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Liver sinusoidal endothelial cells — gate keepers of hepatic immunity

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Abstract

Liver sinusoidal endothelial cells (LSECs) line the low shear, sinusoidal capillary channels of the liver and are the most abundant non-parenchymal hepatic cell population. LSECs do not simply form a barrier within the hepatic sinusoids but have vital physiological and immunological functions, including filtration, endocytosis, antigen presentation and leukocyte recruitment. Reflecting these multifunctional properties, LSECs display unique structural and phenotypic features that differentiate them from capillary endothelium present within other organs. It is now clear that LSECs play a critical role in maintaining immune homeostasis within the liver and in mediating the immune response during acute and chronic liver injury. In this Review, we outline how LSECs influence the immune microenvironment within the liver and discuss their contribution to immune-mediated liver diseases and the complications of fibrosis and carcinogenesis.

Author: Please provide 4-6 key points. This is a feature that should comprise a bullet-pointed list of the contents of the article (4-6 points, each 1 sentence max, max 30 words long). These points should provide the reader with a quick overview of the content, and should also act as a reminder once the article has been read.

1. Liver sinusoidal endothelial cells (LSECs) that line the hepatic sinusoids play important physiological roles and mediate the filtration and scavenger functions of the liver.
2. LSECs also have innate and adaptive immunological functions including antigen presentation and maintaining the balance between tolerance and effector immune responses.
3. In inflammatory liver diseases they influence the composition of hepatic immune populations by mediating diapedesis of leukocyte subsets via distinct combinations of adhesion molecules and chemokines.
4. LSECs play a crucial role in the cellular cross talk which regulates progressive chronic liver disease leading to fibrosis and carcinogenesis.
5. The role of LSECs in initiating immune responses and contributing to progressive liver disease make them a potential therapeutic target for treating inflammatory liver diseases.

[H1]Introduction

Sinusoidal endothelial cells line what constitutes a unique vascular bed in the liver, which receives blood from both the hepatic artery and portal veins into the hepatic parenchyma (Fig 1). Studies of these cells isolated from animals usually refer to them as liver sinusoidal endothelial cells (LSECs), whereas isolated human cells have also been referred to as human hepatic sinusoidal endothelial cells (HSECs). For the purpose of this Review we use the term LSEC. The exposure of these sinusoidal endothelial cells to blood originating from both the gut and systemic circulation means they are ideally situated to remove and recycle blood-borne proteins and lipids. In combination with Kupffer cells (liver-resident macrophages), LSECs constitute the most powerful scavenger system in the body[1]. This activity is facilitated by the presence of fenestrae in LSECs, their lack of a classical basement membrane and their expression of promiscuous scavenger receptors combined with the most potent endocytic capacity in the body[2]. Thus virus particles[3], advanced glycation end products[4] and modified LDL[5] can be cleared from the circulation within minutes by this route.

Endothelial cells in different vascular beds are generated from common early embryological precursors, and have broadly similar histological appearance and functional roles throughout the body. However, extensive variations in phenotype and function arise as a consequence of local microenvironmental signals dependent on anatomical localisation[6]. The vascular architecture in the human liver is acquired by 17–25 weeks of gestation, but different vessels within the liver have distinct embryonic origins. Thus, portal vessels derive from vitelline veins, whereas sinusoids develop from capillary vessels of the septum transversum and acquire their distinctive fenestrated phenotype by week 20 of gestation[7] under the control of GATA-4[8]. From this point onward, sinusoidal endothelial cells remain functionally and phenotypically distinct from the other vascular endothelial cells in the liver microenvironment and assume a phenotype that has many similarities with lymphatic endothelial cells[9]. The unique characteristics of LSECs are presented in Box 1. Both lymphatic and sinusoidal endothelial cells have minimal basement membranes and loosely organised cell junctions [10] and share a complement of receptors such as LYVE-1[11], Prox-1[12], podoplanin[13] and L-SIGN[14]. It has been shown that the phenotype of sinusoidal endothelial cells alters across the liver acinus; a study of human liver tissue published in 2017 demonstrated that zone 1 LSECs are $CD36^{hi}$ and $Lyve\text{-}1^{lo}$ whereas zone 2 and zone 3 LSECs are $CD36^{lo}$, $LYVE\text{-}1^{hi}$ AND $CD32^{hi}$ [15]. The presence of fenestrations or membranous pores organised into sieve plates is a feature that also distinguishes LSEC from the other hepatic endothelial populations.[2]

Fenestrations are not unique to hepatic endothelial cells and are also found in endothelium in endocrine glands such as the pancreas[16], the kidney[17], spleen[18] and bone marrow[19] and are sometimes observed in tumour vasculature[20]. However, unlike other fenestrated endothelial populations such as those in the kidney, hepatic fenestrations lack a diaphragm or basal lamina and are grouped into organised sieve plates, rendering LSEC highly permeable. Many studies have implicated VEGF as an essential factor for regulation of fenestrations[21], but dynamic changes in hepatic fenestration number and size can occur rapidly in response to agents such as alcohol[22], dietary constituents[23] and fasting[24] or calorie restriction[25].The fenestrations act as a ‘dynamic filter’ [26]to permit the access of macromolecules to parenchymal cells and in addition these pores might allow circulating viruses to gain access to hepatocytes[27]. Evidence from animal studies suggests that fenestrations can constitute up to 40% of the cell and that the size, distribution and clustering of the pores in sieve plates varies with the zonal distribution of the endothelium[28] and across the endothelial surface. Up to a third of these pores are organised into complex labyrinths and many are associated with components of microtubules[29], caveoli and coated pits to form a transport network that could impose additional regulation on the traffic of material into the cells[30], enabling them to govern the movement of materials to and from the liver parenchyma.

