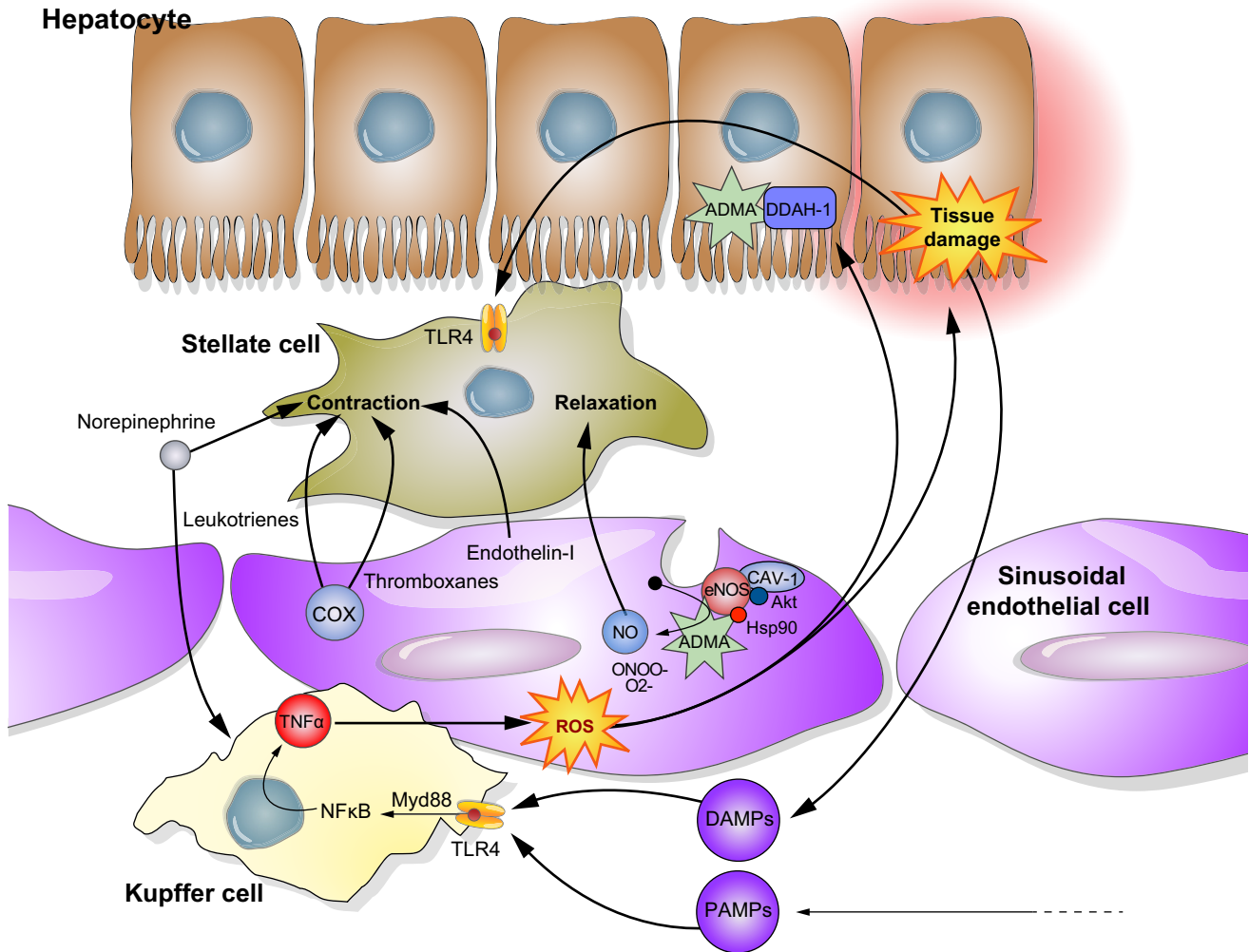
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Fig. 2. Nitric oxide (NO) regulates intrahepatic vascular tone, through maintaining hepatic stellate cells (HSCs) in a quiescent phenotype and promoting vasodilatation through cGMP signaling. Asymmetric dimethylarginine (ADMA) is a paracrine, competitive inhibitor of NO synthesis by endothelial nitric oxide synthase (eNOS), and is metabolized in the hepatocyte by dimethylarginine dimethylaminohydrolase-1 (DDAH-1). Inflammation leads to ROS generation by KCs, which inhibits DDAH-1 activity thereby leading to eNOS inhibition by ADMA and decreased local NO production. Other molecules such as Caveolin and Akt also contribute to inhibition of eNOS activity. ROS also interact with free NO generating further reactive nitrogen species (RNS) contributing to local tissue damage and propagating innate immune signaling through DAMPs. The activated SEC also produces further vasoactive mediators such as endothelin-1 and thromboxanes/leukotrienes, which increase HSC contractility thereby increasing intrahepatic resistance. Stimulation of Kupffer cells and stellate cells by pathogen associated and damage associated molecular patterns (PAMPs and DAMPs) further accentuates inflammation and generation of ROS, which acts in a feed-forward cycle exacerbating HSC activation and severity of portal hypertension.

