

# Liver sinusoidal endothelial cells - gatekeepers of hepatic immunity

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1 **Liver sinusoidal endothelial cells — gate keepers of hepatic immunity**2 **Shishir Shetty, Patricia Lalor & David H. Adams\***

3

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11

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  
15 population. LSECs do not simply form a barrier within the hepatic sinusoids but have  
16 vital physiological and immunological functions, including filtration, endocytosis,  
17 antigen presentation and leukocyte recruitment. Reflecting these multifunctional  
18 properties, LSECs display unique structural and phenotypic features that differentiate  
19 them from capillary endothelium present within other organs. It is now clear that LSECs  
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.

25

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

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

## 20 **[H1]Introduction**

21 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 body<sup>1</sup>. 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 body<sup>2</sup>. Thus virus particles<sup>3</sup>, advanced glycation end  
34 products<sup>4</sup> and modified LDL<sup>5</sup> can be cleared from the circulation within minutes by this  
35 route.

36 Endothelial cells in different vascular beds are generated from common early  
37 embryological precursors, and have broadly similar histological appearance and  
38 functional roles throughout the body. However, extensive variations in phenotype and  
39 function arise as a consequence of local microenvironmental signals dependent on  
40 anatomical localisation<sup>6</sup>. The vascular architecture in the human liver is acquired by  
41 17–25 weeks of gestation, but different vessels within the liver have distinct embryonic

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 gestation<sup>7</sup> under the control of GATA-4<sup>8</sup>. 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 cells<sup>9</sup>. 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<sup>10</sup> and share a complement of receptors such as LYVE-1<sup>11</sup>, Prox-1<sup>12</sup>,  
10 podoplanin<sup>13</sup> and L-SIGN<sup>14</sup>. 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<sup>hi</sup> and Lyve-1<sup>lo</sup> whereas zone 2 and  
13 zone 3 LSECs are CD36<sup>lo</sup>, LYVE-1<sup>hi</sup> AND CD32<sup>hi</sup><sup>15</sup>. 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.<sup>2</sup>

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

1

## 2 **[H1]Balancing tolerance and immune response**

3 The permissive nature of sinusoidal endothelium probably evolved to handle the  
4 constant exposure of the liver to microbial and food antigens derived from the  
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  
13 by pattern recognition receptors<sup>31</sup>. Pattern recognition receptors are highly  
14 evolutionarily conserved and include the Toll-like Receptor (TLR) family and the  
15 scavenger receptors<sup>31</sup>. An example of how the liver regulates inflammatory and  
16 immune responses is seen in the recognition of the TLR-4 ligand lipopolysaccharide  
17 (LPS) by KCs and LSEC. Chronic exposure of both KCs and LSEC to LPS leads to an  
18 LPS-refractory state, and in LSECs specifically LPS exposure is associated with  
19 reduced nuclear translocation of nuclear-factor-kappa-light-chain enhancer of  
20 activated B cells (NF- $\kappa$ B) and subsequent reduced leukocyte adhesion<sup>32</sup>. 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  
25 classical antigen presenting cells, thereby contributing to an organ-specific response  
26 to antigens and the tolerogenic environment of the liver<sup>33</sup>.

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  
30 conserved<sup>34</sup>. In contrast to TLRs, they were believed to be functionally redundant and  
31 to perform silent uptake of ligands. However, gathering evidence suggests that this is  
32 not the case and that scavenger receptors have an important cell-specific role in  
33 immune responses<sup>34</sup>. They have been shown to promote potent pro-inflammatory and  
34 anti-inflammatory signalling as well as directly interacting with TLRs. Membrane bound  
35 scavenger receptors recognise their extracellular ligands which leads to internalisation

1 of the ligand, termed endocytosis, trafficking from the cell membrane to intracellular  
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  
4 scavenger receptors on LSECs is the mannose receptor (MR)<sup>1,35,36</sup>. Others include the  
5 homologous scavenger receptors stabilin-1 and stabilin-2<sup>37</sup> and related molecules  
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  
8 node-specific intercellular adhesion molecule 3- grabbing non-integrin (L-SIGN)<sup>38,39</sup>.  
9 The members of the C-type lectin group are involved in varied functions, from cell-cell  
10 interaction to uptake of serum glycoproteins. A third lectin with a similar structure to  
11 DC-SIGN and L-SIGN has been identified and designated the liver and lymph node  
12 sinusoidal endothelial cell C-type lectin (LSEctin)<sup>40</sup>. This lectin has been shown to be  
13 co-expressed with L-SIGN and is encoded in the same cluster of lectin encoding genes  
14 as DC-SIGN and L-SIGN.

