Role of liver sinusoidal endothelial cells in liver diseases

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Abstract | Liver sinusoidal endothelial cells (LSECs) form the wall of the hepatic sinusoids. Unlike other capillaries, they lack an organized basement membrane and have cytoplasm that is penetrated by open fenestrae, making the hepatic microvascular endothelium discontinuous. LSECs have essential roles in the maintenance of hepatic homeostasis, including regulation of the vascular tone, inflammation and thrombosis, and they are essential for control of the hepatic immune response. On a background of acute or chronic liver injury, LSECs modify their phenotype and negatively affect neighbouring cells and liver disease pathophysiology. This Review describes the main functions and phenotypic dysregulations of LSECs in liver diseases, specifically in the context of acute injury (ischaemia–reperfusion injury, drug-induced liver injury and bacterial and viral infection), chronic liver disease (metabolism-associated liver disease, alcoholic steatohepatitis and chronic hepatotoxic injury) and hepatocellular carcinoma, and provides a comprehensive update of the role of LSECs as therapeutic targets for liver disease. Finally, we discuss the open questions in the field of LSEC pathobiology and future avenues of research.

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The hepatic sinusoid is composed of specialized cells that communicate with each other to maintain liver function[1,2]. Hepatocytes, organized in hexagonal lobules, represent the parenchymal cells of the liver, separated from the thin-walled sinusoidal endothelium by the space of Disse. Hepatic stellate cells (HSCs) are located in this space, where they contribute to maintain sinusoidal tone and liver stiffness by the release of proinflammatory and anti-inflammatory cytokines and extracellular matrix (ECM) components. The monocyte-derived resident macrophages, also known as Kupffer cells, reside in the sinusoidal lumen and are the first defence line of the liver's immune system. Hepatic sinusoids are assembled by liver sinusoidal endothelial cells (LSECs)[1].

LSECs are characterized by a lack of a basement membrane and the presence of open fenestrae (or transcellular pores) without a diaphragm that form a permeable barrier that enables direct communication between hepatocytes and access to oxygen, micronutrients and macronutrients from the bloodstream[3]. LSECs are involved in the regulation of the vascular tone and the secretion of molecules with vasoactive properties, such as nitric oxide (NO)[4]. Importantly, they also act as antigen-presenting cells (APCs), regulating immune homeostasis through the release of cytokines and the activation of immune cell signalling pathways[5,6]. In this context, LSECs have been found to be extremely efficient scavenger cells[7–9], actively participating in the clearance of antigens reaching the liver sinusoid and contributing to the maintenance of the tolerogenic state[10,11]. In addition, LSECs actively modulate intrahepatic coagulation through diverse mechanisms, including direct generation of procoagulant and anticoagulant factors[12,13], recruitment and activation of neutrophils[14,15] and interaction with platelets[16] (FIG. 1).

Considering the key roles of LSECs in maintaining intrahepatic microcirculation homeostasis, this Review aims to describe the biology of LSECs and their pathological deregulations occurring in acute and chronic liver injury and in hepatocellular carcinoma (HCC), and to provide a comprehensive update of the therapeutic options for liver diseases targeting this cell type.

Features of LSECs

LSECs and the fenestrated sinusoid. Liver sinusoidal fenestrae were observed for the first time by transmission electron microscopy in 1970 when Eddie Wisse confirmed the organization of LSEC fenestrae in clusters or sieve plates in rats[17]. This new visualization enabled the differentiation of LSECs from other cell types, including Kupffer cells and other vascular endothelial cells. In the 1970s, the visualization of liver sinusoidal fenestrae by scanning electron microscopy enabled the description and measurement of fenestrae distribution

Key points

- Liver sinusoidal endothelial cells (LSECs) form the vascular wall of the hepatic microcirculatory system, the hepatic sinusoid, and exhibit unique phenotypic characteristics, including open fenestrae and lack of a basement membrane.
- In health, LSECs have key roles maintaining hepatic homeostasis and are critical for several processes, including immune regulation, control of inflammation, modulation of vascular tone and regulation of the coagulation cascade.
- LSECs become rapidly dedifferentiated during acute and chronic liver injuries, acquiring vasoconstrictor, proinflammatory and prothrombotic properties; this process, termed 'capillarization', contributes to the activation and dedifferentiation of other hepatic cells.
- LSEC capillarization plays a key part in the pathophysiology of major liver diseases, including ischaemia–reperfusion injury, drug-induced liver injury, chronic liver disease and hepatocellular carcinoma; several LSEC molecular targets have been proposed as treatments.

throughout the liver sinusoid[18,19]. In 2008, the diameter of healthy sinusoidal fenestrae was found to range between 100 and 200 nm, depending on the species, showing a larger fenestrae diameter in humans than in rodents[20]. Moreover, it was observed that the diameter of fenestrae varies along the sinusoid depending on the oxygen concentration, with the smallest diameter and number of fenestrae in the periportal zone[21].

The development and regulation of fenestrae is still poorly understood, with different hypotheses postulated for the formation of opened fenestrae in the hepatic sinusoidal endothelium. In 1986, Steffan et al.[22] described alterations in the actin cytoskeleton of murine LSECs as the major driver of this process. Their data were corroborated some years later through the modulation of key regulatory proteins of the actin cytoskeleton, such as RHO-like GTPase, endothelin 1, NO and calcium[23–25]. However, different processes of cell membrane fusion and membrane invaginations were also previously suggested as possible mechanisms in the formation of LSEC fenestrae. One study, in mice, suggested membrane fusion with small transmembrane pores followed by an increase in the size of the pores as a possible mechanism[26]. Another study, in golden hamsters, described a trabecular meshwork, or connective tissue mesh, as responsible for fenestrae formation[27].

Diaphragms are formed by thin fibrils and are dynamic and active gates responsible for regulating the entry of soluble molecules into the parenchyma[28]; they are very common in the endothelial cells of the lungs, kidneys and spleen, among other organs[29]. The peculiar lack of a diaphragm in LSEC fenestrae might be explained by the low expression of plasmalemma vesicle-associated protein (PLVAP; also known as PV1)[30,31], which is encoded by the *PLVAP* gene and has been described as a major a component of the diaphragm in other endothelial cells[29]. Indeed, Bankston et al.[32] showed that fetal rat LSECs exhibit a diaphragm until fenestrae open after 17 days of gestation. The formation of LSEC fenestrae required PV1 for their biogenesis[31] as well as for their opening[33]. The main function of PV1 was indeed found to be the formation of a diaphragm, when the required components (for example, actin cytoskeleton and cytochalasin B, among others) are available[30,34]. When these structures are not accessible, PV1 is transported to the cell surface to be internalized and degraded by lysosomes[35]. However, it has been described that adult rodent LSECs lose diaphragms but PV1 is still expressed in cells from periportal, midlobular and pericentral sinusoids[36]. Thus, fenestrae formation is not only dependent on PV1 expression, suggesting new protein complexes such as vascular endothelial growth factor (VEGF) receptor–neuropilin 1 as a focus for future investigations[36].

Phenotypic markers of LSECs in health. The definition of specific phenotypic markers of healthy LSECs remains controversial. Although previous studies attempted to find specific phenotypic markers of LSECs, and indeed proposed a variety of cell membrane receptors, scavenging proteins and different cellular components[9] (TABLE 1), the current gold-standard method to identify healthy LSECs is still the visualization of sinusoidal fenestrae by different methods, such as scanning electron microscopy[37,38], transmission electron microscopy[39,40] or newer techniques of super-resolution optical microscopy[41] and atomic force microscopy[42,43]. The development of technologies based on single-cell sequencing has enabled unbiased examination of the cellular transcriptome in human and rodent livers[44–47], and opened the possibility to impartially define LSEC phenotypic markers. For example, MacParland et al.[46] identified three endothelial cell populations in healthy human livers depending on the liver zonation, enabling their classification according to differences in enriched gene expression.

LSECs were identified in the central venous zone with enriched expression of *CD32B* (also known as *FCGR2B*), *LYVE1* and *STAB2*, whereas non-LSECs positioned in portal arterial and venous zones showed low or no expression of these three markers[46]. PECAM1 expression occurs in primary rat LSECs subjected to culturing and is also upregulated in LSECs isolated from human liver with cirrhosis and dysplasia[48,49], and is sometimes considered a marker of LSEC capillarization[50]. Some of the LSEC-specific markers were also described as potential genes to discriminate central and midzonal LSECs from those in the periportal zone[47,51]. Thus, new transcriptome approaches based on single-cell sequencing technology could be a useful tool to describe new gene markers to identify healthy LSECs. This approach will be even more important when one is trying to define LSEC markers in liver diseases, as healthy phenotypic makers might not be equally expressed and disease-specific markers might arise[46]. In this regard, current panels for identification of zonated LSECs might be further defined in upcoming years.

The capillarization process. During acute and chronic liver injury, all hepatic cells experience dysregulations that result in phenotypic and functional modifications. LSECs become capillarized in injury, a term associated with loss of fenestrae and development of a basement membrane, phenotype descriptors similar to common capillary endothelium[1,3]. In addition, LSECs lose their protective properties, acquiring vasoconstrictor, pro-inflammatory