KC-independent process [39]. By contrast, in more advanced cirrhosis, KCs play a more prominent role in the development of hepatic inflammation and oxidative stress, leading to increased intrahepatic resistance. In alcoholic liver disease (ALD), TLR signaling on KCs leads to the production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-8, initiating both hepatic and systemic inflammation [41]. A further downstream effect of TLR activation on KCs is the production of reactive oxygen species (ROS) [42]. KCs also produce vasoactive mediators, predominantly from the cyclooxygenase-thromboxane A2 pathway, in response to PAMPs. LPS administration to cirrhotic rats leads to production of thromboxane A2 and cysteinyl leukotrienes, and augmented portal hypertension. Moreover, both KC depletion and treatment with the leukotriene antagonist montelukast abrogate portal hypertension in this model [19,43]. There is also evidence of KC activation in humans – in cirrhotic patients a serum

marker of KC activation, sCD163, has been shown to closely correlate with HVPG, severity of liver disease and risk of VH [44].

A further downstream effect of innate immune signaling and local oxidative stress is on SEC function. As noted above, local intrahepatic NO production is decreased in cirrhosis, although expression of the enzyme eNOS in SECs remains normal or increased. ROS generation in cirrhosis is due to both increased production from KCs, as well as decreased activity of elimination systems such as superoxide dismutase [45]. Indeed, gene transfer of superoxide dismutase has been shown to lower portal pressure in rodent models of cirrhosis [46]. Oxidative stress leads to decreased NO bioavailability through a number of mechanisms – ROS directly interacts with NO leading to the formation of peroxynitrite and other reactive nitrogen species [47]. Additionally ROS leads directly to eNOS dysfunction through eNOS 'uncoupling' and decreased eNOS phosphorylation, as well as increasing

the formation of eNOS inhibitors [47]. Plasma levels of the NOS inhibitor ADMA are elevated in cirrhosis, and are elevated further in ACLF due to AH [30]. Moreover, hepatic levels of ADMA correlate with HVPG in patients with ACLF, associated with decreased hepatic expression of dimethylarginine dimethylaminohydrolase-1 (DDAH-1) the metabolizing enzyme for ADMA. The enzyme DDAH-1 is sensitive to oxidative stress [48], hence ROS production by activated KCs will lead to decreased DDAH-1 expression and activity, and thereby increased levels of the eNOS inhibitor ADMA, thus decreasing local NO generation. Additionally, hepatic expression of the eNOS inhibitor caveolin-1, and the eNOS trafficking protein NOSTRIN, are increased in ACLF and AH compared to patients with decompensated cirrhosis alone [31,49]. These proteins also decrease eNOS activity and NO production from SECs. Conversely, pre-treatment of cirrhotic rats with recombinant HDL, which neutralizes circulating LPS, leads to a reduction in LPS-induced systemic inflammation, improvement in eNOS-mediated NO generation, and abrogation of portal hypertension [50].

