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Liver sinusoidal endothelial cells - gatekeepers of hepatic immunity

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1 Liver sinusoidal endothelial cells — gate keepers of hepatic immunity

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12 Abstract

13 Liver sinusoidal endothelial cells (LSECs) line the low shear, sinusoidal capillary 14 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 15 vital physiological and immunological functions, including filtration, endocytosis, 16 antigen presentation and leukocyte recruitment. Reflecting these multifunctional 17 18 properties, LSECs display unique structural and phenotypic features that differentiate them from capillary endothelium present within other organs. It is now clear that LSECs 19 20 play a critical role in maintaining immune homeostasis within the liver and in mediating 21 the immune response during acute and chronic liver injury. In this Review, we outline 22 how LSECs influence the immune microenvironment within the liver and discuss their 23 contribution to immune-mediated liver diseases and the complications of fibrosis and 24 carcinogenesis.

Author: Please provide 4-6 key points. This is a feature that should comprise a bulletpointed 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.

- Liver sinusoidal endothelial cells (LSECs) that line the hepatic sinusoids play important physiological roles and mediate the filtration and scavenger functions of the liver.
- LSECs also have innate and adaptive immunological functions including antigen presentation and maintaining the balance between tolerance and effector immune responses.
- 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.
- LSECs play a crucial role in the cellular cross talk which regulates
 progressive chronic liver disease leading to fibrosis and carcinogenesis.
- 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.

20 [H1]Introduction

Sinusoidal endothelial cells line what constitutes a unique vascular bed in the liver, 22 which receives blood from both the hepatic artery and portal veins into the hepatic 23 parenchyma (Fig 1). Studies of these cells isolated from animals usually refer to them 24 as liver sinusoidal endothelial cells (LSECs), whereas isolated human cells have also 25 been referred to as human hepatic sinusoidal endothelial cells (HSECs). For the 26 purpose of this Review we use the term LSEC. The exposure of these sinusoidal 27 endothelial cells to blood originating from both the gut and systemic circulation means 28 they are ideally situated to remove and recycle blood-borne proteins and lipids. In 29 combination with Kupffer cells (liver-resident macrophages), LSECs constitute the 30 most powerful scavenger system in the body¹. This activity is facilitated by the 31 presence of fenestrae in LSECs, their lack of a classical basement membrane and 32 their expression of promiscuous scavenger receptors combined with the most potent 33 endocytic capacity in the body². Thus virus particles³, advanced glycation end 34 products⁴ and modified LDL⁵ can be cleared from the circulation within minutes by this 35 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 localisation⁶. The vascular architecture in the human liver is acquired by 17–25 weeks of gestation, but different vessels within the liver have distinct embryonic

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1 origins. Thus, portal vessels derive from vitelline veins, whereas sinusoids develop 2 from capillary vessels of the septum transversum and acquire their distinctive 3 fenestrated phenotype by week 20 of gestation⁷ under the control of GATA-4⁸. From 4 this point onward, sinusoidal endothelial cells remain functionally and phenotypically 5 distinct from the other vascular endothelial cells in the liver microenvironment and 6 assume a phenotype that has many similarities with lymphatic endothelial cells⁹. The 7 unique characteristics of LSECs are presented in Box 1. Both lymphatic and sinusoidal 8 endothelial cells have minimal basement membranes and loosely organised cell 9 junctions ¹⁰ and share a complement of receptors such as LYVE-1¹¹, Prox-1¹², 10 podoplanin¹³ and L-SIGN¹⁴. It has been shown that the phenotype of sinusoidal 11 endothelial cells alters across the liver acinus; a study of human liver tissue published 12 in 2017 demonstrated that zone 1 LSECs are CD36^{hi} and Lyve-1^{lo} whereas zone 2 and 13 zone 3 LSECs are CD36^{lo}, LYVE-1^{hi} AND CD32^{hi 15}. The presence of fenestrations or 14 membranous pores organised into sieve plates is a feature that also distinguishes 15 LSEC from the other hepatic endothelial populations.²

16 Fenestrations are not unique to hepatic endothelial cells and are also found in endothelium in endocrine glands such as the pancreas¹⁶, the kidney¹⁷, spleen¹⁸ and 17 bone marrow¹⁹ and are sometimes observed in tumour vasculature²⁰. However, unlike 18 19 other fenestrated endothelial populations such as those in the kidney, hepatic 20 fenestrations lack a diaphragm or basal lamina and are grouped into organised sieve 21 plates, rendering LSEC highly permeable. Many studies have implicated VEGF as an essential factor for regulation of fenestrations²¹, but dynamic changes in hepatic 22 fenestration number and size can occur rapidly in response to agents such as 23 alcohol²², dietary constituents²³ and fasting²⁴ or calorie restriction²⁵. The fenestrations 24 act as a 'dynamic filter' ²⁶to permit the access of macromolecules to parenchymal cells 25 and in addition these pores might allow circulating viruses to gain access to 26 hepatocytes²⁷. Evidence from animal studies suggests that fenestrations can 27 constitute up to 40% of the cell and that the size, distribution and clustering of the pores 28 in sieve plates varies with the zonal distribution of the endothelium²⁸ and across the 29 30 endothelial surface. Up to a third of these pores are organised into complex labyrinths and many are associated with components of microtubules²⁹, caveoli and coated pits 31 to form a transport network that could impose additional regulation on the traffic of 32 material into the cells³⁰, enabling them to govern the movement of materials to and 33 from the liver parenchyma. 34