### 15 **[H3] Innate immunity**

16 Several of the C-type lectin receptor family members have been directly implicated in  
17 viral uptake. Both DC-SIGN and L-SIGN have been shown to interact with the Ebola  
18 virus and HIV, as well as the coronavirus<sup>41,42</sup>. Both these receptors have also been  
19 shown to be expressed on LSECs and bind the E2 glycoprotein of the hepatitis C virus  
20 (HCV) and facilitate hepatocyte infection<sup>39</sup>. LSEctin has also been implicated in the  
21 uptake of SARS coronavirus and HCV<sup>43,44</sup>. The ability of LSEC to bind multiple viruses  
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  
24 viruses<sup>45</sup>. In a mouse model of adenovirus infection, 90% of virus is found in LSECs  
25 and 10% in KCs within a minute of intravenous viral infusion<sup>45</sup>. A study published in  
26 2017 reported that HIV-like particles are taken up by mouse LSECs at a rate of 100  
27 million particles per min.<sup>3</sup> The transit of viruses internalised by LSECs is less well  
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 hours<sup>46</sup>. LSECs enable direct entry of certain viruses such as Ebola,  
31 whereas with other viruses, such as HCV and HBV, LSECs promote hepatotropism by  
32 facilitating parenchymal cell infection<sup>47</sup>. Rapid uptake of virus can also lead to  
33 redistribution to other cells, for instance in animal models of HBV viral particles are  
34 preferentially taken up by LSECs and subsequently passed on to infect underlying  
35 hepatocytes<sup>48</sup>. In the case of HCV, innate sensing of viral infection by LSEC leads to  
36 downstream signalling and release of paracrine signals such as the pro-viral molecule

1 bone morphogenetic protein 4, which enhances viral infection of hepatocytes<sup>49</sup>. On the  
2 other hand, direct sensing of HCV RNA in LSECs also leads to the release of IFN I/III  
3 rich exosomes that inhibit HCV replication<sup>50</sup>. The balance of such responses will  
4 determine whether virus infection is established or prevented, thereby emphasising  
5 the critical role that LSEC play in hepatotropic viral infections.

### 6 **[H3]Adaptive immunity**

7 LSEC not only regulate innate immune responses but also directly regulate adaptive  
8 immune responses through antigen presentation to T cells (FIG. 2). Knolle's group  
9 demonstrated that LSECs can cross-present antigen to CD8<sup>+</sup> T cells<sup>51</sup> by using  
10 scavenger receptors, notably the mannose receptor, to take up, process and transfer  
11 antigen to MHC class I<sup>52</sup>. The presentation of antigen, including oral antigens, by  
12 LSECs drives a tolerogenic response in naïve CD8<sup>+</sup> T cells mediated by upregulation  
13 on LSECs of the co-inhibitory molecule programmed death-ligand 1 (PD-L1), also  
14 known as CD274 or B7 homolog 1, which can activate its receptor PD-1 of naïve T  
15 cells<sup>51,53,54</sup>. Endocytosis of antigens by the mannose receptor on LSECs has been  
16 shown to promote CD8<sup>+</sup> T cell tolerance<sup>55</sup>, including tolerance to tumour antigens<sup>56</sup>.  
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  
20 LSECs in which antigens at varying concentrations were delivered to LSECs for cross  
21 presentation to CD8<sup>+</sup> T cells, high antigen concentrations led to a shift from tolerogenic  
22 to effector T cell differentiation<sup>57</sup> as a consequence of enhanced TCR signalling that  
23 overcame PD-1 mediated tolerogenic responses. This response is also affected by  
24 local levels of IL-2. Furthermore, rapid activation of CD8<sup>+</sup> T cells by LSECs occurs in  
25 the presence of IL-6 trans-signalling and this activation not only drives rapid effector T  
26 cell differentiation but also primes T cells to respond to other inflammatory signals and  
27 leads to sustained effector responses<sup>58</sup>.