Hepatocyte cell death through oxidative injury is also likely to propagate local innate immune signaling through the production of damage associated molecular patterns (DAMPs) [51]. These intracellular molecules are responsible for the induction of 'sterile' inflammation following tissue injury, and act through similar downstream pathways to PAMPs, through TLR4 signaling. There is also direct cross-talk between PAMP and DAMP pathways, since bacterial LPS also directly stimulates the release of DAMPs such as HMGB1 [52]. Therefore, the induction of local liver injury through BT and innate immunopathology sets into a motion a feed-forward cycle of PAMP and DAMP mediated inflammation leading to further oxidative stress and vascular dysfunction.

Thus paracrine communication and matrix-cell interactions in the sinusoidal niche are key regulators of cellular phenotype and functional status. The SEC-HSC 'cross-talk' has been proposed as a means of regulating both SEC and HSC activation, since these cells each maintains the other's differentiated phenotype. Local NO production by differentiated SECs promotes HSC quiescence, and activation of the VEGF-NO pathway in hepatocytes and HSCs maintains SECs in a quiescent differentiated state [53,54]. Disruption of paracrine communication in this sinusoidal niche propagates fibrosis and endothelial dysfunction in the cirrhotic liver, hence strategies to deliver or increase intrahepatic NO are unlikely to be sophisticated enough to halt this process without further understanding of the signalling pathways involved in these cellular processes.

Systemic circulatory dysfunction and splanchnic vasodilatation

Portal hypertension is further augmented by vasodilatation of the splanchnic vascular bed and increased portal venous inflow to the liver. Pre-clinical models suggest that the primary pathophysiological event is the development of intrahepatic resistance, which signals to the splanchnic and systemic vasculature leading to increased expression of VEGF and eNOS in the mesenteric circulation [55]. Thus, unlike the intrahepatic circulation, there is an excess of local NO production, and decreased responsiveness of the mesenteric circulation to vasoconstrictors.

The relative contribution of different NOS isoforms to the enhanced systemic and splanchnic NO production in cirrhosis remains controversial. Data from rodent studies seem to vary depending on whether a pre-sinusoidal model of portal hypertension has been used, such as partial portal vein ligation (PPVL), or a model of cirrhosis. In the PPVL model, it is clear from studies using knockout mice that eNOS is responsible for the major part of the vasodilatation of cirrhosis, rather than inducible NOS (iNOS) [56,57]. However, these animals may be less representative of the pathophysiology of advanced cirrhosis, with less systemic inflammation and immune dysfunction. In rodents with biliary cirrhosis and portal hypertension, aortic iNOS expression is induced by the administration of bacterial LPS [58]. Moreover, the role of iNOS expression in perivascular cells has recently been investigated – the adventitial layer of mesenteric vessels in cirrhotic rats has been shown to contain increased number of activated macrophages expressing iNOS [59]. Thus paracrine effects of iNOS activation in inflammatory cells may increase mesenteric flow in advanced cirrhosis, and thereby augment portal hypertension. This is in direct contrast to the intrahepatic circulation, where despite upregulation of hepatic iNOS expression following LPS administration, specific iNOS antagonists have little effect on liver blood flow and typically ameliorate liver injury, suggesting that iNOS does not play a role in maintaining liver perfusion following injury [60,61].

There is indirect evidence in humans for gut-derived bacteria exacerbating systemic circulatory dysfunction in cirrhosis. Patients with advanced cirrhosis demonstrate increased systemic NO production and endotoxinemia following TIPS insertion [62]. Plasma from these patients, when incubated with HUVEC cells, leads to decreased eNOS activity but increased iNOS activity, suggesting that portal venous bacterial products cause increased systemic NO production and circulatory dysfunction. There is further indirect evidence from improvement in vascular dysfunction in cirrhosis with antibiotics. Norfloxacin use in cirrhosis has been shown to significantly decrease endotoxin levels, increase mean arterial blood pressure and systemic vascular resistance, and decrease NO-mediated forearm vasodilatation [12,22]. In cirrhotic rats, aortic eNOS phosphorylation by Akt is decreased by norfloxacin treatment, associated with downregulation of TNF-α and IL-6 [63].