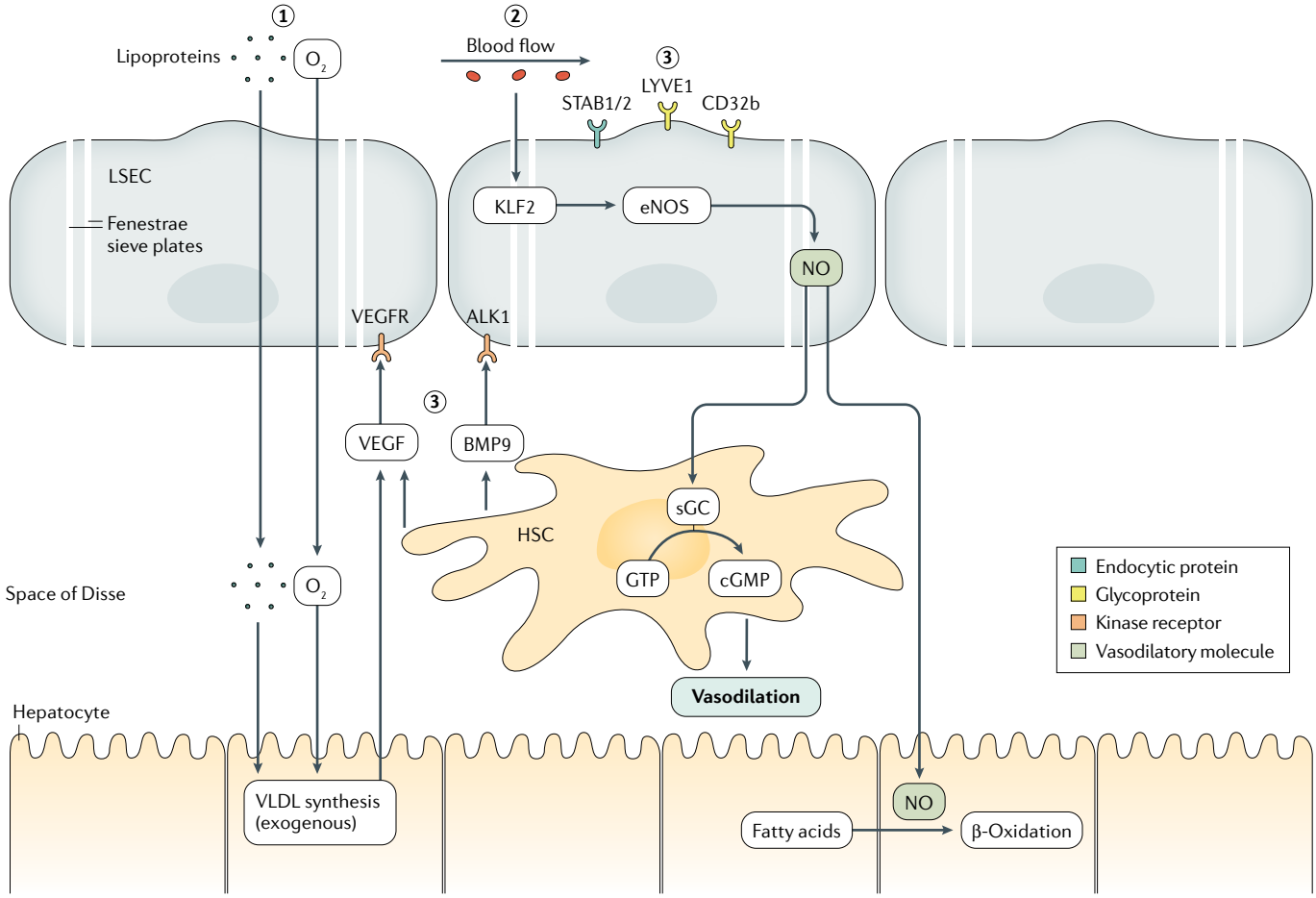

Fig. 1 | **LSECs under physiological conditions maintain liver homeostasis.** Specific phenotypic features of liver sinusoidal endothelial cells (LSECs) such as lack of a basement membrane and fenestration enable the direct communication and exchange with hepatocytes of oxygen and micronutrients and macronutrients such as lipoproteins in the form of chylomicrons (step 1). Shear stress activates the transcription factor Krüppel-like factor 2 (KLF2), regulating endothelial nitric oxide synthase (eNOS) expression and synthesis of nitric oxide (NO). This vasodilatory molecule maintains the quiescence of hepatic stellate cells (HSCs) and sinusoidal vasodilation through cyclic GMP (cGMP) formation, as well as enabling fatty acid β-oxidation in hepatocytes (step 2). Healthy LSECs express endocytic proteins (stabilin 1/2 (STAB1/2)), glycoproteins (lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1) and Fcγ receptor IIb (FcγRIIb; also known as CD32b)) and different membrane receptors (vascular endothelial growth factor receptor (VEGFR) and activin receptor-like kinase 1 (ALK1)), which contribute to liver homeostasis (step 3). BMP9, bone morphogenetic protein 9; sGC, soluble guanylate cyclase; VEGF, vascular endothelial growth factor.

and prothrombotic functions. Additionally, loss of fenestrae and basement membrane deposition impedes the appropriate oxygenation of hepatocytes, resulting in apoptosis and necrosis and, ultimately, the secretion of damage-associated-molecular-patterns (DAMPs)[52,53]. Consequently, HSCs become activated by DAMPs and LSEC-derived factors, producing an excess of ECM and promoting fibrosis development[54]. During the capillarization process, and in response to direct and indirect paracrine interactions with other sinusoidal cells[55], Kupffer cells also polarize to a proinflammatory phenotype and activate the immune response and inflammation process by secretion of several cytokines[56]. These interconnected dysregulations demonstrate that LSECs play an important part in sinusoidal paracrine interactions between all hepatic cells[3]. Thus, the maintenance or restoration of a healthy phenotype in LSECs is an essential step to prevent or relieve liver diseases.

Similarly, and although not a disease per se, the ageing process also affects the LSEC phenotype, promoting partial dedifferentiation[39]. Studies have characterized this process, which is termed 'pseudocapillarization', defining a decline in fenestrae porosity, the development of a basement membrane and a reduction in vasodilatory capacity and paracrine activation of HSCs as major characteristics[39,57–59]. It is important to note that pseudocapillarization of LSECs does not compromise microcirculatory function in healthy ageing, but markedly exacerbates the development of liver disease on acute or chronic injuries in aged individuals[60,61].

Although in vitro capillarization, the spontaneous dedifferentiation process occurring in LSECs during cell culture on plastic, is not a biological representation of real LSEC loss of fenestrae, some in vitro studies have suggested that VEGF[62] and Hedgehog signalling[63] are important pathways in this process. On the other hand,

Table 1 | **LSEC markers and functions in health and disease**

Marker	Definition and function	Changes in disease	Refs
CD4	Interaction with MHC class II and receptor for HIV	Not reported	279
CD11b	Also known as αM integrin; adhesion of monocytes, macrophages and granulocytes, taking up of complement-coated particles and pathogens	Not reported	280
CD11c	Also known as αX integrin; adhesion of neutrophils and monocytes to stimulated endothelial cells, phagocytosis of complement-coated particles	Expression reduced after treatment with endotoxin in mice	280
CD13	Extracellular peptidase	Not reported	279
CD14	Endotoxin receptor, LPS-binding protein	Not reported	279
CD16	Low-affinity Fc receptor for IgG	Not reported	279
CD31	Also known as PECAM1; endothelial tissue marker, cytoplasmic expression	Expression Increased in human cirrhosis and liver dysplasia; expression reduced in human HCC	48,49, 80,263
CD32b	Fcγ receptor for soluble IgG–antigen complexes	Expression reduced during in vitro LSEC capillarization in rats and in human CLD; lost in human HCC ECs	264,279, 281,282
CD33	Also known as SIGLEC3; mediates cell–cell interactions and maintains immune cells in a resting state	Not reported	283
CD34	Haematopoietic stem cell marker; functions in endocytosis	Expression reduced in human HCC ECs and related to HCC occurrence; expression increased in cirrhosis and HCV-associated CLD, as well as in angiogenesis processes related to liver fibrosis and HCC progression in mice	263, 284–287
CD36	Scavenger receptor class B, collagen receptor, thrombospondin receptor	Not reported	288
CD40	Co-stimulatory molecule in antigen presentation	Expression increased in human fulminant liver failure	289–291
CD45	Also known as PTPRC; leukocyte antigen	Not reported	51
CD46	Cofactor for inactivation of complement C3b and C4b by serum factor I; provides protection of the host cell from damage by complement	Not reported	292
CD54	Also known as ICAM1; binds to integrins of type CD11a/CD18	In animals and humans, expression increased under inflammatory stimuli and acute hepatitis, and expression reduced in HCC ECs	263, 293–296
CD80	Co-stimulatory molecule in antigen presentation	Expression increased in human fulminant liver failure	291,297
CD86	Co-stimulatory molecule in antigen presentation	Expression increased in human fulminant liver failure	291,297
CD91	LDL receptor-related protein 1	Not reported	298
CD105	Also known as endoglin; involved in regulation of angiogenesis	Not reported	299
CD106	Also known as VCAM1; mediates leukocyte–endothelial cell adhesion	Expression increased in human alcohol-induced cirrhosis and upregulated in rats under inflammatory stimuli	294,300
CD146	Cell adhesion and cohesion of the endothelial monolayer in vascular tissue	Expression decreased in the mouse fibrotic liver	301,302
CD204	Scavenger receptor class A (also known as MSR1); involved in endocytosis of modified LDLs	Not reported	303
CD206	Mannose receptor (also known as MRC1)	Expression increased under inflammatory stimuli in rats	304,305
CD209	Pathogen receptor (also known as DC-SIGN)	Not reported	306
CD299	Pathogen receptor (also known as CLEC4M, L-SIGN and CD209L)	Expression reduced in cirrhosis in humans. Increased serum-soluble levels in patients with colon cancer with liver metastases	307–309
LSECtin	Liver and lymph node sinusoidal endothelial C-type lectin; interacts with CD44 to inactivate T cell responses and with L-SIGN in response to HCV; a receptor for Ebola virus	In rats and humans, expression reduced in cirrhosis and HCC. Increased serum-soluble levels in patients with colon cancer with liver metastases	162, 308–312
LYVE1	Lymphatic vessel endothelial hyaluronan receptor 1	In humans, expression decreased in cirrhotic LSECs and absent in HCC	49,309, 313
Stabilin 1/2	Angiogenesis, lymphocyte homing, cell adhesion and scavenger receptor	In humans, lost during LSEC capillarization and HCC progression	46,264, 309,314
HLA-DR	Human leukocyte antigen DR; antigen-presenting molecule	Not reported	315
TLRs	Toll-like receptors	Not reported	164,316
VEGFR3	Vascular endothelial growth factor receptor 3	Not reported	317,318

CLD, chronic liver disease; CLEC4M, C-type lectin domain family 4 member M; DC-SIGN, dendritic cell-specific ICAM3-grabbing non-integrin; HCC, hepatocellular carcinoma; HCC EC, hepatocellular carcinoma-associated endothelial cell; ICAM1, intercellular adhesion molecule 1; LPS, lipopolysaccharide; LSEC, liver endothelial sinusoidal cell; L-SIGN, liver/lymph node-specific ICAM3-grabbing non-integrin; MRC1, macrophage mannose receptor 1-like protein 1; MSR1, macrophage scavenger receptor types I and II; PECAM1, platelet endothelial adhesion molecule 1; PTPRC, receptor-type tyrosine-protein phosphatase C; SIGLEC3, sialic acid-binding immunoglobulin-like lectin 3; VCAM1, vascular cell adhesion molecule 1.

Desroches-Castan et al.[64] demonstrated that loss of fenestrae is the earliest event occurring in *Bmp9*-depleted mice, followed by hepatic inflammation and fibrosis, suggesting the functional axis between *BMP9*, *GATA4* (REFS[65–67]) and *PLVAP* as an important mechanism for loss of fenestrae in LSECs.

Role of LSECs in acute liver injury

Ischaemia–reperfusion injury and liver regeneration. Ischaemia–reperfusion injury (IRI) causes critical damage to the liver and is the result of the interruption of blood delivery to the organ that occurs during different surgical procedures, such as liver transplantation or hepatic resection[68–70]. Different cellular and molecular mechanisms are involved in liver function during IRI[70–72]. LSECs are fundamental modulators during this acute liver injury and, together with hepatocytes, they are highly susceptible to IRI damage[69,73] (FIG. 2). During liver transplantation, organ acquisition involves both warm and cold IRI with contrasting consequences for cells: while hepatocytes undergo greater damage than LSECs during warm ischaemia, LSECs are more susceptible to damage during cold ischaemia, with half of them becoming non-functional after 48 hours of injury in preclinical rat studies[69,74].

The initial step of IRI is a consequence of tissue hypoxia accompanied by the lack of operating blood flow into the organ, which immediately influences the functionality of the parenchymal and non-parenchymal liver cell microenvironments[69]. In the course of the ischaemic stage, rat LSECs become rounder, plasma membranes become discontinuous and their nuclear membranes vacuolate[71]. This event is accompanied by metabolic alterations and loss of ATP supply[69,75], which combined with low tissue levels of NO and high production of endothelin, thromboxane A_2 (TXA_2) and reactive oxygen species (ROS), induce alterations in LSECs and the microvascular circulation[76,77]. A key master regulator of these events in rat and human LSECs is the mechanosensing transcription factor Krüppel-like factor 2 (KLF2), which under static (no-flow) conditions is downregulated and, consequently, so are its derived transcriptional programmes[77,78]. Indeed, KLF2 reduction during ischaemia leads to decreased endothelial NO synthase (eNOS) expression and activity, ultimately leading to deficient NO production, sinusoidal vasoconstriction and increase of hepatic vascular resistance during the reperfusion stage[69,77,79]. Also during reperfusion, LSECs increase their scavenging function in response to the increased numbers of ROS generated following the reestablishment of oxygen supply to the liver[69].