[H1]Balancing tolerance and immune response

The permissive nature of sinusoidal endothelium probably evolved to handle the constant exposure of the liver to microbial and food antigens derived from the gastrointestinal tract via the portal vein. The liver needs to ensure that damaging immune responses are not precipitated against harmless antigens, whilst at the same time being able to eliminate invading pathogens. The first site of exposure to these antigens occurs within the hepatic sinusoids and both Kupffer cells (KCs) and LSECs are important players in taking up and eliminating soluble antigens entering via the portal vein and in determining the nature of any immune response such antigens trigger.

The initial critical step in an immune response is the innate pathway of antigen uptake by pattern recognition receptors[31]. Pattern recognition receptors are highly evolutionarily conserved and include the Toll-like Receptor (TLR) family and the scavenger receptors[31]. An example of how the liver regulates inflammatory and immune responses is seen in the recognition of the TLR-4 ligand lipopolysaccharide (LPS) by KCs and LSEC. Chronic exposure of both KCs and LSEC to LPS leads to an LPS-refractory state, and in LSECs specifically LPS exposure is associated with reduced nuclear translocation of nuclear-factor-kappa-light-chain enhancer of activated B cells (NF-κB) and subsequent reduced leukocyte adhesion[32]. This mechanism prevents the liver being in a constantly activated inflamed state in response to the constant exposure to bacterial products from the gut. Studies of other TLRs demonstrate that LSEC can respond to signals mediated via TLR1-4, 6, 8 and 9, but their activation has cell-specific responses that are restricted compared with classical antigen presenting cells, thereby contributing to an organ-specific response to antigens and the tolerogenic environment of the liver[33].

A unique characteristic of LSEC is their expression of high levels of several scavenger receptors compared with conventional endothelium. Scavenger receptors are a diverse family of pattern recognition receptors that, like TLRs, are highly evolutionarily conserved[34]. In contrast to TLRs, they were believed to be functionally redundant and to perform silent uptake of ligands. However, gathering evidence suggests that this is not the case and that scavenger receptors have an important cell-specific role in immune responses[34]. They have been shown to promote potent pro-inflammatory and anti-inflammatory signalling as well as directly interacting with TLRs. Membrane bound scavenger receptors recognise their extracellular ligands which leads to internalisation of the ligand, termed endocytosis, trafficking from the cell membrane to intracellular compartments such as the endosomes. The high levels of scavenger receptors on LSEC give them a high endocytic capacity. One of the most extensively studied scavenger receptors on LSECs is the mannose receptor (MR)[1,35,36]. Others include the homologous scavenger receptors stabilin-1 and stabilin-2[37] and related molecules such as C-type lectins, including the type-2 receptor subclass dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) and liver/lymph node-specific intercellular adhesion molecule 3- grabbing non-integrin (L-SIGN)[38,39]. The members of the C-type lectin group are involved in varied functions, from cell–cell interaction to uptake of serum glycoproteins. A third lectin with a similar structure to DC-SIGN and L-SIGN has been identified and designated the liver and lymph node sinusoidal endothelial cell C-type lectin (LSECtin)[40]. This lectin has been shown to be co-expressed with L-SIGN and is encoded in the same cluster of lectin encoding genes as DC-SIGN and L-SIGN.

[H3] Innate immunity

Several of the C-type lectin receptor family members have been directly implicated in viral uptake. Both DC-SIGN and L-SIGN have been shown to interact with the Ebola virus and HIV, as well as the coronavirus[41,42]. Both these receptors have also been shown to be expressed on LSECs and bind the E2 glycoprotein of the hepatitis C virus (HCV) and facilitate hepatocyte infection[39]. LSECtin has also been implicated in the uptake of SARS coronavirus and HCV[43,44]. The ability of LSEC to bind multiple viruses through their diverse endocytic receptors gives them a crucial role in the response to viral infections and a specific role in mediating rapid clearance of blood-borne viruses[45]. In a mouse model of adenovirus infection, 90% of virus is found in LSECs and 10% in KCs within a minute of intravenous viral infusion[45]. A study published in 2017 reported that HIV-like particles are taken up by mouse LSECs at a rate of 100 million particles per min.[3] The transit of viruses internalised by LSECs is less well understood whereas after receptor mediated endocytosis of circulating matrix breakdown products the subsequent transit from early endosomes to late endosomes takes several hours[46]. LSECs enable direct entry of certain viruses such as Ebola, whereas with other viruses, such as HCV and HBV, LSECs promote hepatotropism by facilitating parenchymal cell infection[47]. Rapid uptake of virus can also lead to redistribution to other cells, for instance in animal models of HBV viral particles are preferentially taken up by LSECs and subsequently passed on to infect underlying hepatocytes[48]. In the case of HCV, innate sensing of viral infection by LSEC leads to downstream signalling and release of paracrine signals such as the pro-viral molecule bone morphogenetic protein 4, which enhances viral infection of hepatocytes[49]. On the other hand, direct sensing of HCV RNA in LSECs also leads to the release of IFN I/III rich exosomes that inhibit HCV replication[50]. The balance of such responses will determine whether virus infection is established or prevented, thereby emphasising the critical role that LSEC play in hepatotropic viral infections.