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2 [H1]Balancing tolerance and immune response

3 The permissive nature of sinusoidal endothelium probably evolved to handle the constant exposure of the liver to microbial and food antigens derived from the 4 5 gastrointestinal tract via the portal vein. The liver needs to ensure that damaging 6 immune responses are not precipitated against harmless antigens, whilst at the same 7 time being able to eliminate invading pathogens. The first site of exposure to these 8 antigens occurs within the hepatic sinusoids and both Kupffer cells (KCs) and LSECs 9 are important players in taking up and eliminating soluble antigens entering via the 10 portal vein and in determining the nature of any immune response such antigens 11 trigger.

12 The initial critical step in an immune response is the innate pathway of antigen uptake by pattern recognition receptors³¹. Pattern recognition receptors are highly 13 evolutionarily conserved and include the Toll-like Receptor (TLR) family and the 14 scavenger receptors³¹. An example of how the liver regulates inflammatory and 15 immune responses is seen in the recognition of the TLR-4 ligand lipopolysaccharide 16 (LPS) by KCs and LSEC. Chronic exposure of both KCs and LSEC to LPS leads to an 17 18 LPS-refractory state, and in LSECs specifically LPS exposure is associated with reduced nuclear translocation of nuclear-factor-kappa-light-chain enhancer of 19 20 activated B cells (NF-kB) and subsequent reduced leukocyte adhesion³². This 21 mechanism prevents the liver being in a constantly activated inflamed state in 22 response to the constant exposure to bacterial products from the gut. Studies of other 23 TLRs demonstrate that LSEC can respond to signals mediated via TLR1-4, 6, 8 and 24 9, but their activation has cell-specific responses that are restricted compared with classical antigen presenting cells, thereby contributing to an organ-specific response 25 to antigens and the tolerogenic environment of the liver³³. 26

27 A unique characteristic of LSEC is their expression of high levels of several scavenger 28 receptors compared with conventional endothelium. Scavenger receptors are a 29 diverse family of pattern recognition receptors that, like TLRs, are highly evolutionarily conserved³⁴. In contrast to TLRs, they were believed to be functionally redundant and 30 to perform silent uptake of ligands. However, gathering evidence suggests that this is 31 32 not the case and that scavenger receptors have an important cell-specific role in immune responses³⁴. They have been shown to promote potent pro-inflammatory and 33 34 anti-inflammatory signalling as well as directly interacting with TLRs. Membrane bound 35 scavenger receptors recognise their extracellular ligands which leads to internalisation

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of the ligand, termed endocytosis, trafficking from the cell membrane to intracellular 1 2 compartments such as the endosomes. The high levels of scavenger receptors on 3 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 4 homologous scavenger receptors stabilin-1 and stabilin-2³⁷ and related molecules 5 6 such as C-type lectins, including the type-2 receptor subclass dendritic cell-specific 7 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}. 8 The members of the C-type lectin group are involved in varied functions, from cell-cell 9 10 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 11 sinusoidal endothelial cell C-type lectin (LSECtin)⁴⁰. This lectin has been shown to be 12 co-expressed with L-SIGN and is encoded in the same cluster of lectin encoding genes 13 14 as DC-SIGN and L-SIGN.

15 [H3] Innate immunity

Several of the C-type lectin receptor family members have been directly implicated in 16 17 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 18 shown to be expressed on LSECs and bind the E2 glycoprotein of the hepatitis C virus 19 (HCV) and facilitate hepatocyte infection³⁹. LSECtin has also been implicated in the 20 uptake of SARS coronavirus and HCV^{43,44}. The ability of LSEC to bind multiple viruses 21 22 through their diverse endocytic receptors gives them a crucial role in the response to 23 viral infections and a specific role in mediating rapid clearance of blood-borne viruses⁴⁵. In a mouse model of adenovirus infection, 90% of virus is found in LSECs 24 and 10% in KCs within a minute of intravenous viral infusion⁴⁵. A study published in 25 2017 reported that HIV-like particles are taken up by mouse LSECs at a rate of 100 26 million particles per min.³ The transit of viruses internalised by LSECs is less well 27 28 understood whereas after receptor mediated endocytosis of circulating matrix 29 breakdown products the subsequent transit from early endosomes to late endosomes 30 takes several hours⁴⁶. LSECs enable direct entry of certain viruses such as Ebola, whereas with other viruses, such as HCV and HBV, LSECs promote hepatotropism by 31 facilitating parenchymal cell infection⁴⁷. Rapid uptake of virus can also lead to 32 redistribution to other cells, for instance in animal models of HBV viral particles are 33 preferentially taken up by LSECs and subsequently passed on to infect underlying 34 hepatocytes⁴⁸. In the case of HCV, innate sensing of viral infection by LSEC leads to 35 36 downstream signalling and release of paracrine signals such as the pro-viral molecule bone morphogenetic protein 4, which enhances viral infection of hepatocytes⁴⁹. 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 replication⁵⁰. 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.