Systemic and splanchnic vasodilatation may also augment portal hypertension through systemic vasoactive systems, such as endocannabinoid (EC) and renin-angiotensin signaling. The EC system has been shown to contribute to vasodilatation in cirrhosis – anandamide has been shown to mediate splanchnic and systemic vasodilatation in cirrhotic rats through endothelial CB1 receptors [64,65]. Furthermore, in the intrahepatic circulation, anandamide causes a CB1-mediated, dose-dependent increase in vasoconstrictor eicosanoids and intrahepatic resistance [66]. Since LPS is a stimulus for EC generation from platelets and macrophages [67], the EC system may be a major contributor to splanchnic vasodilatation through gut-derived LPS – this hypothesis requires further consideration. A further consequence of systemic circulatory dysfunction is activation of the renin-angiotensin system, which potentiates intrahepatic resistance through angiotensin-mediated increases in hepatic ROS formation and HSC contractility [35,68].

Aside from excess NO generation in the splanchnic circulation, data from eNOS and iNOS knockout mice suggests that factors other than NO are also involved in the pathogenesis of arterial

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vasodilatation in cirrhosis [57]. Microparticles (MPs), membrane vesicles that can affect vascular and inflammatory signaling pathways, have recently been shown to be increased in the plasma of cirrhotic patients, and correlate with severity of liver disease and inflammation [69]. Moreover, these MPs impaired the response of cultured rat aortic rings to vasoconstrictors. Thus, MP signaling represents a further tier of complexity in the regulation of inflammation and vascular function.

Novel therapeutic approaches for portal hypertension – a glimpse of the future

Accordingly, our perspective of portal hypertension and vascular dysfunction in cirrhosis is evolving from a linear process associated with progressive fibrosis and the development of complications, to a dynamic interplay between innate inflammatory responses and the sinusoidal niche, exacerbated by systemic inflammation and endothelial dysfunction. This shift in perspective, along with the parallel development of advances in immunological and genomic technologies, has opened avenues for the identification of novel therapeutic targets. Shakespeare's Hamlet spoke of the future as the "undiscover'd country", and this allegory applies to opportunities to improve current therapies for the patient with advanced cirrhosis.

The clinical use of antibiotics in cirrhosis represents one such paradigm shift. Rifaximin, a non-absorbable antibiotic, has been demonstrated to have a clinically significant beneficial effect when used in combination with lactulose for preventing recurrent HE [70]. As described above, BT in cirrhosis leads to stimulation of TLR4-mediated signaling in HSCs, KCs, and SECs. Interactions between these cells in the sinusoidal niche lead to activation of HSCs, pro-inflammatory cytokine production by KCs, and angiogenesis by activated SECs. As such, inhibitors of these signaling pathways, either through selective gut decontamination or TLR-4 antagonism, are attractive targets for portal hypertension in the context of inflammation and ACLF. Rifaximin decreases fibrosis, angiogenesis, and portal pressure following BDL injury in mice [21]. Similarly, TLR-4 knockout mice are protected from fibrosis and portal hypertension following BDL [39]. With regard to systemic TLR-4 antagonists, a randomized controlled trial of the TLR-4 antagonist eritoran in severe sepsis did not reduce mortality compared with placebo [71]. A greater understanding of downstream signaling from TLR-4 in the sinusoidal niche may facilitate other targets in this pathway. For example, fibronectin has been found to be a paracrine mediator from the activated HSC to the SEC, leading to a pro-angiogenic phenotype [21].

The nuclear bile-acid receptor FXR pathway has also been the subject of considerable attention over recent years. Bile acid (BA) signaling, through the FXR pathway in the liver and intestine, maintains homeostasis of the bile acid pool and prevents cholestatic liver injury [72]. However, BAs also have important effects on lipid and glucose metabolism, inflammation, and vascular function. There is an FXR-responsive element in the DDAH-1 gene, and FXR agonists have been shown to dose-dependently increase DDAH-1 expression in hepatocytes [73]. Indeed, the selective FXR agonist obeticholic acid has been shown to increase hepatic DDAH-1 expression in cirrhotic rats, leading to improvements in systemic and hepatic vascular dynamics [74]. Early results from an ongoing multi-centre phase 2a trial of obeticholic

acid in portal hypertension show a trend towards a reduction in portal pressure [75].