[H3]Adaptive immunity

LSEC not only regulate innate immune responses but also directly regulate adaptive immune responses through antigen presentation to T cells (FIG. 2). Knolle's group demonstrated that LSECs can cross-present antigen to $CD8^+$ T cells[51] by using scavenger receptors, notably the mannose receptor, to take up, process and transfer antigen to MHC class I[52]. The presentation of antigen, including oral antigens, by LSECs drives a tolerogenic response in naïve $CD8^+$ T cells mediated by upregulation on LSECs of the co-inhibitory molecule programmed death-ligand 1 (PD-L1), also known as CD274 or B7 homolog 1, which can activate its receptor PD-1 of naïve T cells [51,53,54]. Endocytosis of antigens by the mannose receptor on LSECs has been shown to promote $CD8^+$ T cell tolerance[55], including tolerance to tumour antigens[56]. However, it is crucial that rapid effector responses can be generated locally to harmful pathogens; consistently, LSEC-driven T cell activation changes in response to antigen load and local inflammatory factors. For example, in a culture model with mouse LSECs in which antigens at varying concentrations were delivered to LSECs for cross presentation to $CD8^+$ T cells, high antigen concentrations led to a shift from tolerogenic to effector T cell differentiation[57] as a consequence of enhanced TCR signalling that overcame PD-1 mediated tolerogenic responses. This response is also affected by local levels of IL-2. Furthermore, rapid activation of $CD8^+$ T cells by LSECs occurs in the presence of IL-6 trans-signalling and this activation not only drives rapid effector T cell differentiation but also primes T cells to respond to other inflammatory signals and leads to sustained effector responses[58].

LSEC also express MHC class II molecules that enable them to present antigens to $CD4^+$ T cells[59]. However, the low levels of co-stimulatory molecules on LSECs means that rather than driving naïve $CD4^+$ T cell differentiation to T helper cells[60] they promote the development of regulatory T cells[61]. *In vivo* studies have shown that these tolerogenic properties of LSEC can control autoimmunity. Circulating inflammatory $CD4^+$ T cells (Th1 and Th17 cells) were shown to interact repeatedly with liver sinusoidal endothelium and this interaction successfully suppressed inflammatory cytokine release in mice[62]. The induction of autoantigen-specific T regulatory cells by

LSECs was also shown to have important systemic effects by ameliorating damage in mouse models of autoimmune CNS disease[63,64]. This finding has therapeutic implications for systemic as well as local immunity and has led to development of nanotechnology-based strategies to deliver autoantigen to LSECs as part of tolerance induction protocols[64] C-type lectins also contribute to the unique ability of LSECs to control T cell differentiation. Thus, LSECtin on LSECs inhibits T cell activation and effector functions through its interaction with CD44 on activated T cells [65].

[H1] LSECs in inflammatory liver disease

In addition to their roles as pathogen recognition and antigen presenting cells, LSECs also have a critical role in regulating the recruitment of leukocytes into liver tissue (Box 2). A key step in the progression of liver injury or infection, regardless of aetiology, is the development of hepatitis as a consequence of the recruitment of leukocytes from the circulation. The balance and retention of immune subsets within the liver determines whether injury resolves, persists or progresses to either liver failure or chronic hepatitis and cirrhosis[66]. Leukocyte recruitment from the blood occurs as a consequence of a multistep adhesion cascade that enables leukocytes flowing in the circulation to be captured by activated endothelial cells and then to migrate through the endothelium towards sites of infection or injury[67]. The cascade consists of sequential steps mediated by interactions between receptors on the surface of leukocytes and endothelial cells. The general paradigm applies to all vascular beds, but tissue and inflammation specific interactions provide powerful local regulation of where, when and which leukocytes are recruited. The steps in the cascade are broadly described as rolling or tethering, in which the leukocyte is captured from the circulation and induced to roll on the endothelial surface. In most vascular beds this step is mediated by a family of receptors termed selectins but other receptors are involved under specific circumstances, such as in the hepatic sinusoids[68]. Leukocyte rolling is followed by activation of leukocyte integrins in response to tissue-derived chemoattractant cytokines (chemokines) sequestered in the endothelial glycocalyx[69,70], which leads to firm adhesion mediated by integrins binding to immunoglobulin superfamily members on the endothelial surface. This adhesion is followed by intravascular crawling of the adherent leukocyte on the endothelium, before the final step of transmigration in which the leukocyte migrates across the endothelium, through the post-endothelial tissue and into the liver parenchyma. The transmigration step is mediated by a complex series of receptor–ligand interactions with cytoskeletal changes in both the endothelial cells and the leukocytes and which enable the cell to cross the endothelium without disrupting the vascular barrier[71].

Cell recruitment to the liver has several features that are distinct from the general adhesion cascade (Figure 3). Recruitment of the majority of leukocytes occurs within the sinusoidal channels of the liver, in contrast to most other organs in which recruitment occurs within the post capillary venules[72]. Furthermore, the recruitment of leukocytes subsets to the liver is regulated by specific combinations of typical and atypical adhesion molecules reflecting the unique phenotype and structure of LSECs, and the anatomy and rheology of the sinusoids (Box 1). The sinusoids are narrow, in places no wider than a flowing leukocyte, and characterised by low shear stress. These properties mean that the initial recruitment step does not require rolling and in most circumstances is selectin-independent. As a consequence, sinusoidal endothelium expresses minimal levels of selectins *in vivo*[72,73]. A summary of the key adhesion factors is outlined in Table 1.

[H3]Immunoglobulin superfamily

The conventional endothelial adhesion molecules that mediate firm adhesion of leukocytes, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)[70,74], are expressed at high levels on inflamed LSECs[75]. Their role in lymphocyte recruitment to the liver has been confirmed in both *in vitro* and *in vivo* assays. VCAM-1, which binds the integrin $\alpha_4\beta_1$ expressed on lymphocytes, has an important role in capturing lymphocytes from blood flow and mediating stabilisation[66,76]. ICAM-1 binds to $\alpha_L\beta_2$ integrin to support firm adhesion within the hepatic sinusoids[72]. Another family member is the mucosal vascular addressin cell adhesion molecule-1 (MAdCAM-1), which binds to the integrin $\alpha_4\beta_7$ and plays a major part in lymphocyte homing to the gut via mucosal vessels[77]. Our group demonstrated that this receptor was also upregulated in the liver in some chronic liver diseases, in which it promotes the recruitment of T cells activated in the gut that express high levels of $\alpha_4\beta_7$, thereby contributing to the link between IBD and inflammatory liver disease[78,79]. Although many of these immunoglobulin superfamily members are regulated by pro-inflammatory cytokines, their adhesive function is also dependent on the formation of cell-surface platforms regulated by the tetraspanin family of receptors, which form microdomains and associate laterally with ICAM-1 and VCAM-1[80,81]. For example, our group confirmed that the tetraspanin CD151 associates with VCAM-1 in LSECs and regulates lymphocyte adhesion under shear stress[82].