6 [H3]Adaptive immunity

LSEC not only regulate innate immune responses but also directly regulate adaptive 7 immune responses through antigen presentation to T cells (FIG. 2). Knolle's group 8 demonstrated that LSECs can cross-present antigen to CD8⁺ T cells⁵¹ by using 9 10 scavenger receptors, notably the mannose receptor, to take up, process and transfer antigen to MHC class 1⁵². The presentation of antigen, including oral antigens, by 11 LSECs drives a tolerogenic response in naïve CD8⁺ T cells mediated by upregulation 12 13 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 14 cells ^{51,53,54}. Endocytosis of antigens by the mannose receptor on LSECs has been 15 shown to promote CD8⁺ T cell tolerance⁵⁵, including tolerance to tumour antigens⁵⁶. 16 17 However, it is crucial that rapid effector responses can be generated locally to harmful 18 pathogens; consistently, LSEC-driven T cell activation changes in response to antigen 19 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 20 presentation to CD8⁺ T cells, high antigen concentrations led to a shift from tolerogenic 21 to effector T cell differentiation⁵⁷ as a consequence of enhanced TCR signalling that 22 23 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 24 25 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 26 leads to sustained effector responses⁵⁸. 27

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29 LSEC also express MHC class II molecules that enable them to present antigens to CD4⁺ T cells⁵⁹. However, the low levels of co-stimulatory molecules on LSECs means 30 that rather than driving naïve CD4⁺ T cell differentiation to T helper cells⁶⁰ they promote 31 the development of regulatory T cells⁶¹. In vivo studies have shown that these 32 tolerogenic properties of LSEC can control autoimmunity. Circulating inflammatory 33 CD4⁺ T cells (Th1 and Th17 cells) were shown to interact repeatedly with liver 34 sinusoidal endothelium and this interaction successfully suppressed inflammatory 35 cytokine release in mice⁶². The induction of autoantigen-specific T regulatory cells by 36

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 protocols⁶⁴ 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 cells⁶⁵.

8 [H1] LSECs in inflammatory liver disease

9 In addition to their roles as pathogen recognition and antigen presenting cells, LSECs 10 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 11 12 the development of hepatitis as a consequence of the recruitment of leukocytes from 13 the circulation. The balance and retention of immune subsets within the liver 14 determines whether injury resolves, persists or progresses to either liver failure or chronic hepatitis and cirrhosis⁶⁶. Leukocyte recruitment from the blood occurs as a 15 consequence of a multistep adhesion cascade that enables leukocytes flowing in the 16 17 circulation to be captured by activated endothelial cells and then to migrate through the endothelium towards sites of infection or injury⁶⁷. The cascade consists of 18 19 sequential steps mediated by interactions between receptors on the surface of 20 leukocytes and endothelial cells. The general paradigm applies to all vascular beds, 21 but tissue and inflammation specific interactions provide powerful local regulation of 22 where, when and which leukocytes are recruited. The steps in the cascade are broadly 23 described as rolling or tethering, in which the leukocyte is captured from the circulation 24 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 25 under specific circumstances, such as in the hepatic sinusoids⁶⁸. Leukocyte rolling is 26 27 followed by activation of leukocyte integrins in response to tissue-derived 28 chemoattractant cytokines (chemokines) sequestered in the endothelial glycocalyx^{69,70}, which leads to firm adhesion mediated by integrins binding to 29 30 immunoglobulin superfamily members on the endothelial surface. This adhesion is 31 followed by intravascular crawling of the adherent leukocyte on the endothelium, 32 before the final step of transmigration in which the leukocyte migrates across the 33 endothelium, through the post-endothelial tissue and into the liver parenchyma. The 34 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 barrier⁷¹.

Cell recruitment to the liver has several features that are distinct from the general 3 4 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 5 recruitment occurs within the post capillary venules⁷². Furthermore, the recruitment of 6 leukocytes subsets to the liver is regulated by specific combinations of typical and 7 8 atypical adhesion molecules reflecting the unique phenotype and structure of LSECs, 9 and the anatomy and rheology of the sinusoids (Box 1). The sinusoids are narrow, in 10 places no wider than a flowing leukocyte, and characterised by low shear stress. These 11 properties mean that the initial recruitment step does not require rolling and in most 12 circumstances is selectin-independent. As a consequence, sinusoidal endothelium expresses minimal levels of selectins in vivo^{72,73}. A summary of the key adhesion 13 factors is outlined in Table 1. 14