The function of LSECs as an inducer of local tolerance in homeostasis is disrupted in IRI as they are exposed to the actions of surrounding Kupffer cells, infiltrating neutrophils and lymphocytes, hepatocytes, HSCs and platelets[68–70]. Hepatocytes and HSCs act to maintain their normal phenotype with the production of VEGF, which contributes to downstream determination of the LSEC phenotype[80]. In general, when an injury occurs, proinflammatory cytokines (for example, TNF or IL-1) and ROS released by activated Kupffer cells and neutrophils induce LSEC NF-κB activation and expression of adhesion molecules such as P-selectin, favouring platelet attachment, or the upregulation of intercellular adhesion molecule 1 (ICAM1), E-selectin and IL-8, enabling neutrophil infiltration and extravasation[81–83]. The injury also causes the discharge of DAMPs by hepatic cells, such as HMGB1 or endogenous DNA[84], which implies an activation feedback for Kupffer cells. Moreover, proinflammatory secreted cytokines enable the new recruitment of $CD4^+$ T lymphocytes. These cells promote a further inflammatory state, with the production of cytokines such as interferon-γ, lymphotoxin-α and granulocyte–macrophage colony-stimulating factor, which will intensify Kupffer cell activation and neutrophil recruitment to the sinusoid[75,85,86].

Platelets work as a double-edge sword in IRI. On the one hand, infiltration of platelets in liver tissue will favour LSEC apoptosis as they induce microthrombi in hepatic vessels and, in addition, they produce platelet-activating factor, which is also induced by LSECs, which will amplify neutrophil local production of ROS[87]. On the other hand, platelets can produce factors such as serotonin, NO and calpain, but also platelet-activating factor, that contribute to the induction of hepatic regeneration in murine models[88,89].

LSEC mechanisms counteracting ischaemia–reperfusion injury. Autophagy facilitates the elimination of damaged cellular material via lysosomal degradation, and it helps to control ROS production[90]. It has been suggested that autophagy might be beneficial in liver damage prevention in IRI, as inducers of autophagy, such as the HMG-CoA reductase inhibitor simvastatin, protect from IRI in non-steatotic and steatotic rat livers, avoiding the reduction of the vasoprotective action induced by RAB7–KLF2-mediated transcriptional programmes in LSECs[77,79,91,92]. Statin-derived LSEC vasoprotection in the context of IRI has been demonstrated in preclinical models of ageing, further reinforcing the importance of LSECs in this clinically relevant situation[60]. Also, hypoxia events occurring during IRI induce the transcription of hypoxia-inducible factors in damaged liver, which in turn promote the transcription of cellular protective genes such as *Hmox1*, the gene encoding haem oxygenase 1 (HO1) in mouse hepatocytes[93]. Indeed, HO1 has been shown to be protective for LSECs in vitro, as levels of proinflammatory cytokines are attenuated and LSEC survival rate is increased[93].

Role of LSECs in liver regeneration after acute injury. After liver injury, the hepatic production of VEGF acts as an inducer of proliferation of bone marrow-derived sinusoidal progenitor cells. These cells also produce hepatocyte growth factor to induce hepatocyte proliferation, and will replenish the sinusoids, differentiating into mature LSECs. The contribution from mature LSECs alone is not enough to induce liver regeneration[94,95].

Data suggest that endocannabinoid overproduction by hepatocytes, Kupffer cells and LSECs exerts a protective role in hepatic IRI and liver regeneration through activation of CB_2 receptor. This receptor is expressed in human and murine LSECs, and its stimulation with the

agonist JWH133 induced a reduction in the expression of ICAM1 induced by TNF, and in adhesion of neutrophils to LSECs in vitro[96,97]. Adipokines are cytokines produced mainly by adipose tissue and have been implicated in liver regeneration, especially in ischaemic steatotic livers in humans and animal models[98,99]. Leptin, adiponectin and especially IL-6 have been found to promote murine hepatic regeneration after IRI[99], although their specific roles remain elusive.

Although the arachidonic acid-derived TXA_2 contributes to inflammation and platelet accumulation after an acute liver injury[100], it has been demonstrated that TXA_2 receptor has a positive role in liver regeneration through enhancing macrophage recruitment[101]. These data suggest that inhibition of the TXA_2 pathway in acute liver injury should be considered, taking into account that it might be beneficial to prevent or alleviate IRI without hepatectomy[102] but detrimental in the case of liver surgery.

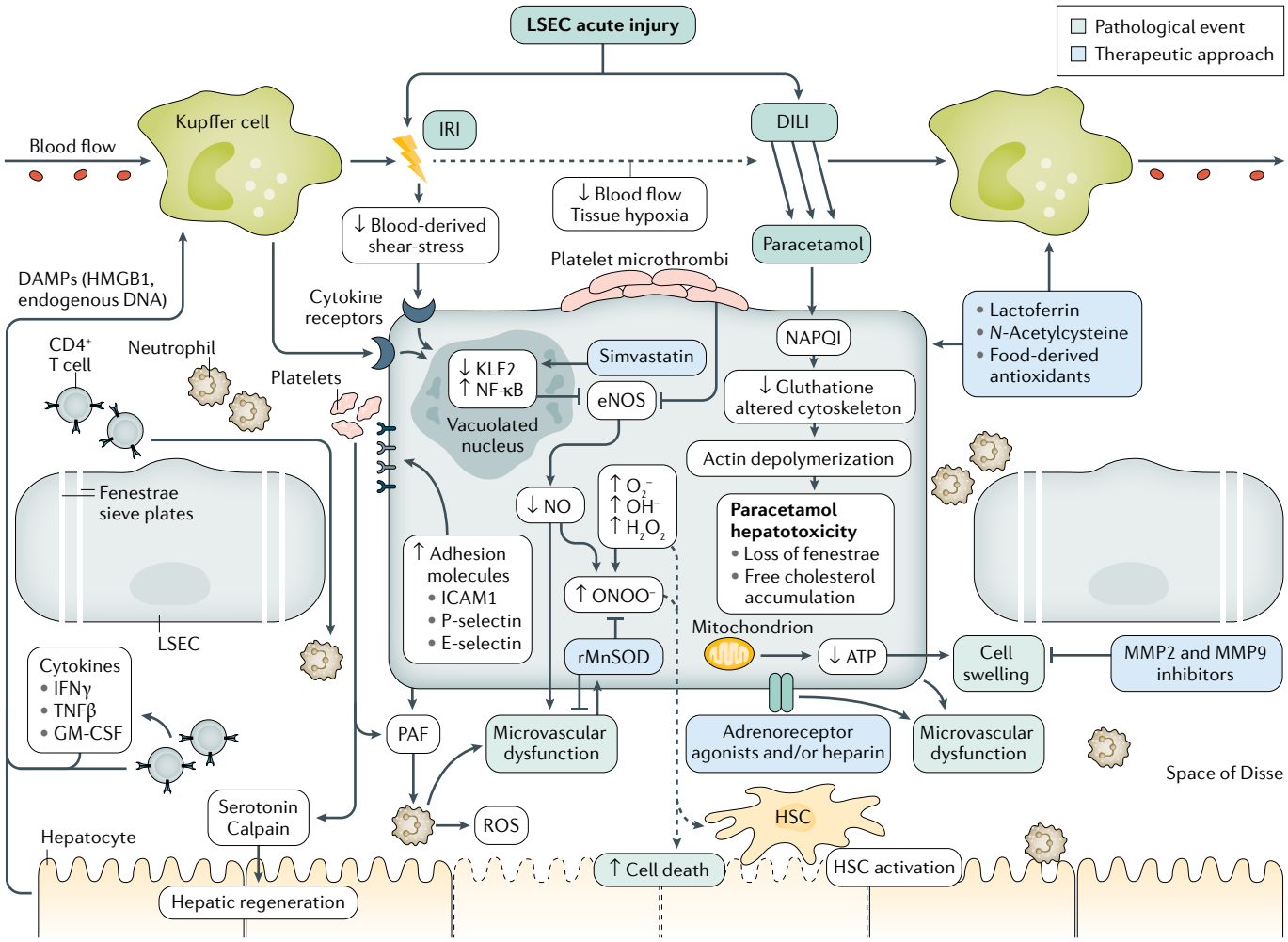

Fig. 2 | **Pathobiology of LSECs in acute liver injury.** The effects on liver sinusoidal endothelial cells (LSECs) under ischaemia–reperfusion injury (IRI) and drug-induced liver injury (DILI) are shown. During IRI, blood flow interruption induces tissue hypoxia and LSECs become round and metabolically altered, with reduction of ATP supply and vacuolation of nuclei. Disruption of blood flow-derived shear stress also promotes depletion of the transcription factor Krüppel-like factor 2 (KLF2) in LSECs, which leads to reduction in its vasoprotective target genes including the endothelial nitric oxide synthase (eNOS) gene. Simvastatin, through the inhibition of the small GTPase RAC1, induces KLF2 expression, thereby maintaining endothelial homeostasis during IRI. In addition, surrounding neutrophils produce reactive oxygen species (ROS), and Kupffer cells also secrete proinflammatory cytokines, causing microvascular circulation alteration and the recruitment of $CD4^+$ T lymphocytes. These cells increase cytokine production, intensifying the inflammatory context. Kupffer cells also receive the feedback stimulation of hepatocyte-released damage-associated molecular patterns (DAMPs) induced by cellular hypoxia. Expression of adhesion molecules by LSECs in response to IRI favours platelet adhesion and formation of vessel microthrombi. Platelet-activating factor (PAF) production by both LSECs and platelets induces neutrophil activation and increased production of ROS. Mediators such as serotonin or calpain are secreted by platelets, favouring hepatic regeneration. In the case of DILI, hepatotoxicity is initiated by reactive metabolites such as paracetamol-derived *N*-acetyl-*p*-benzoquinone imine that induce the reduction of glutathione and actin depolymerization in LSECs, which, together with the accumulation of free cholesterol in endolysosomes and fenestrae disruption forming gaps and enabling extravasation of macrophages and neutrophils, lead to the development of microcirculatory dysfunction. Lactoferrin, *N*-acetylcysteine, matrix metalloproteinase (MMP) inhibitors, adrenoreceptor agonists and heparin help to ameliorate tissue damage during DILI. GM-CSF, granulocyte–macrophage colony-stimulating factor; HMGB1, high mobility group protein B1; HSC, hepatic stellate cell; ICAM1, intercellular adhesion molecule 1; IFNγ, interferon-γ; NF-κB, nuclear factor-κB; NO, nitric oxide; rMnSOD, recombinant manganese superoxide dismutase; TGFβ, transforming growth factor-β.

New therapeutic strategies targeting LSECs in ischaemia–reperfusion injury. Different substrates have been explored as potential targets to reduce IRI-induced LSEC damage (TABLE 2). One study demonstrated the benefits of using a novel recombinant form of the antioxidant human manganese superoxide dismutase on cold storage and warm reperfusion in primary cultured LSECs and liver grafts from rats and human samples[103]. In 2018, another study showed that SEW2871, a selective agonist of sphingosine 1-phosphate receptor 1, increases LSEC survival and improves vasorelaxation and the maintenance of vascular integrity in a mouse model of warm IRI[104]. Yadav et al.[105] used bosentan, an antagonist of endothelin receptors, and observed that mouse LSECs preserve their mitochondrial viability and have reduced DNA damage when treated with this compound. Preclinical studies in rats have also proposed inhibition of hepatic matrix metalloproteinase 9 (MMP9) as a novel therapeutic to ameliorate IRI through the recruitment of sinusoidal progenitor cells, both in steatotic and non-steatotic livers[106,107].