[H3]Atypical adhesive and migratory routes

In addition to conventional adhesion molecules, our group and others have demonstrated that LSECs use atypical adhesion molecules to regulate leukocyte recruitment. Vascular adhesion protein-1 (VAP-1) is a membrane bound amine oxidase that was originally shown to mediate lymphocyte binding to high endothelial venules, the specialised post-capillary venules found in lymph nodes[83]. Further studies confirmed that VAP-1 was expressed at high levels in chronic liver disease and mediated adhesion and transmigration across LSEC[84]. Models of *in vitro* and *in vivo* inflammatory liver injury corroborated its role in mediating recruitment during liver inflammation. VAP-1 has unique properties generated by its enzyme activity that can upregulate expression of adhesion molecules and chemokines in LSECs, thereby amplifying leukocyte recruitment[85-88]. The scavenger receptor family of endothelial receptors also contribute to leukocyte recruitment to the liver. Stabilin-2 was shown to regulate lymphocyte adhesion to LSECs via the integrin $\alpha_M\beta_2$[89] and its homologue, stabilin-1, also known as common lymphatic endothelial and vascular endothelial cell receptor (CLEVER-1), was originally shown to mediate recruitment across lymphatic endothelium[90]. Similarly expression of stabilin-1 is upregulated in chronic liver disease and hepatocellular carcinoma, in which it mediates transmigration of lymphocytes across LSECs under shear stress[91].

Following adherence, leukocytes crawl across the endothelial surface before undergoing transmigration usually via endothelial junctions, termed the 'paracellular' route[92,93]. Several studies have demonstrated that lymphocyte interactions with LSECs within the sinusoids trigger important immune effector mechanisms[94,95], which might influence the infiltration and positioning of cells within the liver in inflammatory liver diseaes[75]. Thus, it is important to understand how the process of transendothelial migration through LSECs is regulated. Visualization of this process using confocal imaging of lymphocytes migrating across LSECs under shear stress demonstrated that approximately 50% of cells took a 'transcellular' route of migration, and migrated directly through the endothelial body[91], as opposed to the conventional paracellular route. This transcellular migration route involved the formation of ICAM-1 rich channels to facilitate lymphocyte migration. Although transcellular migration has been noted in some other specialized endothelial beds, its function and molecular basis remain poorly understood[96]. Transendothelial migration is a multi-step process involving different combinations of receptors that enables preferential recruitment of particular leucocyte subsets, as described in the following sections. An additional step in migration was described in 2016, in which lymphocytes migrate into LSECs and then crawl within the endothelial cell to the cell junction, through which they enter the adjacent endothelial cell[97]. This process, which we term 'intracellular crawling', is dependent on IFNγ and could not be detected when LSEC were stimulated by other interferon family cytokines. We found that IFNγ treatment did alter the cytoskeleton of LSECs which might promote 'intracellular crawling'. This process was also facilitated by the unique junctional complexes between LSECs. The functional consequences of intracellular crawling are yet to be elucidated but could have an important role in lymphocyte positioning in liver tissue.

[H3]Chemokines

Chemokines are a family of small secreted proteins ranging from 67-127 amino acids in size that bind to heparin sulphate on proteoglycans[98,99]. They play central parts in leukocyte migration during homeostasis (in development and localization in secondary lymphoid organs) as well as within tissues during inflammatory responses by binding to G protein-coupled receptors on the surface of leukocytes. Upregulation of several chemokines on liver vasculature has been demonstrated in a range of chronic inflammatory liver diseases, including alcoholic liver disease, primary sclerosing cholangitis and chronic rejection[100-104]. In these conditions, chemokines seem to be compartmentalized to the sinusoidal vasculature and portal vessels and have a substantial influence on immune cell localization and subsequent disease progression [101,102,105]. T cell migration across sinusoidal endothelium is mediated by the interferon-inducible chemokines CXCL9 and particularly CXCL10, which bind the receptor CXCR3[106,107]. In other diseases, including chronic HCV infection, the chemokine CXCL16, which exists in a transmembrane form, is expressed on sinusoidal endothelium, hepatocytes and bile ducts, enabling it to regulate the recruitment and retention of $CXCR6^+$ effector T cells within the liver[108,109]. Subsets of Natural Killer (NK) and NK T cells express high levels of CXCR6 that enable them to interact with CXCL16 on sinusoidal endothelium; this interaction promotes active migration along the sinusoids as part of a process of ongoing immune surveillance and patrolling[110]. Studies in mouse liver endothelial cells have shown that a vital property of chemokine-mediated recruitment is the transcytosis of chemokines from the basolateral side to the luminal side of sinusoidal endothelial cells[111]. This process is clathrin-dependent and promotes the transendothelial migration of lymphocytes across LSECs, and inhibition of this pathway reduces $CD4^+$ Tcell recruitment during liver injury[112].

[H1]Immune subset recruitment

The balance of immune subsets determines the progression and outcome of immune responses within the liver: persistent effector responses will drive chronic inflammatory conditions, whereas excessive immunosuppressive immune subset populations promote pathogen escape and tumour formation[113-115]. In addition to the key mediators of immune cell recruitment discussed earlier, there is now evidence that immune cell subsets utilise distinct combinations of these factors to migrate through the hepatic sinusoids under specific circumstances.