15 **[H3]Immunoglobulin superfamily**

The conventional endothelial adhesion molecules that mediate firm adhesion of 16 leukocytes, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion 17 molecule-1 (VCAM-1)^{70,74}, are expressed at high levels on inflamed LSECs⁷⁵. Their 18 19 role in lymphocyte recruitment to the liver has been confirmed in both in vitro and in 20 *vivo* assays. VCAM-1, which binds the integrin $\alpha_4\beta_1$ expressed on lymphocytes, has 21 an important role in capturing lymphocytes from blood flow and mediating stabilisation^{66,76}. ICAM-1 binds to $\alpha_1\beta_2$ integrin to support firm adhesion within the 22 hepatic sinusoids⁷². Another family member is the mucosal vascular addressin cell 23 24 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 vessels⁷⁷. Our group demonstrated 25 26 that this receptor was also upregulated in the liver in some chronic liver diseases, in 27 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}. 28 29 Although many of these immunoglobulin superfamily members are regulated by pro-30 inflammatory cytokines, their adhesive function is also dependent on the formation of 31 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 32 33 group confirmed that the tetraspanin CD151 associates with VCAM-1 in LSECs and regulates lymphocyte adhesion under shear stress⁸². 34

1 [H3]Atypical adhesive and migratory routes

In addition to conventional adhesion molecules, our group and others have 2 3 demonstrated that LSECs use atypical adhesion molecules to regulate leukocyte recruitment. Vascular adhesion protein-1 (VAP-1) is a membrane bound amine 4 oxidase that was originally shown to mediate lymphocyte binding to high endothelial 5 venules, the specialised post-capillary venules found in lymph nodes⁸³. Further studies 6 7 confirmed that VAP-1 was expressed at high levels in chronic liver disease and mediated adhesion and transmigration across LSEC⁸⁴. Models of *in vitro* and *in vivo* 8 inflammatory liver injury corroborated its role in mediating recruitment during liver 9 10 inflammation. VAP-1 has unique properties generated by its enzyme activity that can upregulate expression of adhesion molecules and chemokines in LSECs, thereby 11 amplifying leukocyte recruitment⁸⁵⁻⁸⁸. The scavenger receptor family of endothelial 12 receptors also contribute to leukocyte recruitment to the liver. Stabilin-2 was shown to 13 regulate lymphocyte adhesion to LSECs via the integrin $\alpha_M \beta_2^{89}$ and its homologue, 14 stabilin-1, also known as common lymphatic endothelial and vascular endothelial cell 15 receptor (CLEVER-1), was originally shown to mediate recruitment across lymphatic 16 endothelium⁹⁰. Similarly expression of stabilin-1 is upregulated in chronic liver disease 17 18 and hepatocellular carcinoma, in which it mediates transmigration of lymphocytes across LSECs under shear stress⁹¹. 19

20 Following adherence, leukocytes crawl across the endothelial surface before 21 undergoing transmigration usually via endothelial junctions, termed the 'paracellular' route^{92,93}. Several studies have demonstrated that lymphocyte interactions with LSECs 22 within the sinusoids trigger important immune effector mechanisms^{94,95}, which might 23 influence the infiltration and positioning of cells within the liver in inflammatory liver 24 diseaes⁷⁵. Thus, it is important to understand how the process of transendothelial 25 migration through LSECs is regulated. Visualization of this process using confocal 26 27 imaging of lymphocytes migrating across LSECs under shear stress demonstrated that approximately 50% of cells took a 'transcellular' route of migration, and migrated 28 directly through the endothelial body⁹¹, as opposed to the conventional paracellular 29 route. This transcellular migration route involved the formation of ICAM-1 rich channels 30 31 to facilitate lymphocyte migration. Although transcellular migration has been noted in some other specialized endothelial beds, its function and molecular basis remain 32 poorly understood⁹⁶. Transendothelial migration is a multi-step process involving 33 different combinations of receptors that enables preferential recruitment of particular 34 35 leucocyte subsets, as described in the following sections. An additional step in 36 migration was described in 2016, in which lymphocytes migrate into LSECs and then

1 crawl within the endothelial cell to the cell junction, through which they enter the adjacent endothelial cell⁹⁷. This process, which we term 'intracellular crawling', is 2 3 dependent on IFNy and could not be detected when LSEC were stimulated by other interferon family cytokines. We found that IFNy treatment did alter the cytoskeleton of 4 5 LSECs which might promote 'intracellular crawling'. This process was also facilitated by the unique junctional complexes between LSECs. The functional consequences of 6 intracellular crawling are yet to be elucidated but could have an important role in 7 lymphocyte positioning in liver tissue. 8