28

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

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

## 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  
11 2). A key step in the progression of liver injury or infection, regardless of aetiology, is  
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  
15 chronic hepatitis and cirrhosis<sup>66</sup>. Leukocyte recruitment from the blood occurs as a  
16 consequence of a multistep adhesion cascade that enables leukocytes flowing in the  
17 circulation to be captured by activated endothelial cells and then to migrate through  
18 the endothelium towards sites of infection or injury<sup>67</sup>. The cascade consists of  
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  
25 mediated by a family of receptors termed selectins but other receptors are involved  
26 under specific circumstances, such as in the hepatic sinusoids<sup>68</sup>. Leukocyte rolling is  
27 followed by activation of leukocyte integrins in response to tissue-derived  
28 chemoattractant cytokines (chemokines) sequestered in the endothelial  
29 glycocalyx<sup>69,70</sup>, which leads to firm adhesion mediated by integrins binding to  
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



1 with cytoskeletal changes in both the endothelial cells and the leukocytes and which  
2 enable the cell to cross the endothelium without disrupting the vascular barrier<sup>71</sup>.

3 Cell recruitment to the liver has several features that are distinct from the general  
4 adhesion cascade (Figure 3). Recruitment of the majority of leukocytes occurs within  
5 the sinusoidal channels of the liver, in contrast to most other organs in which  
6 recruitment occurs within the post capillary venules<sup>72</sup>. Furthermore, the recruitment of  
7 leukocytes subsets to the liver is regulated by specific combinations of typical and  
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  
13 expresses minimal levels of selectins *in vivo*<sup>72,73</sup>. A summary of the key adhesion  
14 factors is outlined in Table 1.

### 15 **[H3]Immunoglobulin superfamily**

16 The conventional endothelial adhesion molecules that mediate firm adhesion of  
17 leukocytes, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion  
18 molecule-1 (VCAM-1)<sup>70,74</sup>, are expressed at high levels on inflamed LSECs<sup>75</sup>. Their  
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  
22 stabilisation<sup>66,76</sup>. ICAM-1 binds to  $\alpha_L\beta_2$  integrin to support firm adhesion within the  
23 hepatic sinusoids<sup>72</sup>. Another family member is the mucosal vascular addressin cell  
24 adhesion molecule-1 (MAdCAM-1), which binds to the integrin  $\alpha_4\beta_7$  and plays a major  
25 part in lymphocyte homing to the gut via mucosal vessels<sup>77</sup>. Our group demonstrated  
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  
28 of  $\alpha_4\beta_7$ , thereby contributing to the link between IBD and inflammatory liver disease<sup>78,79</sup>.  
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  
32 microdomains and associate laterally with ICAM-1 and VCAM-1<sup>80,81</sup>. For example, our  
33 group confirmed that the tetraspanin CD151 associates with VCAM-1 in LSECs and  
34 regulates lymphocyte adhesion under shear stress<sup>82</sup>.

### 1 [H3]Atypical adhesive and migratory routes

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

20 Following adherence, leukocytes crawl across the endothelial surface before  
21 undergoing transmigration usually via endothelial junctions, termed the 'paracellular'  
22 route<sup>92,93</sup>. Several studies have demonstrated that lymphocyte interactions with LSECs  
23 within the sinusoids trigger important immune effector mechanisms<sup>94,95</sup>, which might  
24 influence the infiltration and positioning of cells within the liver in inflammatory liver  
25 diseases<sup>75</sup>. Thus, it is important to understand how the process of transendothelial  
26 migration through LSECs is regulated. Visualization of this process using confocal  
27 imaging of lymphocytes migrating across LSECs under shear stress demonstrated that  
28 approximately 50% of cells took a 'transcellular' route of migration, and migrated  
29 directly through the endothelial body<sup>91</sup>, as opposed to the conventional paracellular  
30 route. This transcellular migration route involved the formation of ICAM-1 rich channels  
31 to facilitate lymphocyte migration. Although transcellular migration has been noted in  
32 some other specialized endothelial beds, its function and molecular basis remain  
33 poorly understood<sup>96</sup>. Transendothelial migration is a multi-step process involving  
34 different combinations of receptors that enables preferential recruitment of particular  
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  
2 adjacent endothelial cell<sup>97</sup>. This process, which we term 'intracellular crawling', is  
3 dependent on IFN $\gamma$  and could not be detected when LSEC were stimulated by other  
4 interferon family cytokines. We found that IFN $\gamma$  treatment did alter the cytoskeleton of  
5 LSECs which might promote 'intracellular crawling'. This process was also facilitated  
6 by the unique junctional complexes between LSECs. The functional consequences of  
7 intracellular crawling are yet to be elucidated but could have an important role in  
8 lymphocyte positioning in liver tissue.