Drug-induced liver injury. The harm to the liver caused by commonly used drugs is referred to as drug-induced liver injury (DILI)[108]. 'Intrinsic drug-induced liver injury' is the term used to identify direct, rapid and dose-dependent injury after drug exposure, and it includes the response to hepatic toxic effects from drugs such as paracetamol. Paracetamol toxicity is responsible for approximately 50% of acute liver failure in many countries[109–112].

Before their metabolism and clearance in hepatocytes, drugs are transported through sinusoidal blood by several mechanisms, including organic anion and cation transporter proteins of the basolateral membrane, passive diffusion and other transporter proteins, such as Na^+–taurocholate co-transporting polypeptides or prostaglandin transporters[113]. Thus, although the final outcome of DILI is hepatocyte loss of specific function and cell death, the direct toxic stress is also delivered to other targets considered important in the initiation and progression of overt tissue damage, such as LSECs[114,115]. Several lines of evidence in experimental models have demonstrated the role of LSECs in the pathogenesis of paracetamol-induced liver injury (FIG. 2). The formation of *N*-acetyl-*p*-benzoquinone imine, a reactive metabolite that depletes hepatic glutathione and initiates paracetamol toxicity, is preceded by an early hepatic microcirculation dysfunction[116,117]. Platelet aggregation to the sinusoidal wall contributes to this endothelial disruption. Moreover, experimental paracetamol hepatotoxicity is exacerbated by free cholesterol accumulation in LSEC endolysosomes[118]. The use of α1-adrenoceptor antagonists or heparin to ameliorate microvascular function[119,120], the use of MMP inhibitors to prevent LSEC damage[121] and the improvement in haemodynamics by NO donors[122] have all demonstrated an ability to attenuate paracetamol toxicity in animal models.

In addition to LSEC toxicity caused by dacarbazine[123], cyclophosphamide[124] or azathioprine[125], other toxicants, such as pyrrolizidine alkaloids, lipopolysaccharide and galactosamine, have also been found to cause LSEC injury in rodents[9,126]. LSEC behaviour in response to these toxicants is similar, although the mechanisms of toxicity have been more intensively evaluated for paracetamol in mouse models[116,127]. LSECs swell minutes after exposure to paracetamol, compromising their scavenger activity[128]. Fenestrae disruption forms gaps in LSECs, similar to those induced by pyrrolizidine alkaloids in early stages of hepatic veno-occlusive disease[128], and favours sinusoid disintegration and the reduction of blood flow. These paracetamol-elicited processes are further exacerbated when combined with ethanol binging[128]. Although sinusoid neutrophil accumulation and priming are initial consequences of acute liver damage, and neutrophil extravasation is considered to potentially worsen tissue injury, the use of galactosamine plus endotoxin versus endotoxin alone in mouse models of liver injury has revealed that the gap formation in LSECs is neither dependent on this neutrophil priming nor secondary to leukocyte migration. In the contrary, the large gaps facilitate neutrophil extravasation and the interaction between sinusoid-accumulated neutrophils and damaged hepatocytes[126].

As the administration of NO donors and the use of MMP2 and MMP9 inhibitors minimize endothelial injury in vivo, it is speculated that DILI from the aforementioned toxicants affects the LSEC cytoskeleton, which is key in preserving the fenestrae[129]. These molecules have also been associated with the depletion of glutathione levels in LSECs in vitro[116,127]. Some evidence supporting this assumption comes from the fact that LSEC injury is increased when eNOS is inhibited, whereas LSEC injury is decreased when inducible NO synthase is inhibited[129]. Also, oxidative stress resulting in the release of free radicals such as superoxide has been associated with LSEC injury in animal models[129].

Drug-induced liver injury treatment and LSECs. The current treatment for paracetamol-induced liver injury is use of the antioxidant *N*-acetylcysteine (NAC), which can restore the depleted glutathione following paracetamol overdosing when administered within 2–10 hours of ingestion[130]. NAC is a donor of sulfhydryl groups, explaining its central role in the restoration of cell glutathione[130]. NAC has been shown to inhibit αV integrin, β3 integrin and laminin expression in ROS-mediated palmitate injury in cultured human LSECs[131], as well as in human LSECs damaged by long-term high glucose stimulation[132]. However, its brief window of efficacy and its adverse effects have boosted research on other food-derived antioxidants[133,134]. For example, curcumin, honey, silymarin, α-lipoic acid, sulforaphane, ginger, hibiscus, lupeol, sesame, resveratrol, aloe vera, artichoke leaf and apigenin have all been considered as treatments for paracetamol-induced hepatotoxicity as they reduce paracetamol-derived increases in the levels of aminotransferases, lipid peroxidation and inflammatory cytokines in animal models[135]. The mechanism of action of each of these converges to replenish glutathione and ROS scavengers, and to modulate the antioxidant enzymes, diminishing the oxidative stress (TABLE 2).

Although the use of NAC has been shown to increase the secretion of cyclic GMP in LSECs from rats with

Table 2 | **Summary of therapies for acute liver injury targeting the sinusoidal endothelium**

Study population	Treatment	Results	Refs
Ischaemia–reperfusion injury and regeneration			
C57Bl/6J mice and human LSECs	CB_2 receptor agonist: JWH133 (20 mg/kg in vivo and 0–4 μM in vitro)	Decrease of hepatic inflammation and oxidative stress; reduction of levels of liver adhesion molecules	96
Wistar rats (lean and with NAFLD) and primary rat LSECs	Simvastatin (10 μM and 1 mg/kg in vivo and 1 μM in vitro)	Vasoprotection via KLF2; upregulation of eNOS and increase of NO bioavailability; prevention of endothelial dysfunction; amelioration of hepatic injury	77,79,91
Sprague Dawley rats (lean and with NAFLD) and primary rat LSECs	Recombinant MnSOD (50–150 μg/kg in vivo and 0.15 μM in vitro)	Amelioration of hepatic and LSEC oxidative stress; maintenance of NO levels in LSECs; prevention of endothelial dysfunction	103
C57Bl/6 mice and primary mouse LSECs	A_{2A} receptor agonist: CGS21680 (0.5 mg/kg in vivo and 5 μM in vitro)	Protection of LSECs phenotype and amelioration of oxidative stress in LSECs	319
C57Bl/6 mice (lean and with NAFLD)	Atorvastatin (5 mg/kg)	Upregulation of hepatic eNOS; decrease of hepatic inflammation and microparticle release; amelioration of hepatic injury	320
Primary rat LSECs	Simvastatin (5 μM)	Upregulation of KLF2; activation of autophagy and improvement of LSEC viability	92
Primary mouse LSECs	Recombinant adenovirus encoding mouse HO1	Reduction of levels of proinflammatory cytokines and increased LSEC survival	93
C57Bl/6 mice and primary mouse LSECs	S1P1R agonist: SEW2871 (25 mg/kg in vivo and 20 μM in vitro)	Increase in LSEC survival and improvement of vasorelaxation, reduction of intrahepatic inflammation	104
Primary mouse LSECs	ET1 receptor antagonist: bosentan (10^{-5} M in vitro)	Reduction of oxidative stress and DNA damage	105
Lewis rats (lean and with NAFLD) and primary rat LSECs	MMP9 inhibitor with ASOs (20 mg/kg), MMP2/MMP9 inhibitor (100 μg/kg)	Preservation of LSEC integrity; improvement of liver regeneration by recruitment and engraftment of progenitor cells; increased hepatic VEGF expression; amelioration of hepatic injury	106,107
Wistar rats	Telluric acid (50 μg/kg)	Upregulation of hepatic eNOS; amelioration of oxidative stress and ischaemia–reperfusion injury	321
Rats	Apelin 13 (2 μg/kg)	Upregulation of hepatic eNOS; amelioration of hepatic injury	322
C57BL/6 mice (wild type and knockout)	NOD1 antagonist-loaded nanoparticles: ALINO73 (5 mg/kg)	Amelioration of hepatic injury; reduction of levels of adhesion molecules	323
Wistar rats (young and aged) and primary aged rat LSECs	Simvastatin (25 mg/kg in vivo and 1 μM in vitro)	Amelioration of microvascular dysfunction; improvement of LSEC fenestrae; reduction of hepatic oxidative stress	60
Drug-induced liver injury (paracetamol)			
CD1 mice	V-PYRRO/NO (5.4 mg/ml)	Prevention of toxic injury progression; amelioration of oxidative stress	122
C57Bl/6 mice	MMP2/MMP9 inhibitor (5 mg/kg)	Attenuation of liver microcirculatory dysfunction; reduction of infiltration of red blood cells	121
CD1 mice	α_1-Adrenoceptor antagonist (prazosin at 35.7 μM)	Prevention of microcirculatory dysfunction	120
BALB/cJ and C57Bl/6 mice and primary mouse LSECs	Lactoferrin (50 mg/kg)	Attenuation of hepatic microcirculation dysfunction by upregulation of eNOS	137
Drug-induced liver injury (SOS)			
Sprague Dawley rats and primary rat LSECs	Doxycycline (5, 10 or 15 mg/kg), MMP2/MMP9 inhibitor (100 or 200 μg/h)	Prevention of SOS development	324
Sprague Dawley rats and primary rat LSECs	V-PYRRO/NO (1.06–2.12 μmol/kg)	Increase in hepatic vein NO levels; prevention of LSEC damage and SOS development	141
Crl:CD1 mice and primary mouse LSECs	Recombinant thrombomodulin (4 mg/kg)	Amelioration of LSEC phenotype and upregulation of eNOS	143
C57Bl/6 mice and primary mouse LSECs	TXA_2 agonist: U46619 (100 μM)	Reduction of levels of liver adhesion molecules and MMPs	145

Table 2 (cont.) | **Summary of therapies for acute liver injury targeting the sinusoidal endothelium**

Study population	Treatment	Results	Refs
Bacterial and viral infections			
Primary mouse LSECs	TLR3 agonist: poly(I:C) (100 µg/mL in vitro)	Suppression of HBV replication	174
C57Bl/6 and DbGagL TCR transgenic mice, and primary mouse LSECs	TLR1/2 agonist: P3C (10 µg/mL in vitro)	$CD8^+$ T cell immunity activation	173
Wistar rats plus LPS	Simvastatin (25 mg/kg)	Prevention of endothelial dysfunction and eNOS downregulation	325
Fulminant hepatitis preclinical model in mice	Perforin 1 inhibitor: SN34960 (150 mg/kg)	Reduction of $CD8^+$ T cell accumulation in periportal zone; amelioration of sinusoidal perfusion and liver failure	326

ASO, antisense oligonucleotide; eNOS, endothelial nitric oxide synthase; ET1, endothelin 1; HO1, haem oxygenase 1; KLF2, Krüppel-like factor 2; LPS, liposaccharide; LSEC, liver sinusoidal endothelial cells; MMP, matrix metalloproteinase; MnSOD, manganese superoxide dismutase; NAFLD, nonalcoholic fatty liver disease; NO, nitric oxide; NOD1, nucleotide-binding oligomerization domain 1; P3C, palmitoyl-3-cysteine-serine-lysine-4; S1P1R, sphingosine 1-phosphate receptor 1; SOS, sinusoidal obstruction syndrome; TCR, T cell receptor; TLR, Toll-like receptor; TXA_2, thromboxane A_2; VEGF, vascular endothelial growth factor; V-PYRRO, O^2-vinyl 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate.

obstructive jaundice[136], specifically treating LSEC injury to prevent DILI-associated hepatocyte damage has been proposed. For instance, lactoferrin has been shown to effectively protect against paracetamol-induced LSEC injury in mice. Lactoferrin elicits the activation of Kupffer cell-derived protective mediators as a mechanism for inhibiting paracetamol-induced LSEC damage and mitigating hepatic microcirculatory dysfunction[137].