9 [H3]Chemokines

10 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 11 leukocyte migration during homeostasis (in development and localization in 12 13 secondary lymphoid organs) as well as within tissues during inflammatory responses 14 by binding to G protein-coupled receptors on the surface of leukocytes. Upregulation 15 of several chemokines on liver vasculature has been demonstrated in a range of chronic inflammatory liver diseases, including alcoholic liver disease, primary 16 sclerosing cholangitis and chronic rejection¹⁰⁰⁻¹⁰⁴. In these conditions, chemokines 17 18 seem to be compartmentalized to the sinusoidal vasculature and portal vessels and 19 have a substantial influence on immune cell localization and subsequent disease progression ^{101,102,105}. T cell migration across sinusoidal endothelium is mediated by 20 the interferon-inducible chemokines CXCL9 and particularly CXCL10, which bind the 21 receptor CXCR3^{106,107}. In other diseases, including chronic HCV infection, the 22 23 chemokine CXCL16, which exists in a transmembrane form, is expressed on sinusoidal endothelium, hepatocytes and bile ducts, enabling it to regulate the 24 recruitment and retention of CXCR6⁺ effector T cells within the liver^{108,109}. Subsets of 25 Natural Killer (NK) and NK T cells express high levels of CXCR6 that enable them to 26 27 interact with CXCL16 on sinusoidal endothelium; this interaction promotes active migration along the sinusoids as part of a process of ongoing immune surveillance 28 and patrolling¹¹⁰. Studies in mouse liver endothelial cells have shown that a vital 29 30 property of chemokine-mediated recruitment is the transcytosis of chemokines from the basolateral side to the luminal side of sinusoidal endothelial cells¹¹¹. This process 31 is clathrin-dependent and promotes the transendothelial migration of lymphocytes 32 across LSECs, and inhibition of this pathway reduces CD4⁺ Tcell recruitment during 33 liver injury¹¹². 34

1 [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 formation¹¹³⁻¹¹⁵. 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.

9 [H3]T cells

T helper cells are divided into multiple functional subsets based on the cytokines they 10 11 secrete and dependent on the microenvironment in which they are activated by 12 antigens. In a concanavalin-A liver mouse inflammation model, Th1 recruitment 13 through the sinusoids was mediated by $\alpha_4\beta_1$ integrin interactions whereas Th2 cells used VAP-1⁸⁵. Both effector Th17 and regulatory T (Treg) cells found in the liver 14 15 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, 16 17 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 18 CCR5, CCR4 and in some cases CCR10¹¹⁸⁻¹²⁰. Tregs were also shown to use a distinct 19 combination of adhesion receptors, involving CLEVER-1/stabilin, ICAM-1 and VAP-1, 20 to migrate across human LSECs under flow⁹¹, whereas recruitment of CD8⁺ T cells to 21 22 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 23 24 cholangitis (PSC) associated with IBD, LSECs present the chemokine CCL25, which 25 can trigger CCR9⁺ gut homing lymphocyte interactions with MAdCAM-1 to promote recruitment of mucosal T cells^{104,123}. 26

These distinct mechanisms of migration across LSECs are probably influenced by 27 epithelial responses to tissue injury^{118,124}, stromal signals¹²⁵ and cooperative 28 29 interactions between several cell types in the sinusoid. For instance, in a model of HBV 30 infection, effector CD8⁺ T cells were shown to arrest in the sinusoids by interacting with platelets adherent to the sinusoidal surface via hyaluronan dependent mechanisms⁹⁵. 31 32 Subsequently, the CD8⁺ T cells crawled along the sinusoids, probing through the LSEC 33 fenestrae for viral antigens presented by underlying hepatocytes. Antigen recognition 34 as a consequence of this probing behaviour led to effector functions by a diapedesisindependent process. A human model of cytomegalovirus (CMV) infection of LSECs 35 led to the recruitment of effector T cells and activated Tregs in an LFA-3 dependent 36

mechanism¹²⁶. 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.

4 [H3]B cells

5 Although B cells are present in substantial numbers in chronically inflamed liver tissue, 6 the molecular mechanism regulating their recruitment from blood into hepatic tissue is 7 poorly understood. Our group demonstrated that B cell recruitment across human 8 LSECs under flow was initially mediated by VCAM-1-dependent capture followed by 9 limited intravascular crawling, compared with T cells¹²⁷. Interestingly, the receptors 10 involved in transmigration of B cells included ICAM-1, VAP-1 and CLEVER-1/stabilin-11 all of which are also involved in Treg transmigration across LSEC.

12 [H3]Neutrophils

Neutrophils are one of the earliest immune cells to be recruited to a site of tissue injury 13 and they are also recruited into the liver via the hepatic sinusoids¹²⁸. It was originally 14 thought that their migration was mediated by simple physical trapping within the narrow 15 16 sinusoidal channels, but work from McDonald et al. implicated a complex multistep 17 recruitment process involving interactions between sinusoidal hyaluronan and CD44 on the neutrophil surface¹²⁹. Whereas neutrophil interactions in post-sinusoidal 18 19 venules followed a conventional rolling mediated by selectins and integrin-mediated 20 adhesion, this was found not to be the case in the sinusoids, where the majority of 21 neutrophil extravasation took place. They found that hyaluronan was highly expressed 22 in liver sinusoids and mediated the recruitment of neutrophils in response to LPS 23 challenge. This interaction was dependent on CD44 binding to hyalorunan rather than 24 the other hyaluronan receptor, receptor for HA-mediated motility (RHAMM). A study 25 published in 2014 also highlighted the importance of TLRs for neutrophil recruitment. 26 TLR2/S100A9 signalling in particular promoted the production of the chemokines CXCL1 and CXCL2, which are known to mediate neutrophil migration, by liver 27 macrophages in acute and chronic mouse models of liver injury¹³⁰. 28