### 9 **[H3]Chemokines**

10 Chemokines are a family of small secreted proteins ranging from 67-127 amino acids  
11 in size that bind to heparin sulphate on proteoglycans<sup>98,99</sup>. They play central parts in  
12 leukocyte migration during homeostasis (in development and localization in  
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  
16 chronic inflammatory liver diseases, including alcoholic liver disease, primary  
17 sclerosing cholangitis and chronic rejection<sup>100-104</sup>. In these conditions, chemokines  
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  
20 progression<sup>101,102,105</sup>. T cell migration across sinusoidal endothelium is mediated by  
21 the interferon-inducible chemokines CXCL9 and particularly CXCL10, which bind the  
22 receptor CXCR3<sup>106,107</sup>. In other diseases, including chronic HCV infection, the  
23 chemokine CXCL16, which exists in a transmembrane form, is expressed on  
24 sinusoidal endothelium, hepatocytes and bile ducts, enabling it to regulate the  
25 recruitment and retention of CXCR6<sup>+</sup> effector T cells within the liver<sup>108,109</sup>. Subsets of  
26 Natural Killer (NK) and NK T cells express high levels of CXCR6 that enable them to  
27 interact with CXCL16 on sinusoidal endothelium; this interaction promotes active  
28 migration along the sinusoids as part of a process of ongoing immune surveillance  
29 and patrolling<sup>110</sup>. Studies in mouse liver endothelial cells have shown that a vital  
30 property of chemokine-mediated recruitment is the transcytosis of chemokines from  
31 the basolateral side to the luminal side of sinusoidal endothelial cells<sup>111</sup>. This process  
32 is clathrin-dependent and promotes the transendothelial migration of lymphocytes  
33 across LSECs, and inhibition of this pathway reduces CD4<sup>+</sup> T cell recruitment during  
34 liver injury<sup>112</sup>.

35

## 1 [H1]Immune subset recruitment

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

## 9 [H3]T cells

10 T helper cells are divided into multiple functional subsets based on the cytokines they  
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  
14 used VAP-1<sup>85</sup>. Both effector Th17 and regulatory T (Treg) cells found in the liver  
15 express high levels of the chemokine receptor CXCR3 and use it to migrate across  
16 LSECs<sup>116,117</sup>. Subsequent signals determine where these cells localise within the liver,  
17 with CCR6+ Th17 cells migrating towards their ligand CCL20 secreted by bile ducts  
18 whilst Tregs respond to different chemokines as a consequence of their expression of  
19 CCR5, CCR4 and in some cases CCR10<sup>118-120</sup>. Tregs were also shown to use a distinct  
20 combination of adhesion receptors, involving CLEVER-1/stabilin, ICAM-1 and VAP-1,  
21 to migrate across human LSECs under flow<sup>91</sup>, whereas recruitment of CD8<sup>+</sup> T cells to  
22 the mouse liver is primarily dependent on ICAM-1 expression by LSECs with a lesser  
23 contribution from VCAM-1<sup>121,122</sup>. In autoimmune hepatitis and primary sclerosing  
24 cholangitis (PSC) associated with IBD, LSECs present the chemokine CCL25, which  
25 can trigger CCR9<sup>+</sup> gut homing lymphocyte interactions with MAdCAM-1 to promote  
26 recruitment of mucosal T cells<sup>104,123</sup>.