[H3]T cells

T helper cells are divided into multiple functional subsets based on the cytokines they secrete and dependent on the microenvironment in which they are activated by antigens. In a concanavalin-A liver mouse inflammation model, Th1 recruitment through the sinusoids was mediated by $\alpha_4\beta_1$ integrin interactions whereas Th2 cells used VAP-1[85]. Both effector Th17 and regulatory T (Treg) cells found in the liver express high levels of the chemokine receptor CXCR3 and use it to migrate across LSECs[116,117]. Subsequent signals determine where these cells localise within the liver, with CCR6+ Th17 cells migrating towards their ligand CCL20 secreted by bile ducts whilst Tregs respond to different chemokines as a consequence of their expression of CCR5, CCR4 and in some cases CCR10[118-120]. Tregs were also shown to use a distinct combination of adhesion receptors, involving CLEVER-1/stabilin, ICAM-1 and VAP-1, to migrate across human LSECs under flow[91], whereas recruitment of $CD8^+$ T cells to the mouse liver is primarily dependent on ICAM-1 expression by LSECs with a lesser contribution from VCAM-1[121,122]. In autoimmune hepatitis and primary sclerosing cholangitis (PSC) associated with IBD, LSECs present the chemokine CCL25, which can trigger $CCR9^+$ gut homing lymphocyte interactions with MAdCAM-1 to promote recruitment of mucosal T cells[104,123].

These distinct mechanisms of migration across LSECs are probably influenced by epithelial responses to tissue injury[118,124], stromal signals[125] and cooperative interactions between several cell types in the sinusoid. For instance, in a model of HBV infection, effector $CD8^+$ T cells were shown to arrest in the sinusoids by interacting with platelets adherent to the sinusoidal surface via hyaluronan dependent mechanisms[95]. Subsequently, the $CD8^+$ T cells crawled along the sinusoids, probing through the LSEC fenestrae for viral antigens presented by underlying hepatocytes. Antigen recognition as a consequence of this probing behaviour led to effector functions by a diapedesis-independent process. A human model of cytomegalovirus (CMV) infection of LSECs led to the recruitment of effector T cells and activated Tregs in an LFA-3 dependent mechanism[126]. In this study, CMV infected human LSEC upregulated LFA-3 at intercellular junctions and during effector T cell recruitment the interaction of LFA-3 with its ligand, CD2 on T cells, contributed to Th1 activation.

[H3]B cells

Although B cells are present in substantial numbers in chronically inflamed liver tissue, the molecular mechanism regulating their recruitment from blood into hepatic tissue is poorly understood. Our group demonstrated that B cell recruitment across human LSECs under flow was initially mediated by VCAM-1-dependent capture followed by limited intravascular crawling, compared with T cells[127]. Interestingly, the receptors involved in transmigration of B cells included ICAM-1, VAP-1 and CLEVER-1/stabilin-1 all of which are also involved in Treg transmigration across LSEC.

[H3]Neutrophils

Neutrophils are one of the earliest immune cells to be recruited to a site of tissue injury and they are also recruited into the liver via the hepatic sinusoids[128]. It was originally thought that their migration was mediated by simple physical trapping within the narrow sinusoidal channels, but work from McDonald *et al.* implicated a complex multistep recruitment process involving interactions between sinusoidal hyaluronan and CD44 on the neutrophil surface[129]. Whereas neutrophil interactions in post-sinusoidal venules followed a conventional rolling mediated by selectins and integrin-mediated adhesion, this was found not to be the case in the sinusoids, where the majority of neutrophil extravasation took place. They found that hyaluronan was highly expressed in liver sinusoids and mediated the recruitment of neutrophils in response to LPS challenge. This interaction was dependent on CD44 binding to hyalorunan rather than the other hyaluronan receptor, receptor for HA-mediated motility (RHAMM). A study published in 2014 also highlighted the importance of TLRs for neutrophil recruitment. TLR2/S100A9 signalling in particular promoted the production of the chemokines CXCL1 and CXCL2, which are known to mediate neutrophil migration, by liver macrophages in acute and chronic mouse models of liver injury[130].

[H3]Monocytes

In addition to the activation of resident Kupffer cells, monocytes and macrophages are also recruited to the liver from the circulation during inflammation or in response to injury. Kupffer cells are yolksac-derived tissue macrophages found within the hepatic sinusoids; they are immobile and probe the environment with pseudopods[131]. The response to liver injury also includes an influx of monocytes, which have a major role in regulating inflammation, regeneration and repair and fibrosis[132]. Furthermore, acute liver injury is associated with an initial influx of GATA6^{+} peritoneal macrophages that enter directly through the mesothelium in a process dependent on CD44 and the DAMP molecule ATP[131]. This entry is followed by the recruitment of CCR2^{+} monocytes from the circulation[133]. The subsequent recruitment signals governing monocyte migration through the sinusoids are less well characterised but several key factors have been determined. The dominant chemokine receptor mediating migration of CD16^{+} monocytes across LSECs is CX(3)CR1 binding to its ligand CX(3)CL1, one of the few transmembrane chemokines, which is restricted to bile ducts in the normal liver but expressed at high levels on inflamed sinusoidal endothelium[134]. In this study, VAP-1 also contributed to adhesion and transendothelial migration of CD16^{+} monocytes across LSECs. The accumulation of CD14^{++}CD16^{+} monocytes has been reported in inflammatory liver disease, which is due in part to the preferential migration of this subset across LSECs compared to CD14^{++}CD16^{-} cells[135]. Monocytes are known to undergo a phenomenon of bidirectional movement across endothelium that involves a reverse migration step[136,137]. This migratory behaviour has been confirmed in LSECs and might have a marked effect on the fate of monocytes and the outcome of liver injury, because monocyte subsets which undergo reverse transmigration are predominantly proinflammatory CD16^{+} monocytes. By contrast, those remaining in the subendothelial space are anti-inflammatory monocytes that suppress T cells and promote endotoxin tolerance[138].