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30 **[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 pseudopods¹³¹. The response to liver injury also includes an influx of monocytes, which have a major role

in regulating inflammation, regeneration and repair and fibrosis¹³². Furthermore, acute 1 2 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 3 DAMP molecule ATP¹³¹. This entry is followed by the recruitment of CCR2⁺ monocytes 4 from the circulation¹³³. The subsequent recruitment signals governing monocyte 5 6 migration through the sinusoids are less well characterised but several key factors have been determined. The dominant chemokine receptor mediating migration of 7 8 CD16⁺ monocytes across LSECs is CX(3)CR1 binding to its ligand CX(3)CL1, one of 9 the few transmembrane chemokines, which is restricted to bile ducts in the normal liver but expressed at high levels on inflamed sinusoidal endothelium¹³⁴. In this study, VAP-10 1 also contributed to adhesion and transendothelial migration of CD16⁺ monocytes 11 across LSECs. The accumulation of CD14⁺⁺CD16⁺ monocytes has been reported in 12 inflammatory liver disease, which is due in part to the preferential migration of this 13 subset across LSECs compared to CD14⁺⁺CD16⁻ cells¹³⁵. Monocytes are known to 14 15 undergo a phenomenon of bidirectional movement across endothelium that involves a reverse migration step^{136,137}. This migratory behaviour has been confirmed in LSECs 16 and might have a marked effect on the fate of monocytes and the outcome of liver 17 18 injury, because monocyte subsets which undergo reverse transmigration are 19 predominantly proinflammatory CD16⁺ monocytes. By contrast, those remaining in the 20 subendothelial space are anti-inflammatory monocytes that suppress T cells and promote endotoxin tolerance¹³⁸. 21

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23 [H1]Interaction with other liver cells

Although we have focused on leukocyte interactions with LSECs, the cross talk 24 25 between LSECs and other liver cell populations will also influence the progression of chronic inflammatory liver diseases. Kupffer cells are found within the hepatic 26 27 sinusoids in close association with LSECs and are also equipped to sense tissue injury 28 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 29 30 the progression of parenchymal liver injury, such as alcoholic liver disease, in which 31 the release of danger signals from damaged hepatocytes stimulates the release of proinflammatory mediators from Kupffer cells¹⁴⁰. Despite poor understanding of the cross-32 talk between LSEC and Kupffer cells, the release of these mediators probably 33 influences LSEC phenotype and activation and leads to subsequent leukocyte 34 recruitment^{141,142}. Furthermore, Kupffer cells can promote LSEC capillarization, 35

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), 3 4 positioned within the Space of Disse. The central role of HSCs in extracellular matrix production in chronic liver disease is well established¹⁴⁵. It is now known that LSECs 5 play an important role in maintaining the guiescence of HSCs and this ability is lost 6 during capillarisation of LSECs, which permits HSC activation and fibrogenesis^{21,146}. 7 8 Activated liver myofibroblasts, derived predominantly from HSCs, also have a role in 9 the subsequent migration and positioning of lymphocytes following their recruitment 10 through LSECs. This process is mediated by distinct combinations of cytokines including IL-6, VEGF and chemokines released by myofibroblasts¹²⁵. 11

12 LSECs also play a key role in maintaining hepatocyte homeostasis. LSEC fenestrations enable bidirectional transport of metabolites between the circulation and 13 14 the liver parenchyma¹. LSECs also facilitate circulating T cells to interact with 15 hepatocytes by allowing T cells to extend cell surface protrusions through LSEC fenestrations¹⁴⁷. In chronic liver injury, microparticles are released from hepatocytes. 16 leukocytes and LSEC and provide another route for cell-cell communication¹⁴⁸. 17 18 Paracrine factors released from hepatocytes influence the expression of adhesion molecules on overlying LSECs and can promote the recruitment of flowing 19 20 lymphocytes from the sinusoids¹²⁴. This mechanism might be particularly important in 21 liver cancer because malignant transformation of hepatocytes enhances their ability to secrete chemokines CXCL10, CCL2 and CCL3 and to upregulate expression of ICAM-22 1 and VAP-1 on co-cultured LSECs^{103,149}. Work from our group has demonstrated that 23 factors secreted by hepatoma cells upregulate the expression of the tetraspanin 24 CD151 in LSECs, which promotes VCAM-1 mediated recruitment of lymphocytes⁸². 25

26 [H1]Therapeutic opportunities

27 The evidence presented here highlights the crucial role played by LSECs in regulating 28 the inflammatory response to liver injury. This importance makes them and the molecules they express attractive therapeutic targets in inflammatory liver 29 disease^{150,151}. VAP-1 is a good example¹⁵², with studies confirming that inhibition of 30 both its enzymatic activity or antibody blockade of its adhesive function reduces 31 hepatic inflammation and fibrosis in mouse liver injury models⁸⁷, and this work has led 32 to a clinical trial of a humanised antibody against VAP-1 that is currently underway 33 34 (BUTEO, NCT02239211) in patients with PSC. Chemokines and adhesion molecules 35 expressed by inflamed LSECs are also potential targets for anti-inflammatory therapy