27 These distinct mechanisms of migration across LSECs are probably influenced by  
28 epithelial responses to tissue injury<sup>118,124</sup>, stromal signals<sup>125</sup> and cooperative  
29 interactions between several cell types in the sinusoid. For instance, in a model of HBV  
30 infection, effector CD8<sup>+</sup> T cells were shown to arrest in the sinusoids by interacting with  
31 platelets adherent to the sinusoidal surface via hyaluronan dependent mechanisms<sup>95</sup>.  
32 Subsequently, the CD8<sup>+</sup> 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 diapedesis-  
35 independent process. A human model of cytomegalovirus (CMV) infection of LSECs  
36 led to the recruitment of effector T cells and activated Tregs in an LFA-3 dependent

1 mechanism<sup>126</sup>. In this study, CMV infected human LSEC upregulated LFA-3 at  
2 intercellular junctions and during effector T cell recruitment the interaction of LFA-3  
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 cells<sup>127</sup>. Interestingly, the receptors  
10 involved in transmigration of B cells included ICAM-1, VAP-1 and CLEVER-1/stabilin-  
11 1 all of which are also involved in Treg transmigration across LSEC.

#### 12 **[H3]Neutrophils**

13 Neutrophils are one of the earliest immune cells to be recruited to a site of tissue injury  
14 and they are also recruited into the liver via the hepatic sinusoids<sup>128</sup>. It was originally  
15 thought that their migration was mediated by simple physical trapping within the narrow  
16 sinusoidal channels, but work from McDonald *et al.* implicated a complex multistep  
17 recruitment process involving interactions between sinusoidal hyaluronan and CD44  
18 on the neutrophil surface<sup>129</sup>. Whereas neutrophil interactions in post-sinusoidal  
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 hyaluronan 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  
27 CXCL1 and CXCL2, which are known to mediate neutrophil migration, by liver  
28 macrophages in acute and chronic mouse models of liver injury<sup>130</sup>.

29

#### 30 **[H3]Monocytes**

31 In addition to the activation of resident Kupffer cells, monocytes and macrophages are  
32 also recruited to the liver from the circulation during inflammation or in response to  
33 injury. Kupffer cells are yolk-sac-derived tissue macrophages found within the hepatic  
34 sinusoids; they are immobile and probe the environment with pseudopods<sup>131</sup>. The  
35 response to liver injury also includes an influx of monocytes, which have a major role

1 in regulating inflammation, regeneration and repair and fibrosis<sup>132</sup>. Furthermore, acute  
2 liver injury is associated with an initial influx of GATA6<sup>+</sup> peritoneal macrophages that  
3 enter directly through the mesothelium in a process dependent on CD44 and the  
4 DAMP molecule ATP<sup>131</sup>. This entry is followed by the recruitment of CCR2<sup>+</sup> monocytes  
5 from the circulation<sup>133</sup>. The subsequent recruitment signals governing monocyte  
6 migration through the sinusoids are less well characterised but several key factors  
7 have been determined. The dominant chemokine receptor mediating migration of  
8 CD16<sup>+</sup> 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  
10 but expressed at high levels on inflamed sinusoidal endothelium<sup>134</sup>. In this study, VAP-  
11 1 also contributed to adhesion and transendothelial migration of CD16<sup>+</sup> monocytes  
12 across LSECs. The accumulation of CD14<sup>++</sup>CD16<sup>+</sup> monocytes has been reported in  
13 inflammatory liver disease, which is due in part to the preferential migration of this  
14 subset across LSECs compared to CD14<sup>++</sup>CD16<sup>-</sup> cells<sup>135</sup>. Monocytes are known to  
15 undergo a phenomenon of bidirectional movement across endothelium that involves a  
16 reverse migration step<sup>136,137</sup>. This migratory behaviour has been confirmed in LSECs  
17 and might have a marked effect on the fate of monocytes and the outcome of liver  
18 injury, because monocyte subsets which undergo reverse transmigration are  
19 predominantly proinflammatory CD16<sup>+</sup> monocytes. By contrast, those remaining in the  
20 subendothelial space are anti-inflammatory monocytes that suppress T cells and  
21 promote endotoxin tolerance<sup>138</sup>.

22

### 23 **[H1]Interaction with other liver cells**

24 Although we have focused on leukocyte interactions with LSECs, the cross talk  
25 between LSECs and other liver cell populations will also influence the progression of  
26 chronic inflammatory liver diseases. Kupffer cells are found within the hepatic  
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  
29 inflammasome pathway in Kupffer cells<sup>139</sup>. Inflammasome activation is a key step in  
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 pro-  
32 inflammatory mediators from Kupffer cells<sup>140</sup>. Despite poor understanding of the cross-  
33 talk between LSEC and Kupffer cells, the release of these mediators probably  
34 influences LSEC phenotype and activation and leads to subsequent leukocyte  
35 recruitment<sup>141,142</sup>. Furthermore, Kupffer cells can promote LSEC capillarization,

1 whereby LSEC morphology becomes more vascular or capillary like with a loss of  
2 fenestrations and a characteristic basement membrane is formed<sup>143,144</sup>.