Statins also have beneficial effects on vascular function in cirrhosis. Simvastatin has been studied in rodents and humans, and has been shown to have a portal pressure lowering effect through a direct effect on eNOS phosphorylation, thereby increasing NO bioavailability from SECs [76]. Simvastatin also has indirect effects on hepatic vascular function by increasing expression of the transcription factor Klf2, which has beneficial downstream effects such as augmenting eNOS expression and decreasing expression of pro-inflammatory vascular adhesion molecules such as VCAM1 [77]. Additionally, statins are generally considered safe in liver disease, and may have other beneficial effects in chronic liver disease, such as decreasing dyslipidaemic liver injury, and slowing the progression of hepatocellular cancer [78]. However, further work is still required before statins can be widely recommended in liver disease. The degree of reduction in portal pressure, either with or without propranolol, remains modest ($\approx$6–10%), and the clinical significance of this magnitude of portal pressure reduction remains to be established.

Tyrosine kinase inhibitors such as sorafenib, used for the treatment of hepatocellular carcinoma, have additional effects on angiogenesis and fibrosis through non-epithelial cells such as HSCs and SECs. Angiogenesis, as a response to tissue injury and wound healing, occurs extensively in cirrhosis and is responsible for the formation of varices and other porto-systemic collaterals. The processes of fibrosis and angiogenesis are considered complementary, since activated HSCs secrete pro-angiogenic mediators such as VEGF and angiopoietin-1 to nearby SECs, facilitating new vessel formation [37]. Activated HSCs are also closely associated with sinusoidal vessels and new vessels, with expansion of contractile HSC filipodia coverage, thus further exacerbating sinusoidal resistance and propagating angiogenesis. Early studies with the multi-kinase inhibitor Sunitinib demonstrated decreased angiogenesis and fibrosis in rodent models of cirrhosis [79]. Similar findings were found with imatinib, another multi-kinase inhibitor, which decreased HSC activation and portal pressure in BDL cirrhotic rats. Sorafenib, which inhibits multiple pathways including Raf, PDGF, and VEGF, also led to decreased liver stiffness and decreased angiogenesis in BDL cirrhotic rats [80]. Human HSC and SEC co-cultures have shown that sorafenib impairs HSC-SEC interaction by blocking PGDF mediated angiopoeitin-1 and fibronectin signaling, leading to decreased fibrosis and angiogenesis, and further demonstrating the importance of paracrine signaling in the sinusoidal niche. The only studies in humans have been uncontrolled observations in patients with HCC, where decreases in HVPG and portal blood flow have been noted [81]. In view of the variable tolerability of sorafenib in patients with cirrhosis and HCC, dose-reduction or novel agents will be required for usage in advanced cirrhosis.

Finally, the role of transcriptional regulation, and small non-coding RNAs in particular, in fine-tuning cellular responses to inflammation is also beginning to be appreciated. For example, regulation of TNF-α production from KCs in ALD involves several microRNAs (miRs) including miR-155 [82]. Similarly, other key genes involved in endothelial function, such as DDAH-1, are regulated by miRs in the context of inflammation and oxidative stress [83]. Targeting of small non-coding RNAs in the liver through antisense oligonucleotides is a significant advance in small molecule drug discovery and delivery. For example, modified locked nucleic acid oligonucleotides targeting miR-122 have

shown safety and efficacy in humans for decreasing replication of hepatitis C virus [84]. These technical advances, along with the knowledge gained from large genomic and transcriptomic sequencing projects such as ENCODE, have enhanced our knowledge of mechanistic RNA targets and expanded the 'druggable' genome [85]. Novel therapies for inflammation and portal hypertension may build on the pathways outline above, but may target transcriptional switches such as small RNAs, since the 'fine-tuning' effect may be more desirable with less toxicity.

Conclusion

Thus, systemic inflammation and portal hypertension are linked by endothelial dysfunction and innate immune interactions within the sinusoidal niche of the injured liver. Our rapidly progressing knowledge of the mechanisms of liver injury, and host responses to injury and inflammation, are leading to advances in the management of portal hypertension in advanced cirrhosis. If we can successfully journey through this 'undiscover'd country', then opportunities beckon for translational research and novel therapeutics in portal hypertension.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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