[H1]Interaction with other liver cells

Although we have focused on leukocyte interactions with LSECs, the cross talk between LSECs and other liver cell populations will also influence the progression of chronic inflammatory liver diseases. Kupffer cells are found within the hepatic sinusoids in close association with LSECs and are also equipped to sense tissue injury from infection and toxins. The release of DAMPs and PAMPs triggers the inflammasome pathway in Kupffer cells [139]. Inflammasome activation is a key step in the progression of parenchymal liver injury, such as alcoholic liver disease, in which the release of danger signals from damaged hepatocytes stimulates the release of pro-inflammatory mediators from Kupffer cells [140]. Despite poor understanding of the cross-talk between LSEC and Kupffer cells, the release of these mediators probably influences LSEC phenotype and activation and leads to subsequent leukocyte recruitment[141,142]. Furthermore, Kupffer cells can promote LSEC capillarization, whereby LSEC morphology becomes more vascular or capillary like with a loss of fenestrations and a characteristic basement membrane is formed[143,144].

The other cell type that populates the sinusoids is the hepatic stellate cell (HSC), positioned within the Space of Disse. The central role of HSCs in extracellular matrix production in chronic liver disease is well established[145]. It is now known that LSECs play an important role in maintaining the quiescence of HSCs and this ability is lost during capillarisation of LSECs, which permits HSC activation and fibrogenesis[21,146]. Activated liver myofibroblasts, derived predominantly from HSCs, also have a role in the subsequent migration and positioning of lymphocytes following their recruitment through LSECs. This process is mediated by distinct combinations of cytokines including IL-6, VEGF and chemokines released by myofibroblasts[125].

LSECs also play a key role in maintaining hepatocyte homeostasis. LSEC fenestrations enable bidirectional transport of metabolites between the circulation and the liver parenchyma[1]. LSECs also facilitate circulating T cells to interact with hepatocytes by allowing T cells to extend cell surface protrusions through LSEC fenestrations[147]. In chronic liver injury, microparticles are released from hepatocytes, leukocytes and LSEC and provide another route for cell–cell communication[148]. Paracrine factors released from hepatocytes influence the expression of adhesion molecules on overlying LSECs and can promote the recruitment of flowing lymphocytes from the sinusoids[124]. This mechanism might be particularly important in liver cancer because malignant transformation of hepatocytes enhances their ability to secrete chemokines CXCL10, CCL2 and CCL3 and to upregulate expression of ICAM-1 and VAP-1 on co-cultured LSECs[103,149]. Work from our group has demonstrated that factors secreted by hepatoma cells upregulate the expression of the tetraspanin CD151 in LSECs, which promotes VCAM-1 mediated recruitment of lymphocytes[82].

[H1]Therapeutic opportunities

The evidence presented here highlights the crucial role played by LSECs in regulating the inflammatory response to liver injury. This importance makes them and the molecules they express attractive therapeutic targets in inflammatory liver disease[150,151]. VAP-1 is a good example[152], with studies confirming that inhibition of both its enzymatic activity or antibody blockade of its adhesive function reduces hepatic inflammation and fibrosis in mouse liver injury models[87], and this work has led to a clinical trial of a humanised antibody against VAP-1 that is currently underway (BUTEO, NCT02239211) in patients with PSC. Chemokines and adhesion molecules expressed by inflamed LSECs are also potential targets for anti-inflammatory therapy in liver disease. For example, patients with PSC have been treated with NI-0801, a humanized monoclonal antibody against CXCL10. Interestingly, the high production rate of CXCL10 by the inflamed liver made it difficult to achieve sustained neutralization of the chemokine *in vivo*, despite evidence that the antibody could “strip” chemokine from the sinusoidal endothelial bed. Although the drug was well tolerated and demonstrated immunological changes, the overall results were negative. (K de Graaf et al. submitted for publication). Thus, therapies directed at the chemokine receptors themselves might have merit, and evidence from early trials using the dual CCR2–CCR5 antagonist cenicriviroc in patients with NASH suggests that such treatment can induce a persistent blockade[153].

There is also a strong rationale to target gut-tropic chemokines in patients with liver diseases associated with IBD. Of particular relevance is PSC, a progressive biliary disease that is associated with IBD in 80% of cases and which affects ~8% of patients with IBD, particularly those with colitis[154]. Under physiological conditions expression of CCL25 and MAdCAM-1 is absent from the liver, but in PSC both proteins are detectable on hepatic endothelium and support the aberrant recruitment of $\alpha_4\beta_7^+$ $CCR9^+$ effector lymphocytes from the gut. Clinical trials are currently being considered to target the $\alpha_4\beta_7$-MAdCAM-1 pathways in PSC using antibodies developed for treating IBD.

The tolerogenic capabilities of LSECs have also been targeted therapeutically. Nanoparticles loaded with autoantigen can be targeted to LSECs as a consequence of their potent scavenging capability; the ability of LSECs to take up molecules using their scavenger receptors is an excellent way of potentially targeting a range of therapies to the liver. Presentation of delivered autoantigens by LSECs to naive T cells results in the generation of autoantigen-specific regulatory T cells that can suppress systemic as well as local autoimmune responses. This strategy could be applied to a wide range of autoimmune and allergic conditions[64]. Targeting LSEC stabilin-1 and stabilin-2 with nanoparticle-based drugs[155] has been suggested as a way to deliver local treatment to manage a range of conditions including ischaemia–reperfusion injury (a specific type of injury that follows liver surgery and transplantation which is a biphasic process involving hypoxia followed by restoration of blood flow and reoxygenation) and NAFLD. Similarly, blockade of LSECtin or the related molecule DC-SIGNR has been shown to reduce the metastasis of colon cancer cells to the liver via impairment of interactions with LSEC in mouse models[156,157].