Hepatic sinusoidal obstruction syndrome. Hepatic sinusoidal obstruction syndrome (SOS) is a form of DILI characterized by the obstruction of the hepatic sinusoids. SOS occurs after toxic administration of certain chemicals, including chemotherapy agents, or in haematopoietic stem cell transplantation (HSCT) due to the depletion of glutathione, increase in the level of von Willebrand factor and thrombus formation as a consequence of cytotoxic agents inherent to HSCT[138,139]. LSECs are first damaged in the centrilobular zone of the hepatic lobule, promoting dedifferentiation of hepatocytes and HSC activation[140]. It has been demonstrated in animals that LSECs have an important role in SOS pathogenesis through NO bioavailability reduction[141] and platelet aggregation[142]. Takada et al.[143] demonstrated that NO expression was decreased in a preclinical model of SOS, and suggested recombinant thrombomodulin as treatment for SOS through coagulation inhibition. Clinical trials have shown anticoagulant therapy is effective for SOS after HSCT[144]. Moreover, TXA_2 receptor agonism could also ameliorate LSEC damage in a preclinical model of SOS[145] (TABLE 2).

Herbal-induced liver injury. Natural remedies, mostly herbal and dietary supplements, have also been associated with liver injury[146,147]. Studies showing this association, which have been summarized elsewhere[148], have led to the term 'herbal-induced liver injury' and to the listing of restricted herbal ingredients by European authorities[149]. The clinical manifestations of herbal-induced liver injury are similar to those of DILI and can range widely from asymptomatic abnormal liver biochemistry to severe liver failure[150]. Although only pyrrolizidine alkaloids have specifically and consistently been associated with liver sinusoidal endothelium damage[125,147,151,152], the general mechanisms affected by several herbal and dietary supplements, such as apoptosis, oxidative stress or immune function, make it plausible that these compounds might also influence LSEC viability. Nevertheless, LSEC susceptibility to herbal-induced liver injury has not been directly confirmed and will require further research.

Finally, it is important to stress that newly developed tools, such as fluidic devices mimicking human liver sinusoids[153], might help test both toxicity and treatment of liver injury caused by drugs, herbal and dietary supplements, improving our knowledge of the specific roles of LSECs in the initiation and maintenance of DILI.

Acute bacterial and viral infections. The role of LSECs as APCs has already been reviewed[154]. Although different studies point to reduced MHC class II expression by LSECs and their lack of ability to activate T cells[155–157], they have been found to work as professional APCs in disease conditions, participating not only in T cell cytotoxic activity[158,159] but also in the activation of T helper cell responses[160–162].

In the steady state, commensal bacteria induce LSEC regulation of pericellular matrix chemokine gradients through MYD88-dependent signalling. As a consequence, immune cells are spatially polarized around periportal regions to effectively protect against systemic bacterial dissemination[163].

During acute liver failure, bacterial and viral infections have also been found to target LSECs, switching their tolerogenic steady state to promote inflammatory activity[162,164] (FIG. 3). LSECs can recruit leukocytes through differential expression of adhesion molecules such as ICAM1 and vascular adhesion protein 1 (VAP1). During inflammation, LSEC expression of ICAM1 increases and other adhesion molecules, such as VCAM1 and PECAM1, are induced[165].

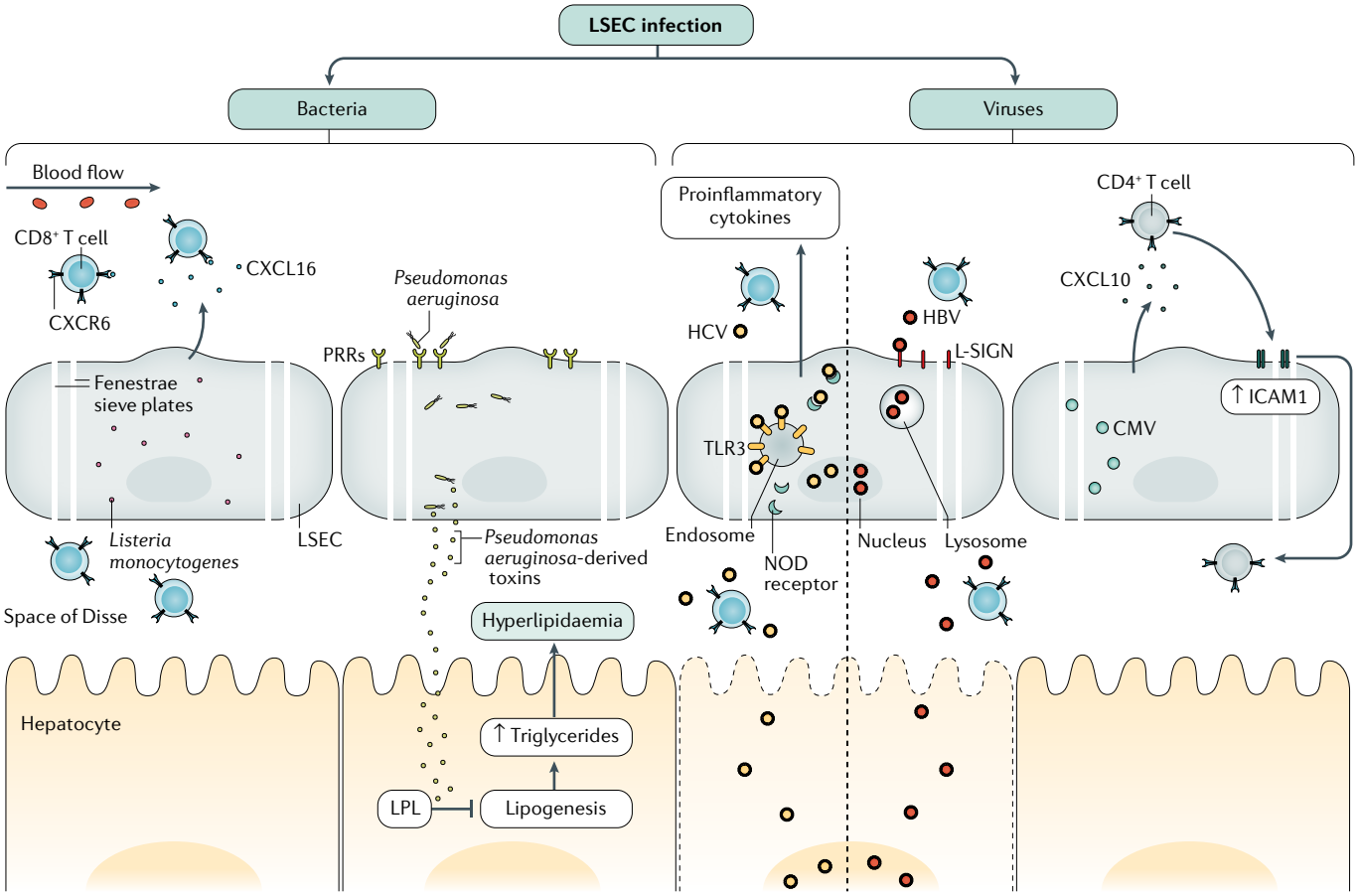

Fig. 3 | **Pathobiology of LSECs in bacterial and viral infection.** During infection by *Listeria monocytogenes*, liver sinusoidal endothelial cells (LSECs) produce CXC-chemokine ligand 16 (CXCL16), which induces the recruitment of cytotoxic CD8⁺ T lymphocytes expressing its receptor CXC-chemokine receptor 6 (CXCR6). Infection of LSECs by *Pseudomonas aeruginosa* is mediated by pathogen recognition receptors (PRRs) and its derived toxins, leading to lipoprotein retention in parenchymal cells and subsequent bacterial sepsis-related hyperlipidaemia. In the course of viral infections, LSECs can detect HBV and HCV by different PRRs, inducing the production of proinflammatory cytokines to recruit cytotoxic CD8⁺ T lymphocytes, and possibly helping in the *trans*-infection of hepatocytes. In cytomegalovirus (CMV) infection, intercellular cell adhesion molecule 1 (ICAM1) expression is increased as is production of CXCL10 to recruit T helper CD4⁺ lymphocytes to the tissue microenvironment. LPL, lipoprotein lipase; L-SIGN, liver/lymph node-specific ICAM3-grabbing non-integrin; NOD, nucleotide oligomerization domain; TLR3, Toll-like receptor 3.

In response to common bacterial CpG oligonucleotides, LSECs are able to mediate signalling through Toll-like receptor 9 (TLR9) in vitro[164]. *Pseudomonas aeruginosa*, one of the most common nosocomial bacteria causing opportunistic infections in liver transplant recipients, causes substantial ultrastructural changes in rat LSECs, such as endothelial thinning and reduction of porosity, that might lead to loss of fenestrae[166]. These structural changes were also described in LSECs in response to bacilli, such as *Bartonella* bacilli[167]. LSEC loss of porosity leads to impaired lipoprotein and chylomicron uptake by the liver, and subsequent hyperlipidaemia, highlighting these cells as key players in sepsis-associated tissue lipoprotein lipase inhibition and increased hepatic triglyceride delivery[168]. Lipopolysaccharide released by Gram-negative bacteria during sepsis is rapidly cleared from circulation. LSECs are involved in this clearance through the HDL-mediated association with lipopolysaccharide[169]. Scavenger receptor B1, which is abundantly expressed by mouse LSECs[170], might be implicated in this process. In response to *Listeria monocytogenes*, LSECs express constitutive CXCR6 ligand CXCL16, indirectly contributing to accumulation of CXCR6⁺ cytotoxic T lymphocytes in mouse livers[171].

LSECs have been outlined as important antiviral players in liver immunology as they contribute to eliminating internalized bacteriophages by lysosomal degradation[172]. They can also overcome T cell suppressive-induced immunity by TLR1 or TLR2 ligand activation after exposure to palmitoyl-3-cysteine-serine-lysine-4. In this context, LSECs can induce antiviral CD8⁺ T specific cell responses[173]. TLR3 (REF.[174]) and NOD1 receptors[175] in mouse LSECs induce proinflammatory responses and activation of T cell-specific antigenic responses against HBV in vitro. Initial scavenging of HBV by LSECs[176] suggests that virus transcytosis across LSECs might constitute a mechanism explaining the described contradiction between highly efficient liver targeting and inefficient virus uptake by cultured hepatocytes[177,178].

LSECs interact with structural envelope protein 2 from HCV through the C-type lectin L-SIGN, although whether this interaction causes HCV lysosomal degradation or *trans*-infection of hepatocytes remains to be elucidated[179]. In this regard, one study showed that in chronic HCV infection, LSECs maintain their phenotype and that capillarization is induced exclusively in the initial stages of fibrosis[180].

LSECs can also be targeted by cytomegalovirus. Cytomegalovirus-infected LSECs increase their expression of trafficking molecules such as ICAM1 and CXCL10. As a consequence, effector $CD4^+$ T cells are recruited and functional activation of different T cell subsets is promoted, leading to hepatic inflammation[181].

Beyond bacterial and viral infections, LSECs have shown a role in parasitic *Plasmodium* infections as they can bind malaria sporozoites, probably by recognizing proteoglycans present along the endothelial surface, and contribute to their liver entry towards hepatic parenchyma[182,183].