3 The other cell type that populates the sinusoids is the hepatic stellate cell (HSC),  
4 positioned within the Space of Disse. The central role of HSCs in extracellular matrix  
5 production in chronic liver disease is well established<sup>145</sup>. It is now known that LSECs  
6 play an important role in maintaining the quiescence of HSCs and this ability is lost  
7 during capillarisation of LSECs, which permits HSC activation and fibrogenesis<sup>21,146</sup>.  
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  
11 including IL-6, VEGF and chemokines released by myofibroblasts<sup>125</sup>.

12 LSECs also play a key role in maintaining hepatocyte homeostasis. LSEC  
13 fenestrations enable bidirectional transport of metabolites between the circulation and  
14 the liver parenchyma<sup>1</sup>. LSECs also facilitate circulating T cells to interact with  
15 hepatocytes by allowing T cells to extend cell surface protrusions through LSEC  
16 fenestrations<sup>147</sup>. In chronic liver injury, microparticles are released from hepatocytes,  
17 leukocytes and LSEC and provide another route for cell–cell communication<sup>148</sup>.  
18 Paracrine factors released from hepatocytes influence the expression of adhesion  
19 molecules on overlying LSECs and can promote the recruitment of flowing  
20 lymphocytes from the sinusoids<sup>124</sup>. This mechanism might be particularly important in  
21 liver cancer because malignant transformation of hepatocytes enhances their ability to  
22 secrete chemokines CXCL10, CCL2 and CCL3 and to upregulate expression of ICAM-  
23 1 and VAP-1 on co-cultured LSECs<sup>103,149</sup>. Work from our group has demonstrated that  
24 factors secreted by hepatoma cells upregulate the expression of the tetraspanin  
25 CD151 in LSECs, which promotes VCAM-1 mediated recruitment of lymphocytes<sup>82</sup>.

## 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  
29 molecules they express attractive therapeutic targets in inflammatory liver  
30 disease<sup>150,151</sup>. VAP-1 is a good example<sup>152</sup>, with studies confirming that inhibition of  
31 both its enzymatic activity or antibody blockade of its adhesive function reduces  
32 hepatic inflammation and fibrosis in mouse liver injury models<sup>87</sup>, and this work has led  
33 to a clinical trial of a humanised antibody against VAP-1 that is currently underway  
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  
4 neutralization of the chemokine *in vivo*, despite evidence that the antibody could “strip”  
5 chemokine from the sinusoidal endothelial bed. Although the drug was well tolerated  
6 and demonstrated immunological changes, the overall results were negative. (K de  
7 Graaf et al. submitted for publication). Thus, therapies directed at the chemokine  
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  
10 treatment can induce a persistent blockade<sup>153</sup>.

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  
15 with IBD, particularly those with colitis<sup>154</sup>. Under physiological conditions expression of  
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<sup>+</sup> 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  
23 of their potent scavenging capability; the ability of LSECs to take up molecules using  
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  
27 systemic as well as local autoimmune responses. This strategy could be applied to a  
28 wide range of autoimmune and allergic conditions<sup>64</sup>. Targeting LSEC stabilin-1 and  
29 stabilin-2 with nanoparticle-based drugs<sup>155</sup> has been suggested as a way to deliver  
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  
35 via impairment of interactions with LSEC in mouse models<sup>156,157</sup>.