1 in liver disease. For example, patients with PSC have been treated with NI-0801, a 2 humanized monoclonal antibody against CXCL10. Interestingly, the high production 3 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" 4 5 chemokine from the sinusoidal endothelial bed. Although the drug was well tolerated and demonstrated immunological changes, the overall results were negative. (K de 6 Graaf et al. submitted for publication). Thus, therapies directed at the chemokine 7 8 receptors themselves might have merit, and evidence from early trials using the dual 9 CCR2-CCR5 antagonist cenicriviroc in patients with NASH suggests that such treatment can induce a persistent blockade¹⁵³. 10

11

12 There is also a strong rationale to target gut-tropic chemokines in patients with liver 13 diseases associated with IBD. Of particular relevance is PSC, a progressive biliary 14 disease that is associated with IBD in 80% of cases and which affects ~8% of patients with IBD, particularly those with colitis¹⁵⁴. Under physiological conditions expression of 15 16 CCL25 and MAdCAM-1 is absent from the liver, but in PSC both proteins are 17 detectable on hepatic endothelium and support the aberrant recruitment of $\alpha_4 \beta_7^+$ 18 CCR9⁺ effector lymphocytes from the gut. Clinical trials are currently being considered 19 to target the $\alpha_4\beta_7$ -MAdCAM-1 pathways in PSC using antibodies developed for treating 20 IBD.

21 The tolerogenic capabilities of LSECs have also been targeted therapeutically. 22 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 23 24 their scavenger receptors is an excellent way of potentially targeting a range of 25 therapies to the liver. Presentation of delivered autoantigens by LSECs to naive T cells 26 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 27 wide range of autoimmune and allergic conditions⁶⁴. Targeting LSEC stabilin-1 and 28 stabilin-2 with nanoparticle-based drugs¹⁵⁵ has been suggested as a way to deliver 29 30 local treatment to manage a range of conditions including ischaemia-reperfusion injury 31 (a specific type of injury that follows liver surgery and transplantation which is a 32 biphasic process involving hypoxia followed by restoration of blood flow and 33 reoxygenation) and NAFLD. Similarly, blockade of LSECtin or the related molecule 34 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}. 35

During cirrhosis and chronic hepatitis, LSECs can undergocapillarisation¹⁵⁸. This 1 2 process is associated with loss of GATA4-dependent signals⁸, upregulation of CD31 and VCAM-1 and loss of fenestrations¹⁵⁸⁻¹⁶⁰. The number of fenestrations per 3 endothelial cell not only decreases with disease¹⁶¹⁻¹⁶³ but also with ageing¹⁶⁴, and this 4 phenotypic change is governed by p19^{ARF} and p53-dependent signalling¹⁶⁵. These 5 6 changes might impede the transfer of materials to or from the parenchyma and 7 contribute towards regional hepatocyte hypoxia. Capillarisation is mechanistically 8 linked to the development of chronic inflammatory disease. In rodent models, it is associated with enhanced antigen presentation and cytotoxic T cell priming during 9 fibrosis¹⁵¹, and in NASH capillarisation precedes and contributes to the transition from 10 simple steatosis to steatohepatitis¹⁵⁹. The changes that occur in LSECs in response to 11 chronic inflammation also affect angiogenic pathways. Neo-angiogenesis is a key 12 feature of chronic liver disease and the majority of neo-vessels arise from portal vein 13 branches and are closely associated with areas of fibrogenesis^{166,167} A key initiating 14 15 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 16 specific; for example, acute injury can induce CXCR7 expression and a regenerative 17 response, whereas chronic injury leads to CXCR4 induction, HSC proliferation and 18 fibrogenesis¹⁷⁰. During ischaemia-reperfusion injury LSEC develop a proinflammatory, 19 prothrombotic phenotype associated with vasoconstriction¹⁷¹. These changes have 20 21 been directly linked to neutrophils because IL-33 released by LSECs during 22 ischaemia-repurfusion injury triggers the release of neutrophil extracellular traps (NETs), which exacerbate acute hepatic injury¹⁷². In chronic injury, the changes in 23 endothelial phenotype that accompany capillarisation and precede fibrosis have been 24 linked to alterations in signalling via the hedgehog gene family¹⁷³ and lead to 25 vasocontriction and increased intrahepatic vascular resistence due to reduced nitric 26 oxide production by LSEC¹⁷⁴. Tumour progression in hepatocellular carcinoma is 27 28 associated with changes in the phenotype of peritumoural LSECs and increased production of angiogenic factors including IL-6^{175,176}. 29

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 models²¹ and it might also be possible to use GATA-4 mediated cellular reprogramming to restore the differentiated phenotype of LSECs and promote fibrosis resolution⁸. 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 transport¹⁷³. 1 2 However, studies testing cessation of VEGF-based cancer therapies also highlight 3 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 4 drugs as cancer treatments. Withdrawal of anti-VEGF- antibody therapy is associated 5 6 with development of hyperpolarised LSECs and promotion of hepatic metastasis¹⁷⁷. 7 Thus, low-dose, non-stop anti-angiogenic therapy might present a future solution to minimise these effects. 8