Role of LSECs in chronic liver disease

LSECs in steatohepatitis. Nonalcoholic steatohepatitis (NASH) is an advanced stage of nonalcoholic fatty liver disease (NAFLD) characterized by inflammation, steatosis, hepatocellular injury and fibrosis[184,185]. Preclinical studies in models of NAFLD and NASH have suggested that LSECs become capillarized in the early stages of NAFLD, even without substantial inflammation or HSC activation[186–189]. A reduction in eNOS activity, accompanied by dysregulation of a variety of capillarization markers, and a defect in cell survival mechanisms have been described in rodent LSECs in NAFLD[187,190,191]. In preclinical models, dysfunctional LSECs affect the intrahepatic microcirculatory status, evidenced by the development of portal hypertension due to increased hepatic vascular resistance, and steatosis progression to NASH. Hepatic haemodynamic dysregulations in preclinical NAFLD and NASH derive from a deficient vasodilatory capacity of capillarized LSECs[188,192,193]. Additionally, evidence from mice suggests that dysfunctional LSECs produce profibrogenic molecules such as transforming growth factor-β[194] that, with the associated reduction in NO bioavailability, promote HSC activation, which results in ECM production and sinusoidal vasocontraction in NASH. It is well known that healthy rat LSECs maintain the HSC quiescent phenotype, whereas capillarized LSECs lose this effect[54].

In addition, reduced permeability of the sinusoids in the early stages of murine NAFLD might impede the hepatic uptake of chylomicrons and retinol[195], which in combination with a reduction in hepatic fatty acid oxidation, the blockage of lipid outflow through sinusoids[196] and the increase in the de novo synthesis of hepatic lipids[197] would favour advanced stages of NASH. Regarding the latter mechanism, preclinical data have shown that targeting hepatic glutaminase 1 (GLS1) results in NASH amelioration through the restoration of VLDL assembly and export[198]. Considering that LSECs exhibit higher expression of GLS1 than hepatocytes[199], it is conceivable that LSEC GLS1 might have a direct role in NASH pathophysiology and might be a novel treatment target for NASH.

In summary, LSEC capillarization precedes NAFLD and can contribute to the progression and perpetuation of chronic liver injury in NASH[186,200] (FIG. 4). The characterization of the dysfunctional paracrine communication between LSECs and parenchymal cells represents an important goal for developing future NAFLD and NASH treatments.

LSECs in chronic viral infection. Chronic viral infection occurs when the host immune response is unable to resolve the acute viral infection phase[201]. Viruses can persist in the liver, promoting chronic liver damage, cirrhosis and HCC. Hepatic immune defence is very effective against acute hepatitis A and hepatitis E virus infection, whereas HBV, HCV or hepatitis D virus infection can progress until chronic infection[202].

Worldwide, HBV infection is the main cause of cirrhosis and HCC[203]. An early innate immune response followed by the adaptive immune response is essential for HBV clearance[204], and it has been demonstrated in mice that HBV induces the host innate immune response to supress HBV replication via TLR signalling in non-parenchymal cells, including LSECs[174]. In this sense, preclinical studies of HBV infection showed that treatments using proinflammatory molecules such as interferon, TNF and TLR ligands to stimulate immune cells and liver parenchymal and non-parenchymal cells induce antiviral mediators such as type I interferon, which ultimately inhibit viral replication[205]. A preclinical study including in vitro and in vivo data showed the use of semaphorin 4D as a promoter of $CD8^+$ T cell response for HBV clearance in LSECs[206] and found that semaphorin 4D was able to activate LSECs as APCs.

LSECs have also been shown in in vitro and in vivo preclinical models to act as APCs to eliminate HCV-infected hepatocytes by release of several cytokines[207,208] and recruitment of $CD8^+$ T cells[5]. Additionally, using primary cells and cell lines of human LSECs and hepatocytes, Rowe et al.[209] described BMP4–VEGFR2–p38 mitogen-activated protein kinase signalling as an important paracrine connection between LSECs and hepatocytes for supporting HCV replication, suggesting a possible future therapeutic strategy. Indeed, preclinical studies in primary human LSECs and in mouse models of HCV infection have demonstrated that treatments with regulators of immune response, such as interferon and TNF, delivered to LSECs are able to eliminate HCV or inhibit its replication[210,211].

The implication of LSECs in chronic viral hepatitis remains partially described but investigations of LSEC dysregulation after acute viral infection will be valuable to understand progression to cirrhosis and to discover new treatments for viral infection progression. It would also be especially relevant to understand the possible effects of direct-acting antiviral agents on LSEC phenotype. In this regard, clinical studies have suggested improvement of endothelial function after direct-acting antiviral treatment in patients with HCV, as shown by reduction in expression of endothelial cell adhesion molecules, including ICAM1 and E-selectin[212,213].

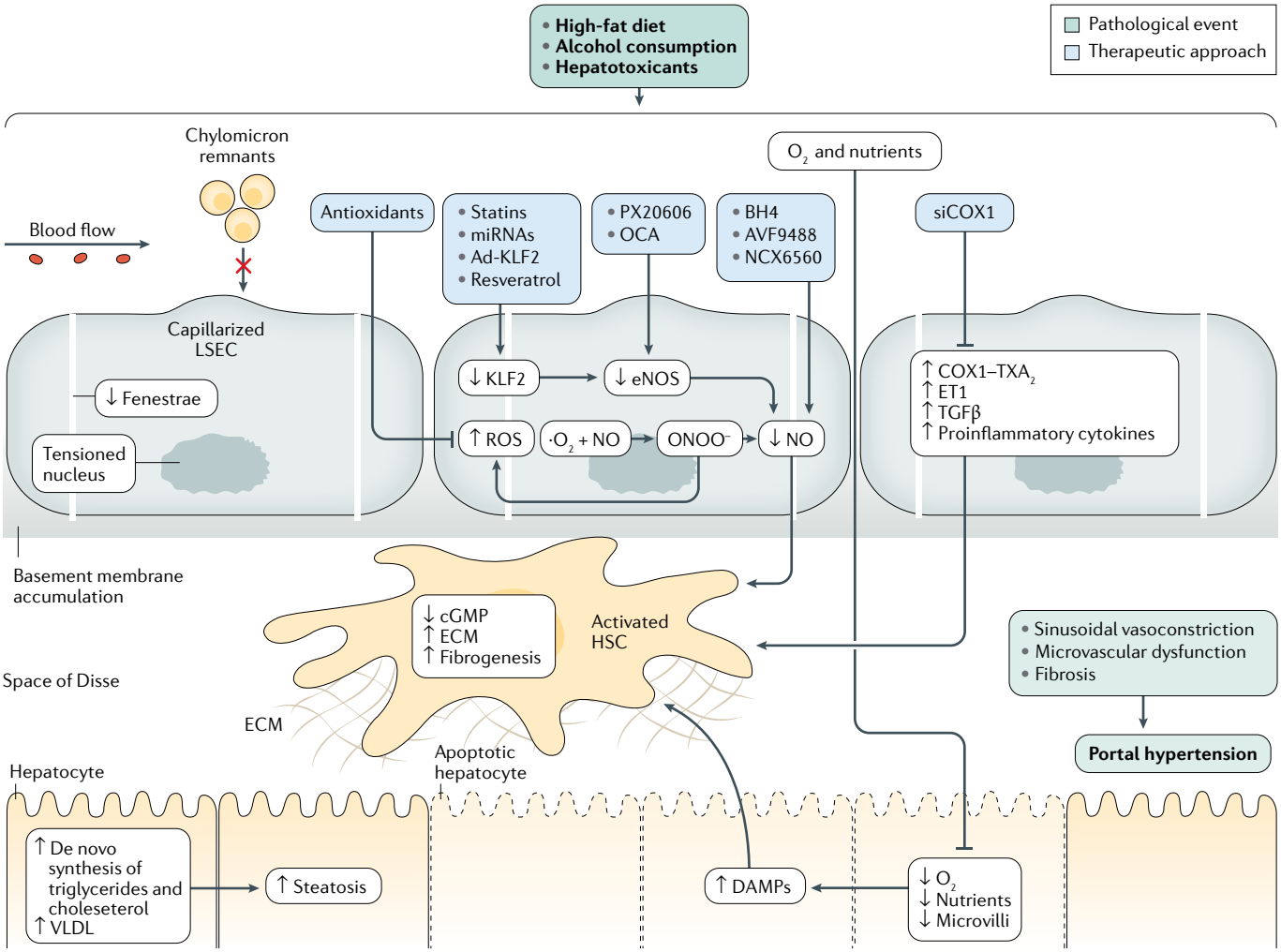

Fig. 4 | **Pathobiology of LSECs in chronic liver disease.** Chronic liver injury leads to a profound dedifferentiation of liver sinusoidal endothelial cells (LSECs), which lose their vasoprotective properties and become vasoconstrictive, proinflammatory and prothrombotic. The main molecular dysregulations observed in LSECs in chronic liver disease are depicted in the figure. These effects include loss of fenestrae and development of basement membrane that impede the exchange of molecules such as lipoproteins and oxygen with hepatocytes, promoting steatosis and parenchymal apoptosis; reduction of nitric oxide (NO) bioavailability by downregulation of Krüppel-like factor 2 (KLF2) and endothelial NO synthase (eNOS) activity, together with increased reactive oxygen species (ROS)-mediated NO scavenging, resulting in hepatic stellate cell (HSC) activation and extracellular matrix (ECM) deposition; and increased vasoconstrictor (such as endothelin 1 (ET1) or thromboxane A_2 (TXA_2)) and proinflammatory cytokine production that further aggravates sinusoidal vasoconstriction. These pathobiological alterations lead to sinusoidal vasoconstriction, microvascular dysfunction, fibrosis and ultimately the development of portal hypertension. Therapeutic approaches targeting these molecular pathways, including inducers of the transcription factor KLF2 (such as statins), antioxidants and inhibitors of prostanoid synthesis are also shown. Ad-KLF2, adenovirus codifying for Krüppel-like factor 2; cGMP, cyclic GMP; COX1, cyclooxygenase 1; DAMP; damage-associated molecular pattern; miRNA, microRNA; OCA, obeticholic acid; siCOX1, cyclooxygenase 1 silencing RNA; TGFβ, transforming growth factor-β.

LSECs in chronic hepatotoxic injury. Different preclinical models have been developed to understand the hepatocellular dysregulations occurring in chronic hepatotoxicity; however, little is known about the effect of long-term alcohol intake on LSECs. In this regard, only one study in a preclinical model of long-term ethanol intake examined LSEC fenestrae by transmission electron microscopy, showing an ethanol-intake time-dependent decrease in sinusoidal porosity[214]. Complementary studies to understand the role of LSEC pathobiology in toxicant-induced liver injury have been performed in preclinical models that recapitulate most of the hepatic and extrahepatic complications of chronic liver disease (CLD), such as carbon tetrachloride (CCl_4) and thioacetamide models[215]. In these models, dysregulation of LSECs starts rapidly after acute liver injury, followed by the loss of fenestrae and reduced porosity in chronic hepatic damage[216–218].

Capillarization is accompanied by the release of several cytokines and soluble factors that rapidly affect neighbouring cells, promoting their dedifferentiation and favouring the development of CLD complications,

including portal hypertension[3,62]. Increased production of vasoconstrictors by LSECs during cirrhosis has been demonstrated in preclinical models[219]. Activation of the cyclooxygenase 1 (COX1)–TXA_2 pathway[219] and endothelin 1 (REF.[220]) in rat chronic liver injury contributes to sinusoidal contraction, aggravating microvascular dysfunction. Studies by Graupera et al.[221] rejected a role for endothelial COX2 modulating the hepatic vascular tone in CLD rats; however, this isoform might indeed play a part in fibrogenesis through its activity in other non-parenchymal cells[222]. Other arachidonic acid-derived eicosanoids, such as leukotrienes[223], also contribute to hepatic microcirculatory dysfunction and portal hypertension in cirrhosis partly through their production by LSECs.