1 During cirrhosis and chronic hepatitis, LSECs can undergo capillarisation<sup>158</sup>. This  
2 process is associated with loss of GATA4-dependent signals<sup>8</sup>, upregulation of CD31  
3 and VCAM-1 and loss of fenestrations<sup>158-160</sup>. The number of fenestrations per  
4 endothelial cell not only decreases with disease<sup>161-163</sup> but also with ageing<sup>164</sup>, and this  
5 phenotypic change is governed by p19<sup>ARF</sup> and p53-dependent signalling<sup>165</sup>. These  
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  
9 associated with enhanced antigen presentation and cytotoxic T cell priming during  
10 fibrosis<sup>151</sup>, and in NASH capillarisation precedes and contributes to the transition from  
11 simple steatosis to steatohepatitis<sup>159</sup>. The changes that occur in LSECs in response to  
12 chronic inflammation also affect angiogenic pathways. Neo-angiogenesis is a key  
13 feature of chronic liver disease and the majority of neo-vessels arise from portal vein  
14 branches and are closely associated with areas of fibrogenesis<sup>166,167</sup>. A key initiating  
15 step is the capillarisation of LSECs, which leads to increased hepatocyte hypoxia and  
16 subsequent release of pro-angiogenic factors<sup>168,169</sup>. The LSEC response is context  
17 specific; for example, acute injury can induce CXCR7 expression and a regenerative  
18 response, whereas chronic injury leads to CXCR4 induction, HSC proliferation and  
19 fibrogenesis<sup>170</sup>. During ischaemia-reperfusion injury LSEC develop a proinflammatory,  
20 prothrombotic phenotype associated with vasoconstriction<sup>171</sup>. These changes have  
21 been directly linked to neutrophils because IL-33 released by LSECs during  
22 ischaemia-reperfusion injury triggers the release of neutrophil extracellular traps  
23 (NETs), which exacerbate acute hepatic injury<sup>172</sup>. In chronic injury, the changes in  
24 endothelial phenotype that accompany capillarisation and precede fibrosis have been  
25 linked to alterations in signalling via the hedgehog gene family<sup>173</sup> and lead to  
26 vasoconstriction and increased intrahepatic vascular resistance due to reduced nitric  
27 oxide production by LSEC<sup>174</sup>. Tumour progression in hepatocellular carcinoma is  
28 associated with changes in the phenotype of peritumoural LSECs and increased  
29 production of angiogenic factors including IL-6<sup>175,176</sup>.

30 These changes in LSECs therefore present opportunities for therapeutic intervention.  
31 For example, pharmacological therapy in the form of a soluble guanylate cyclase  
32 activator which restores fenestrations has been linked to fibrosis regression in rodent  
33 models<sup>21</sup> and it might also be possible to use GATA-4 mediated cellular  
34 reprogramming to restore the differentiated phenotype of LSECs and promote fibrosis  
35 resolution<sup>8</sup>. Similarly, therapies that restore normal hedgehog signalling promote  
36 regression of capillarisation and the reappearance of fenestrations, which suggests a

1 potential pathway for reversal of fibrosis and the restoration of lipid transport<sup>173</sup>.  
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,  
4 and go some way in explaining poor performance of some strategies using anti-VEGF  
5 drugs as cancer treatments. Withdrawal of anti-VEGF- antibody therapy is associated  
6 with development of hyperpolarised LSECs and promotion of hepatic metastasis<sup>177</sup>.  
7 Thus, low-dose, non-stop anti-angiogenic therapy might present a future solution to  
8 minimise these effects.

## 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  
15 inflammation and to prevent or reverse fibrogenesis. In the absence of licenced  
16 antifibrotic therapies, strategies to maintain LSEC differentiation and to inhibit their  
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.

20

1

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48

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

Morphological appearance	<p>Fenestrated, continuous endothelium with minimal basement membrane in normal conditions</p> <p>Fenestrations can be organized into sieve plates and range from around 50-100nm in diameter</p>
Expression of endothelial markers	<p>CD31 present at low levels</p> <p>Von Willebrand factor expression is controversial but can be detected in human LSECs in the context of liver injury</p> <p>CD34 is absent or only expressed at low levels</p> <p>CD105 (endoglin) is present</p> <p>CD36 is present at a much higher level than vascular endothelium</p> <p>E-selectin is absent on unstimulated cells but expression can be induced, albeit at lower levels than vascular endothelium in inflammatory conditions</p>
Endocytic capabilities	<p>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</p>
Expression of scavenger receptors	<p>LSEC have very potent scavenger capabilities by virtue of expression of many scavenger receptors, including Mannose receptor(CD206), FcγRIIb, Stabilin-1 (Clever-1), Stabilin-2, Scavenger receptors B1 and B2, L-SIGN, LYVE-1 and LRP-1</p>
Junctional structure	<p>'Mixed' type of junctions having some features of tight junctions but generally showing lower or absent claudin-5 and occludin compared to vascular endothelium</p> <p>VE-Cadherin can be present in a disease setting</p>
Adhesion molecules	<p>LSEC constitutively express low levels of ICAM-1, ICAM-2 and VCAM-1</p> <p>Selectin expression is considered to be minimal in most circumstances</p> <p>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</p>
Chemokine expression	<p>Minimal chemokine expression is seen on unstimulated LSECs although they will express factors such as CXCL9-</p>