During cirrhosis and chronic hepatitis, LSECs can undergocapillarisation[158]. This process is associated with loss of GATA4-dependent signals[8], upregulation of CD31 and VCAM-1 and loss of fenestrations[158-160]. The number of fenestrations per endothelial cell not only decreases with disease[161-163] but also with ageing[164], and this phenotypic change is governed by p19^{ARF} and p53-dependent signalling[165]. These changes might impede the transfer of materials to or from the parenchyma and contribute towards regional hepatocyte hypoxia. Capillarisation is mechanistically linked to the development of chronic inflammatory disease. In rodent models, it is associated with enhanced antigen presentation and cytotoxic T cell priming during fibrosis[151], and in NASH capillarisation precedes and contributes to the transition from simple steatosis to steatohepatitis[159]. The changes that occur in LSECs in response to chronic inflammation also affect angiogenic pathways. Neo-angiogenesis is a key feature of chronic liver disease and the majority of neo-vessels arise from portal vein branches and are closely associated with areas of fibrogenesis[166,167] A key initiating step is the capillarisation of LSECs, which leads to increased hepatocyte hypoxia and subsequent release of pro-angiogenic factors[168,169]. The LSEC response is context specific; for example, acute injury can induce CXCR7 expression and a regenerative response, whereas chronic injury leads to CXCR4 induction, HSC proliferation and fibrogenesis[170]. During ischaemia-reperfusion injury LSEC develop a proinflammatory, prothrombotic phenotype associated with vasoconstriction[171]. These changes have been directly linked to neutrophils because IL-33 released by LSECs during ischaemia–repurfusion injury triggers the release of neutrophil extracellular traps (NETs), which exacerbate acute hepatic injury[172]. In chronic injury, the changes in endothelial phenotype that accompany capillarisation and precede fibrosis have been linked to alterations in signalling via the hedgehog gene family[173] and lead to vasocontriction and increased intrahepatic vascular resistence due to reduced nitric oxide production by LSEC[174]. Tumour progression in hepatocellular carcinoma is associated with changes in the phenotype of peritumoural LSECs and increased production of angiogenic factors including IL-6[175,176].

These changes in LSECs therefore present opportunities for therapeutic intervention. For example, pharmacological therapy in the form of a soluble guanylate cyclase activator which restores fenestrations has been linked to fibrosis regression in rodent models[21] and it might also be possible to use GATA-4 mediated cellular reprogramming to restore the differentiated phenotype of LSECs and promote fibrosis resolution[8]. Similarly, therapies that restore normal hedgehog signalling promote regression of capillarisation and the reappearance of fenestrations, which suggests a potential pathway for reversal of fibrosis and the restoration of lipid transport[173]. However, studies testing cessation of VEGF-based cancer therapies also highlight how important the development of fenestrations can be in the context of metastasis, and go some way in explaining poor performance of some strategies using anti-VEGF drugs as cancer treatments. Withdrawal of anti-VEGF- antibody therapy is associated with development of hyperpolarised LSECs and promotion of hepatic metastasis[177]. Thus, low-dose, non-stop anti-angiogenic therapy might present a future solution to minimise these effects.

[h1]Conclusions

Sinusoidal endothelial cells have complex interrelated roles in the maintenance of liver homeostasis and are implicated as drivers of inflammation and fibrogenesis in liver disease. Their unique positioning, phenotype and function make them attractive candidates for organ-specific therapy and it is likely that more therapies targeting these cells will be tested in the future as new treatments to reduce liver injury and inflammation and to prevent or reverse fibrogenesis. In the absence of licenced antifibrotic therapies, strategies to maintain LSEC differentiation and to inhibit their ability to recruit harmful pro-inflammatory leukocytes through the selective orchestration of immune cell traffic might provide vital tools to halt the increase in mortality linked to chronic liver failure.

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1

1 **Box 1 |** Unique characteristics of liver sinusoidal endothelium

Morphological appearance	Fenestrated, continuous endothelium with minimal basement membrane in normal conditions Fenestrations can be organized into sieve plates and range from around 50-100nM in diameter
Expression of endothelial markers	CD31 present at low levels Von Willebrand factor expression is controversial but can be detected in human LSECs in the context of liver injury CD34 is absent or only expressed at low levels CD105 (endoglin) is present CD36 is present at a much higher level than vascular endothelium E-selectin is absent on unstimulated cells but expression can be induced, albeit at lower levels than vascular endothelium in inflammatory conditions
Endocytic capabilities	High and rapid clathrin-mediated endocytosis of many substances, ranging from cellular components such as collagen and hyaluronan to acetylated LDL, immune complexes and exogenous antigens such as ovalbumin via key receptors
Expression of scavenger receptors	LSEC have very potent scavenger capabilities by virtue of expression of many scavenger receptors, including Mannose receptor(CD206), FcgRIIb, Stabilin-1 (Clever-1), Stabilin-2, Scavenger receptors B1 and B2,L-SIGN, LYVE-1 and LRP-1
Junctional structure	'Mixed' type of junctions having some features of tight junctions but generally showing lower or absent claudin-5 and occludin compared to vascular endothelium VE-Cadherin can be present in a disease setting
Adhesion molecules	LSEC constitutively express low levels of ICAM-1, ICAM-2and VCAM-1 Selectin expression is considered to be minimal in most circumstances Also more unusual 'adhesion' and scavenger receptors such as Clever-1, VAP-1, DC-SIGN, L-SIGN, LYVE-1 and MAdCAM-1 can contribute to recruitment of immune cells in a disease specific context
Chemokine expression	Minimal chemokine expression is seen on unstimulated LSECs although they will express factors such as CXCL9-

	11, CCL25, CX3CL1 and CXCL16 in response to cytokine stimulation. They can also present chemokines derived from neighbouring or underlying cells to promote binding and migration of immune cell subsets

Box 2 | **The role of LSECs in progression of chronic liver diseases**

Hepatitis C

Recruitment and positioning of effector T cells in hepatitis C through sinusoidal endothelial expression of adhesion molecules ICAM-1, VCAM-1 , VAP-1 and presentation of CXCR3 ligands associated with compartmentalisation within the parenchyma[102,109]. Retention of CXCR6+ T cells through the expression of its ligand CXCL16 [108].