In addition to increased vasoconstrictors, capillarized LSECs exhibit an impairment of the eNOS–NO pathway resulting in endothelial dysfunction and portal hypertension. Rockey et al. demonstrated downregulation pf eNOS activity and NO bioavailability in the cirrhotic rat liver[224], and our group showed that elevated hepatic oxidative stress in preclinical cirrhosis[4,225], and disrupted activity of the transcription factor KLF2 (REF.[216]), further contributes to diminish NO availability, aggravating sinusoidal vasoconstriction[4]. A close interrelation between the NO system and the COX1–TXA_2 pathway in the endothelium associated with cirrhosis further aggravates the imbalance of vasodilators and vasoconstrictors within the liver sinusoid[226] (FIG. 4).

LSECs might also play a role in CLD and portal hypertension through a dysregulation of their antithrombotic capacity. LSEC capillarization, and in particular the loss of the KLF2-dependent vasoprotective pathways (which includes various genes involved in coagulation), might actively contribute to the recruitment and activation of platelets, promoting microthrombosis and fibrin deposition within the sinusoids, leading to episodes of hypoxia, sinusoidal hypertension and even parenchymal extinction[227]. These detrimental endothelium–platelet interactions could be inhibited or reduced with the use of anticoagulants[228,229], but benefit should also be expected by vasoprotective strategies targeting KLF2, such as statins.

Therapeutic approaches targeting LSECs in CLD. Considering their role in CLD, several therapeutic options targeting LSECs have been investigated in the past few years (TABLE 3). Various preclinical studies evaluated statins and demonstrated their beneficial effects on endothelial dysfunction, fibrogenesis and portal hypertension[230]. Simvastatin reduces endothelial dysfunction and portal hypertension through the activation of the transcription factor KLF2 in LSECs[231–233], which promotes beneficial paracrine effects in HSCs. Conversely, the effect of statins on LSEC fenestrae remains unclear. Hunt et al.[234] showed that in vitro treatment with simvastatin did not ameliorate loss of fenestrae in aged capillarized LSECs. Nevertheless, our group[61] reported in aged cirrhotic animals that in vivo simvastatin treatment was able to increase endothelium porosity, with associated amelioration of microvascular dysfunction and portal hypertension.

Short-term or long-term simvastatin treatment also decreased portal pressure in patients with cirrhosis[235,236] without changes in hepatic blood flow, suggesting an increase in hepatic vascular resistance through the increase in NO production. In preclinical late-stage cirrhosis, simvastatin prevented the deleterious effects of acute-on-chronic liver failure, mainly by inhibiting the proinflammatory response and further deterioration of microvascular dysfunction[237]. Ongoing clinical trials will determine its usefulness in advanced CLD[238].

In addition to statins, treatments regulating eNOS activity and NO production, such as AVE 9488 (REF.[239]) and tetrahydrobiopterin[240,241], were also suggested as therapeutic options for ameliorating LSEC dysfunction in animal models of cirrhosis. Similarly, reduction of the levels of vasoconstrictors is also a good therapeutic strategy for LSEC function improvement. Following seminal studies targeting the hepatic COX1–TXA_2 axis[219,242,243], Lin et al.[244] demonstrated that CCl_4-cirrhotic mice treated with small interfering RNA against LSEC-specific COX1 and TXA_2 showed reduced portal pressure and liver fibrosis.

Antioxidant molecules targeting LSECs have been proposed as potential therapeutic options to relieve CLD and its complications. Diverse compounds, including a recombinant form of manganese superoxide dismutase[245], resveratrol[246], docosahexaenoic acid triglyceride[247] and dark chocolate[248,249], have caused improvement in LSEC phenotype and function in preclinical models or in patients, which ultimately ameliorated key components of CLD pathophysiology, including fibrosis, microvascular dysfunction and portal hypertension.

KLF2 is a nuclear transcription factor sensitive to shear stress that confers endothelial vasoprotection. In preclinical models of CLD, liver endothelial KLF2 is upregulated as a compensatory mechanism aimed at promoting the transcription of its vasoprotective target genes, but important post-transcriptional mechanisms inhibit their efficient expression[216]. Thus, further activation of the KLF2 pathway with statins[231,232], resveratrol[250] or microRNAs[251,252] is a strategy for endothelial protection and amelioration of CLD.

In addition to KLF2, other transcription factors have been studied as potential therapeutic strategies for LSEC phenotype modulation. Different agonists of farnesoid X receptor, such as obeticholic acid[253] and PX20606 (REF.[254]), were able to restore eNOS activity in cirrhotic animals, with the associated amelioration of endothelial dysfunction and portal hypertension. Obeticholic acid has also been suggested as a new therapy for NASH[255]. Finally, activation of different peroxisome proliferator-activated receptors (PPARs) by fenofibrate[256] or aleglitazar[257], an agonist of PPARα and PPARγ, resulted in amelioration of hepatic endothelial dysfunction in cirrhotic rats. Moreover, primary isolated cirrhotic rat LSECs treated in vitro with aleglitazar exhibited reduced levels of proangiogenic markers[257]. Also, data from our group support the beneficial effects of the pan-PPAR agonist lanifibranor on LSEC phenotype and hepatic vascular function in preclinical cirrhosis and in primary cells isolated from patients with cirrhosis[258].

Table 3 | **Summary of therapies for cirrhosis and ACLF targeting the sinusoidal endothelium**

Study population	Treatment	Results	Ref.
Cirrhosis			
Patients with cirrhosis	Simvastatin (40 mg)	Increased hepatic vein NO levels and amelioration of postprandial increase in HVPG	235
CCl_4-cirrhotic Wistar rats	COX1 inhibitor: SC-560 (5 μM)	Amelioration of microvascular dysfunction and reduction of hepatic TXA_2 production	243
CCl_4-cirrhotic Wistar rats	Tetrahydrobiopterin (8 mg/kg)	Amelioration of endothelial dysfunction and increase of NO bioavailability	240
CCl_4-cirrhotic Wistar rats and primary CCl_4-rat LSECs	COX inhibitor: indomethacin (10 μM); COX1 inhibitor: SC-560 (5 μM); PGH_2/TXA_2 receptor inhibitor: SQ-29548 (1 μM)	Amelioration of microvascular dysfunction and reduction of hepatic TXA_2 production	219
CCl_4-cirrhotic Wistar rats	Simvastatin (25 mg/kg)	Decrease of PP and increased eNOS activity and NO bioavailability	327
cBDL Sprague Dawley rats	eNOS enhancer: AVE 9488 (1 mg)	Decrease of PP, IHVR and microvascular dysfunction and upregulation of hepatic eNOS	239
CCl_4-cirrhotic Wistar rats	Tetrahydrobiopterin (10 mg/kg)	Decrease of PP and increased eNOS activity and NO bioavailability	241
Patients with cirrhosis	Dark chocolate (0.55 g/kg)	Amelioration of postprandial increase in HVPG and reduction of hepatic oxidative stress	248
CCl_4-cirrhotic Wistar rats and primary CCl_4-rat LSECs	PPARα agonist: fenofibrate (25 mg/kg in vivo and 100 μM in vitro)	Decrease of PP, amelioration of hepatic endothelial dysfunction and increased NO bioavailability in LSECs	256
Primary CCl_4-rat LSECs	Simvastatin (1 μM in vitro)	Upregulation of KLF2 and eNOS and deactivation of HSCs via paracrine signalling	231
CCl_4-cirrhotic Wistar rats and cBDL Sprague Dawley rats	Recombinant MnSOD (15 μg/kg)	Decrease of PP, HVR and hepatic endothelial dysfunction	245
CCl_4-cirrhotic Wistar rats and primary isolated CCl_4-rat LSECs	Resveratrol (10 mg/kg)	Decrease of PP, reduction of hepatic oxidative stress and TXA_2 levels, increased NO production in LSECs and reduction of TXA_2 production in LSECs	246
TAA-cirrhotic Wistar rats and BALB/cByJ mouse primary isolated LSECs	FXR agonist: obeticholic acid (10 mg/kg in vivo and 0.1–10 μM in vitro)	Decrease of PP and HVR and downregulation of profibrotic cytokines in LSECs	328
CCl_4-cirrhotic Wistar rats	Ad-KLF2 (10^{11} adenovirus particles)	Decrease of PP and endothelial function, and up-regulation of eNOS	232
CCl_4-cirrhotic Wistar rats and cBDL Sprague Dawley rats	Atorvastatin (10–15 mg/kg), NCX 6560 (17.5 mg/kg)	Decrease of PP and increased hepatic eNOS activity	233
CCl_4 C57Bl/6 mice and primary isolated CCl_4-mouse LSECs	siCOX1 (0.6 mg/kg in vivo and 100 nM in vitro)	Decrease of PP and reduction of TXA_2 production in LSECs	244
CCl_4-cirrhotic Sprague Dawley rats	FXR agonist: PX20606 (10 mg/kg)	Decrease of PP and upregulation of eNOS	254
CCl_4-cirrhotic Wistar rats	Human amnion-derived stem cells (4×10^6 viable cells)	Decrease of PP, amelioration of microvascular dysfunction, amelioration of LSEC capillarization and reduction of hepatic inflammation	329
TAA-cirrhotic Sprague Dawley rats, cBDL Sprague Dawley rats and primary rat LSECs	PPAR agonist: aleglitazar (0.3 mg/kg in vivo and 100 nM in vitro)	Decrease of PP and decrease of LSEC migration and angiogenic index	257
cBDL-cirrhotic Sprague Dawley rats	AMPK activator: AICAR (200 mg/kg)	Decrease of PP and increase of hepatic eNOS activity and NO levels	330
CCl_4-cirrhotic Wistar rats	Caspase inhibitor: emricasan (10 mg/kg)	Decrease of PP and amelioration of endothelial dysfunction, increased eNOS activity and NO bioavailability and amelioration of LSEC phenotype	331
CCl_4-cirrhotic Wistar rats	UT antagonist: palosuran (300 mg/kg)	Decrease of PP and HVR and upregulation of hepatic p-eNOS	332
HFD-fed Sprague Dawley rats and primary rat LSECs	Statins (10 mg/kg)	Decrease of PP and amelioration of endothelial dysfunction and LSEC capillarization	333
CCl_4-cirrhotic aged Wistar rats	Simvastatin (5 mg/kg)	Decrease of PP and HVR, amelioration of microvascular dysfunction, amelioration of LSEC phenotype and reduction of hepatic inflammation and oxidative stress	61
TAA-cirrhotic Sprague Dawley rats, cBDL-cirrhotic Sprague Dawley rats and primary human liver cells	Pan-PPAR agonist: lanifibranor (100 mg/kg)	Decrease of PP and HVR and amelioration of LSEC phenotype	258

Table 3 (cont.) | **Summary of therapies for cirrhosis and ACLF targeting the sinusoidal endothelium**

Study population	Treatment	Results	Ref.
Cirrhosis (cont.)			
cBDL-cirrhotic Sprague Dawley rats and primary rat LSECs	Simvastatin-loaded nanoparticles (1–5 mg/kg)	Decrease of PP and upregulation of KLF2–eNOS	334
ACLF			
cBDL Sprague Dawley rats plus haemorrhage and resuscitation	Simvastatin (5 mg/kg)	Decrease of microvascular dysfunction and reduction of hepatic inflammation	335
CCl_4-cirrhotic Wistar rats plus LPS, cBDL Sprague Dawley rats plus LPS and TAA-cirrhotic Sprague Dawley rats plus LPS	Simvastatin (5 and 25 mg/kg)	Decrease of PP and HVR, amelioration of microvascular dysfunction, upregulation of hepatic eNOS and reduction of hepatic inflammation and oxidative stress	237

ACLF, acute-on-chronic liver failure; Ad-KLF2, adenovirus codifying for Krüppel-like factor 2; AICAR, 5-aminoimidazole-4-carboxamide riboside; cBDL, common bile duct ligation; COX, cyclooxygenase; eNOS, endothelial nitric oxide synthase; FXR, farnesoid X receptor; HFD: high-fat diet; HSC, hepatic stellate cell; HVR, hepatic vascular resistance; HVPG, hepatic venous pressure gradient; IHVR, intrahepatic vascular resistance; KLF2, Krüppel-like factor 2; LPS, liposaccharide; LSEC, liver sinusoidal endothelial cell; MnSOD, manganese superoxide dismutase; p-eNOS, phosphorylated endothelial nitric oxide synthase; PGH_2, prostaglandin H_2; PP, portal pressure; PPAR, peroxisome proliferator-activated receptor; siCOX1, cyclooxygenase 1 silencing RNA; TAA, thioacetamide; TXA_2, thromboxane A_2; UT, urotensin II receptor.