	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
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#### 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  
 7 endothelial expression of adhesion molecules ICAM-1, VCAM-1, VAP-1 and presentation  
 8 of CXCR3 ligands associated with compartmentalisation within the parenchyma<sup>102,109</sup>.  
 9 Retention of CXCR6+ T cells through the expression of its ligand CXCL16<sup>108</sup>.

##### 10 **Primary sclerosing cholangitis**

11 Aberrant homing of mucosal effector lymphocytes through expression of MAdCAM-1  
 12 and CCL25 on hepatic sinusoidal endothelium<sup>78,104</sup>. VAP-1 regulates the expression of  
 13 MAdCAM-1 by deaminating primary amines and driving a NF-KB dependent pathway  
 14 <sup>86,88</sup>.

##### 15 **Autoimmune hepatitis**

16 Initial T cell mediated damage directed to sinusoidal endothelium as initiating event in  
 17 models of autoimmune hepatitis<sup>141</sup>. Upregulation of adhesion molecules such as  
 18 MAdCAM-1 promotes lymphocyte recruitment<sup>78</sup>. Development of LSEC-reactive  
 19 autoantibodies leads to capillarisation of sinusoidal endothelium and progressive liver  
 20 disease<sup>142</sup>.

##### 21 **Alcoholic liver disease and NAFLD**

22 Defenestration and activation are early changes in models of alcoholic and fatty liver  
 23 disease<sup>159,160</sup>. The presentation of chemokines by sinusoidal endothelium leads to  
 24 recruitment of T cells with compartmentalisation leading to progressive disease<sup>101</sup>.

##### 25 **Fibrosis**

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

##### 30 **Hepatocellular cancer**

31 Endothelial transdifferentiation with loss of several LSEC markers<sup>176</sup>. Presentation of  
 32 CXC and CC chemokines and expression of ICAM-1, VAP-1 and CD151 promotes  
 33 lymphocyte recruitment to HCC<sup>103,149,82</sup>. Stabilin-1 expression might promote Treg  
 34 specific recruitment<sup>91</sup>.

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**Tables:**

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

Adhesion factor	Ligand	Function
ICAM-1	$\alpha$ L $\beta$ 2	Firm adhesion of CD4 cells and CD8 cells, transmigration of Tregs and B cells
VCAM-1	$\alpha$ 4 $\beta$ 1	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	$\alpha$ M $\beta$ 2	Adhesion of lymphocytes
MAdCAM-1	$\alpha$ 4 $\beta$ 7	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 <sup>+</sup> 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



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

3 a | Low power image of a human liver tissue (stained with haematoxylin and eosin)  
4 illustrating the lobular organisation of the liver with zonal architecture indicated  
5 relative to the position of the portal tract. b | Expanded periportal section of the same  
6 image to illustrate the different vascular compartments within the parenchyma. c |  
7 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-presenting cells**

11 a | LSECs express MHC class I receptors and can cross-present antigen to CD8<sup>+</sup>  
12 cytotoxic T cells. At low antigen concentrations this presentation leads to tolerance  
13 and deletion of CD8<sup>+</sup> T cells. b | If antigen concentrations are high then antigen cross  
14 presentation to CD8<sup>+</sup> T cells leads to a memory effector T cell phenotype. c | In the  
15 context of hepatotropic infections such as hepatitis B, CD8<sup>+</sup> T cells adhere to the  
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  
19 present antigen to CD4<sup>+</sup> T cells via expression of MHC class II, which leads to the  
20 induction of suppressor T cells (CD25<sup>hi</sup> regulatory T cells).

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  
33 migration directly into the LSEC body and then migration to the adjacent LSEC,  
34 termed intracellular crawling (6). Release of chemotactic factors from activated  
35 hepatic stellate cells promotes subsequent migration and positioning in liver tissue  
36 (7).

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3 **Online only information**

4 Subject ontology

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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.