Primary sclerosing cholangitis

Aberrant homing of mucosal effector lymphocytes through expression of MAdCAM-1 and CCL25 on hepatic sinusoidal endothelium[78,104]. VAP-1 regulates the expression of MAdCAM-1 by deaminating primary amines and driving a NF-KB dependent pathway [86,88].

Autoimmune hepatitis

Initial T cell mediated damage directed to sinusoidal endothelium as initiating event in models of autoimmune hepatitis[141]. Upregulation of adhesion molecules such as MAdCAM-1 promotes lymphocyte recruitment[78]. Development of LSEC-reactive autoantibodies leads to capillarisation of sinusoidal endothelium and progressive liver disease[142].

Alcoholic liver disease and NAFLD

Defenestration and activation are early changes in models of alcoholic and fatty liver disease[159,160]. The presentation of chemokines by sinusoidal endothelium leads to recruitment of T cells with compartmentalisation leading to progressive disease[101].

Fibrosis

LSECs prevent hepatic stellate activation[21]. This ability to maintain HSC quiescence is lost during capillarisation of LSEC driven by chronic injury[146]. Capillarisation leads to impaired eNOS activity leading to low nitric oxide production[174] and increased hedgehog signalling[173].

Hepatocellular cancer

Endothelial transdifferentiation with loss of several LSEC markers[176]. Presentation of CXC and CC chemokines and expression of ICAM-1, VAP-1 and CD151 promotes lymphocyte recruitment to HCC [103,149,82]. Stabilin-1 expression might promote Treg specific recruitment[91].

Tables:

Table 1 | Mediators of immune cell recruitment across liver sinusoidal endothelial cells

Adhesion factor	Ligand	Function
ICAM-1	αLβ2	Firm adhesion of CD4 cells and CD8 cells, transmigration of Tregs and B cells
VCAM-1	α4β1	Capture and firm adhesion of T and B cells
VAP-1	unknown	Adhesion and transmigration of lymphocytes and monocytes
Stabilin-1 (CLEVER-1)	unknown	Transmigration of CD4 T cells, predominantly Tregs
Stabilin-2	αMβ2	Adhesion of lymphocytes
MAdCAM-1	α4β7	Adhesion of α4β7 subset of T cells
Hyaluronan	CD44	Adhesion of neutrophils during liver injury Promotes platelet adhesion, which in turn enables intrasinusoidal $CD8^+$ T cell docking
CD151	Forms microdomains to support VCAM-1	Firm adhesion of lymphocytes via a VCAM-1 mediated pathway
CXCL9-11	CXCR3	Transendothelial migration of T cells
CXCL16	CXCR6	Mediates T cell recruitment and NKT cell sinusoidal surveillance
CX(3)CL1	CX(3)CR1	Adhesion and transmigration of monocytes

LSEC, liver sinusoidal endothelial cell; NKT, natural killer T; Treg, regulatory T cell

Figure 1: **Microanatomy of the human liver vascular tree**

a | Low power image of a human liver tissue (stained with haematoxylin and eosin) illustrating the lobular organisation of the liver with zonal architecture indicated relative to the position of the portal tract. b | Expanded periportal section of the same image to illustrate the different vascular compartments within the parenchyma. c | Immunohistochemical staining of stabilin-1, which highlights liver endothelial cell distribution within hepatic tissue in a normal liver section.

Figure 2 | **Hepatic sinusoidal endothelial cells as antigen-presentating cells**

a | LSECs express MHC class I receptors and can cross-present antigen to $CD8^+$ cytotoxic T cells. At low antigen concentrations this presentation leads to tolerance and deletion of $CD8^+$ T cells. **b** | If antigen concentrations are high then antigen cross presentation to $CD8^+$ T cells leads to a memory effector T cell phenotype. **c** | In the context of hepatotrophic infections such as hepatitis B, $CD8^+$ T cells adhere to the sinusoids in a platelet-dependent process and then probe for infected hepatocytes through LSEC fenestrae. Detection of infected hepatocyte leads to diapedesis (the process of cells actively crossing capillaries)-independent killing. **d** | LSECs can also present antigen to $CD4^+$ T cells via expression of MHC class II, which leads to the induction of suppressor T cells ($CD25^{hi}$ regulatory T cells).

Figure 3 | **Lymphocyte recruitment within the hepatic sinusoids**

Lymphocytes recruitment involves an adhesion cascade within the hepatic sinusoids that is influenced by the low shear environment and cellular cross talk between parenchymal and non-parenchymal cells. Chronic parenchymal cell damage leads to the release of DAMPs and pro-inflammatory mediators by Kupffer cells which increase adhesion molecule expression by LSECs (1). Lymphocyte recruitment across activated LSEC involves a selectin-independent tethering step (2) followed by integrin activation and firm adhesion to immunoglobulin superfamily members on the LSEC surface (3). This process is influenced by paracrine factors released from hepatocytes. Lymphocytes then crawl along the luminal endothelium (4) until they receive a signal to transmigrate across LSEC either through a paracellular or transcellular route (5). A third route of lymphocyte migration involves intracellular migration directly into the LSEC body and then migration to the adjacent LSEC, termed intracellular crawling (6). Release of chemotactic factors from activated hepatic stellate cells promotes subsequent migration and positioning in liver tissue (7).

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Liver sinusoidal endothelial cells represent the most abundant non-parenchymal hepatic cell population. In this Review, the authors explore the key roles that liver sinusoidal endothelial cells have in regulating hepatic immunity, and their contribution to immune-mediated disease, liver fibrosis and carcinogenesis.