Role of LSECs in liver cancer

Inflammation is a key player in HCC progression and implies the interaction of tumour cells with tumour-associated immune cells[259,260]. HCC is the consequence of proliferative, invasive and survival feature acquisition of preneoplastic lesions, caused by genetic and epigenetic alterations developed in the context of inflammation-sustained liver damage[261].

HCC induces phenotypic changes in surrounding LSECs that contribute to lessen the antitumoural immune response (FIG. 5). During HCC progression, LSECs lose their fenestrae and accumulate a basement membrane[262]. Human LSEC marker profile modification is also evidenced by reduction of ICAM1 expression, and loss of STAB1, STAB2, LYVE1 and CD32b expression[263,264]. On the other hand, LSECs become capable of participating in angiogenesis, procoagulation and fibrinolytic events during tumorigenesis in mice[264], in which platelets might be potentially involved[227]. These changes are representative of transdifferentiation of LSECs and are suggestive of their role in tumour vascularization during HCC development[265].

Programmed cell death 1 ligand 1(PDL1) and PDL2, as well as the co-stimulatory molecules CD80 and CD86, are expressed by LSECs as part of their antigen-presenting functions[266]. These structures constitute ligands for the immune checkpoints PD1 and cytotoxic T lymphocyte antigen 4 (CTLA4) present in T cells, respectively[266]. On interaction, activation of T cells is inhibited and, in turn, they acquire a tolerogenic differentiated state facilitated by the local production of IL-10 (REF.[266]). Although LSECs increase the expression of CD151, which regulates VCAM1 activity and collaborates in T cell recruitment to the tumour[267], the overexpression of PDL1 in LSECs during HCC[268], as observed also in tumour cells[269], induces the inhibition of T cell function and limits T cell antitumoural activity[270].

A role for LSECs in the recruitment of other tissue-derived tumorigenic cells towards the liver has also been described. The expression of β2 integrin is a docking signal for adhesion molecule-driven infiltration of tumorigenic cells, therefore facilitating metastasis progression to the liver of several solid tumours, including colorectal cancer[271]. Accordingly, the blockade of ICAM1 in LSECs reduces tumoural cell adhesion and transmigration in vivo and in vitro[272]. In addition, LSEC immune modulation by melittin nanoparticles favours the immune response against tumoural cells in a mouse spontaneous liver metastatic tumour model[273].

Therapeutic approaches targeting LSECs in HCC. Blockade with drugs targeting neoangiogenesis, cell proliferation, cell survival or cell motility signalling pathway intermediaries is a recurrent strategy considered in HCC treatment[274]. As an example, antibodies recognizing PDL1 in LSECs such as durvalumab are under evaluation[275]. Also, miR-3178 expression is downregulated in tumour endothelial cells compared with LSECs in mice, and its up-regulation might be considered as a therapeutic target in HCC management[276]. The use tyrosine kinase inhibitors in LSECs such as cabozantinib or regorafenib has been demonstrated to improve clinical outcomes in clinical trials in HCC[275]. Finally, as mentioned already, LSECs express ligands to immune checkpoints that are relevant for inhibiting or stimulating T cell responses. Accordingly, monoclonal antibodies to the immune checkpoints CTLA4, such as tremelimumab and ipilimumab, and PD1 such as tislelizumab and camrelizumab, both recognizing T cells, are currently being studied in patients with HCC; these last two agents are now in phase III clinical trials (NCT03412773 and NCT02989922)[277].

Conclusions

Throughout this Review, we have detailed the fundamental aspects of the (patho)biology of the sinusoidal endothelium, and its potential as a therapeutic target in liver diseases. Although our knowledge of this cell type has advanced significantly, much research is still needed. As a conclusion, we highlight three avenues of research that certainly require effort by the hepatologist community. Firstly, it is important to recognize that the role of LSECs in various liver diseases, such as cholestatic or non-cirrhotic vascular disorders, is still largely unknown. In this regard, we need to perform studies

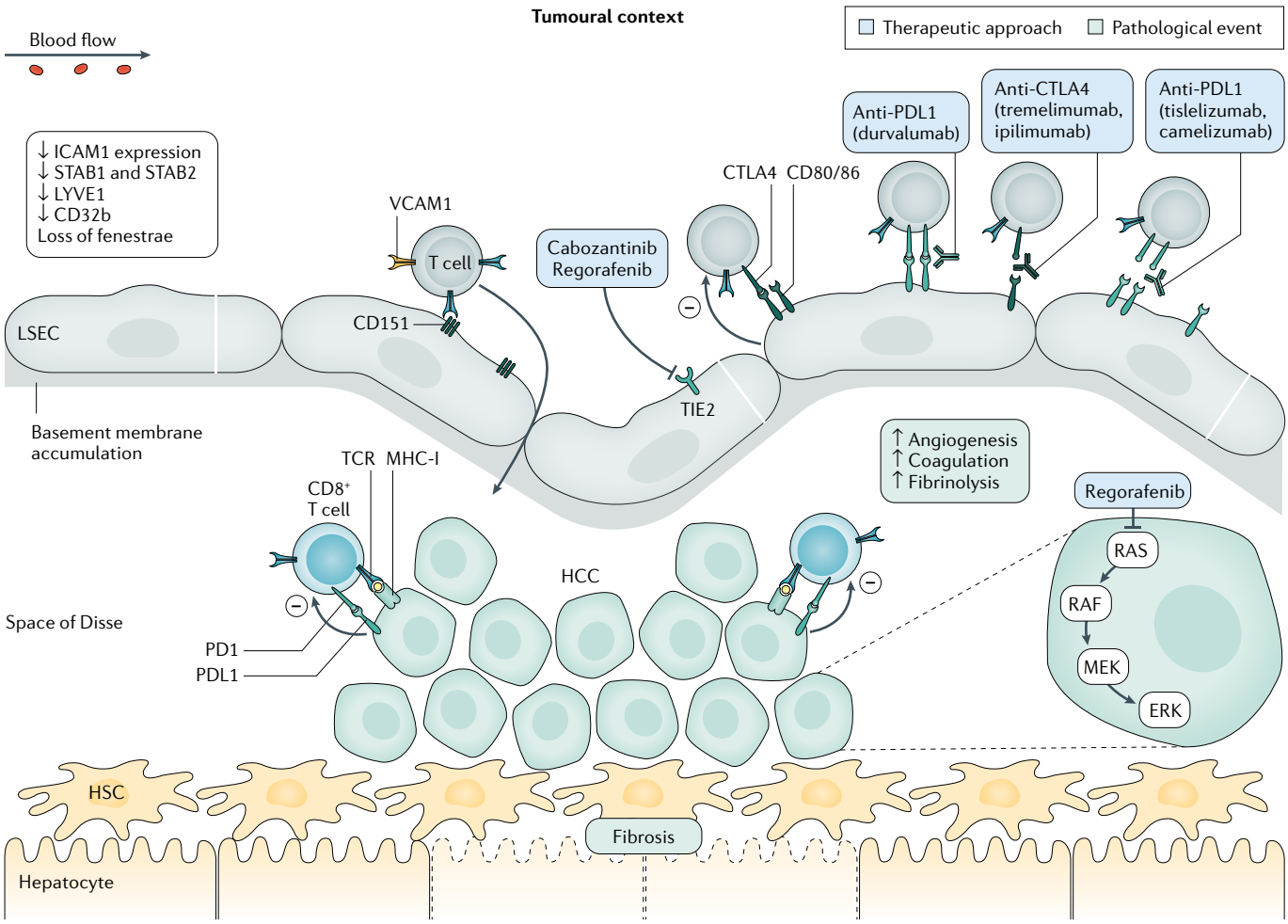

Fig. 5 | **Pathobiology of LSECs in hepatocellular carcinoma.** Liver sinusoidal endothelial cells (LSECs) are capillarized in the tumoural context of hepatocellular carcinoma (HCC) and participate in events such as angiogenesis, coagulation and fibrinolysis. LSECs change their marker expression profile, reducing expression of intercellular adhesion molecule 1 (ICAM1), stabilin 1 (STAB1), STAB2, lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1) and Fcγ receptor IIb (FcγRIIb; also known as CD32b). They undergo a loss of fenestrae and they increase CD151 expression, favouring T cell recruitment via vascular cell adhesion protein 1 (VCAM1). LSECs in HCC also express CD80 and CD86, which can be considered checkpoints for treatment as they are ligands of the T cell inhibitor cytotoxic T lymphocyte antigen 4 (CTLA4). $CD8^+$ antitumoural T cells can be inhibited by interaction of their programmed cell death 1 (PD1) receptor with programmed cell death 1 ligand 1 (PDL1) expressed by the tumour cell. Different blocking monoclonal antibodies to receptors expressed by LSECs are under examination, such as durvalumab for PDL1, tislelizumab and camrelizumab for PD1, and tremelimumab and ipilimumab for CTLA4. Moreover, cabozantinib and regorafenib are being used to block TIE2 expressed by LSECs. ERK, extracellular-signal-regulated kinase; MEK, MAPK/ERK kinase; MHC-I, major histocompatibility complex class I; TCR, T cell receptor.

based on clinical observation, in coordination with basic researchers and using relevant preclinical models. Secondly, future research on LSECs in hepatology should consider the potential of this cell type not only to help understand the pathophysiology of liver disease but also to discover biomarkers of liver microcirculatory dysfunction, development of fibrosis or elevation of portal pressure. As we have detailed, LSECs are positioned at a key location within the sinusoid and liver, and therefore would be able to detect changes in the liver microenvironment (such as a stiffening of the matrix[278] or increase of the sinusoidal resistance) and react by changing its phenotype and its secretome. The latter, derived from LSECs, could modulate neighbouring cells but also pass into the bloodstream and therefore be useful for the discovery of new biomarkers based on liquid biopsy. Finally, it will be important to detail the phenotypic changes that occur in LSECs during the capillarization processes described in this Review, but using more objective and unbiased approaches such as transcriptomic or proteomic sequencing in single cells. These analyses, which ideally will also be performed in human primary cells, will help us better understand the profile of LSECs during their dedifferentiation and, importantly, will provide reliable specific markers in disease. These points should be developed by the multidisciplinary liver sinusoidal community in the years to come.

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