9 [h1]Conclusions

10 Sinusoidal endothelial cells have complex interrelated roles in the maintenance of liver 11 homeostasis and are implicated as drivers of inflammation and fibrogenesis in liver 12 disease. Their unique positioning, phenotype and function make them attractive 13 candidates for organ-specific therapy and it is likely that more therapies targeting these 14 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 15 antifibrotic therapies, strategies to maintain LSEC differentiation and to inhibit their 16 17 ability to recruit harmful pro-inflammatory leukocytes through the selective 18 orchestration of immune cell traffic might provide vital tools to halt the increase in 19 mortality linked to chronic liver failure.

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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	CD31 present at low levels
markers	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
5 5

- 1
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- 3

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

5 **Hepatitis C**

- 6 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 7
- 8 of CXCR3 ligands associated with compartmentalisation within the parenchyma^{102,109}.
- 9 Retention of CXCR6+ T cells through the expression of its ligand CXCL16¹⁰⁸.

10 **Primary sclerosing cholangitis**

- 11 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 12
- 13 MAdCAM-1 by deaminating primary amines and driving a NF-KB dependent pathway 86,88 14

15 Autoimmune hepatitis

- 16 Initial T cell mediated damage directed to sinusoidal endothelium as initiating event in
- models of autoimmune hepatitis¹⁴¹. Upregulation of adhesion molecules such as 17
- MAdCAM-1 promotes lymphocyte recruitment⁷⁸. Development of LSEC-reactive 18
- 19 autoantibodies leads to capillarisation of sinusoidal endothelium and progressive liver disease142.
- 20

21 Alcoholic liver disease and NAFLD

- 22 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 23
- 24 recruitment of T cells with compartmentalisation leading to progressive disease¹⁰¹.

25 **Fibrosis**

- 26 LSECs prevent hepatic stellate activation²¹. This ability to maintain HSC quiescence is
- 27 lost during capillarisation of LSEC driven by chronic injury¹⁴⁶. Capillarisation leads to
- 28 impaired eNOS activity leading to low nitric oxide production¹⁷⁴ and increased hedgehog
- 29 signalling¹⁷³.

30 Hepatocellular cancer

- 31 Endothelial transdifferentiation with loss of several LSEC markers¹⁷⁶. Presentation of
- 32 CXC and CC chemokines and expression of ICAM-1, VAP-1 and CD151 promotes
- 33 lymphocyte recruitment to HCC ^{103,149,82}. Stabilin-1 expression might promote Treg
- 34 specific recruitment⁹¹.
- 35
- 36

3 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	αΜβ2	Adhesion of lymphocytes
MAdCAM-1	α4β7	Adhesion of $\alpha 4\beta 7$ 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

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

34

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

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

- 8 distribution within hepatic tissue in a normal liver section.
- 9

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

11 a | LSECs express MHC class I receptors and can cross-present antigen to CD8⁺ 12 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 13 presentation to $CD8^+$ T cells leads to a memory effector T cell phenotype. **c** | In the 14 context of hepatotrophic infections such as hepatitis B, CD8⁺ T cells adhere to the 15 16 sinusoids in a platelet-dependent process and then probe for infected hepatocytes 17 through LSEC fenestrae. Detection of infected hepatocyte leads to diapedesis (the 18 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 19 induction of suppressor T cells (CD25^{hi} regulatory T cells). 20

21 Figure 3 | Lymphocyte recruitment within the hepatic sinusoids

22 Lymphocytes recruitment involves an adhesion cascade within the hepatic sinusoids 23 that is influenced by the low shear environment and cellular cross talk between 24 parenchymal and non-parenchymal cells. Chronic parenchymal cell damage leads to 25 the release of DAMPs and pro-inflammatory mediators by Kupffer cells which 26 increase adhesion molecule expression by LSECs (1). Lymphocyte recruitment 27 across activated LSEC involves a selectin-independent tethering step (2) followed by 28 integrin activation and firm adhesion to immunoglobulin superfamily members on the 29 LSEC surface (3). This process is influenced by paracrine factors released from 30 hepatocytes. Lymphocytes then crawl along the luminal endothelium (4) until they 31 receive a signal to transmigrate across LSEC either through a paracellular or 32 transcellular route (5). A third route of lymphocyte migration involves intracellular migration directly into the LSEC body and then migration to the adjacent LSEC, 33 34 termed intracellular crawling (6). Release of chemotactic factors from activated hepatic stellate cells promotes subsequent migration and positioning in liver tissue 35 36 (7).

1 2 3	Online only information
4	Subject ontology
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12	Biological sciences / Physiology / Circulation
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14	
15	Liver sinusoidal endothelial cells represent the most abundant non-parenchymal
16	hepatic cell population. In this Review, the authors explore the key roles that liver
17	sinusoidal endothelial cells have in regulating hepatic immunity, and their contribution
18	to immune-mediated disease, liver fibrosis and carcinogenesis.