HHS Public Access

Author manuscript

Semin Liver Dis. Author manuscript; available in PMC 2018 December 22.

Published in final edited form as:

Semin Liver Dis. 2017 November ; 37(4): 377–387. doi:10.1055/s-0037-1617455.

Liver Sinusoidal Endothelial Cell: An Update

Laurie D. DeLeve, MD, PhD[1] and **Ana C. Maretti-Mira, PhD**[1]

[1]Division of Gastrointestinal and Liver Diseases and the USC Research Center for Liver Diseases, Keck School of Medicine of the University of Southern California, Los Angeles, California

Abstract

This update focuses on two main topics. First, recent developments in our understanding of liver sinusoidal endothelial cell (LSEC) function will be reviewed, specifically elimination of blood-borne waste, immunological function of LSECs, interaction of LSECs with liver metastases, LSECs and liver regeneration, and LSECs and hepatic fibrosis. Second, given the current emphasis on rigor and transparency in biomedical research, the update discusses the need for standardization of methods to demonstrate identity and purity of isolated LSECs, pitfalls in methods that might lead to a selection bias in the types of LSECs isolated, and questions about long-term culture of LSECs. Various surface markers used for immunomagnetic selection are reviewed.

Keywords

liver sinusoidal endothelial cell; endocytosis; liver regeneration; liver fibrosis; cell isolation approaches

This review is an update rather than a comprehensive review of liver sinusoidal endothelial cell (LSEC) ultrastructure, biology, and physiology. This update will focus on new findings on LSEC functions reported in the last few years, as well as some of the concerns about isolation and characterization of isolated LSECs that have dogged this field for more than a decade (Table 1).[1] Readers looking for comprehensive reviews of LSEC biology rather than an update have other excellent choices.[2,3]

LSEC Function

Major functions of LSECs include (1) elimination of macromolecules and small particulates from the blood, (2) an immunological role, (3) LSEC interactions with tumor metastases, (4) LSECs as determinants of hepatic fibrosis, and (5) LSECs as drivers of liver regeneration.

Elimination of Macromolecules and Small Particulates from Blood

The reader is referred to dedicated comprehensive reviews on this topic.[3,4] LSECs have higher endocytotic activity than other vascular endothelial cells and are able to rapidly

Address for correspondence: Laurie D. DeLeve, MD, PhD, Division of Gastrointestinal and Liver Diseases and the USC Research Center for Liver Diseases, Keck School of Medicine of University of Southern California, 2011 Zonal Avenue, HMR 603, Los Angeles, CA 90089 (deleve@med.usc.edu).

endocytose cellular and extracellular components, modified macromolecules of mammalian origin, and nonmammalian ligands[3] through several families of endocytotic receptors: stabilin-1, stabilin-2, scavenger receptors, mannose receptor, and FcγRIIb2. Although much of the clearance of blood-borne waste was previously attributed to Kupffer cells, in recent decades it has become clear that LSECs and Kupffer cells play a complementary role in this process. LSECs clear macromolecules and nanoparticles by clathrin-mediated endocytosis and Kupffer cells clear larger particles by phagocytosis.

Viral clearance—Both LSECs and Kupffer cells contribute to viral clearance. Older in vitro studies demonstrated LSEC uptake of duck hepatitis B virus,[5] but newer studies of viral clearance have examined in vivo uptake, which allowed investigators to compare the respective contributions of LSECs and Kupffer cells. Ninety percent of adenovirus cleared by the mouse localized to LSECs and only 10% to Kupffer cells.[6] Similarly, studies that examined clearance of HIV-like particles in the mouse found that 90% of HIV-like particles were cleared by LSECs and only 10% by Kupffer cells. Astonishingly, the sinusoid is capable of eliminating 100 million HIV-like particles per minute.[7] Studies with virus-like particles of two nonenveloped human DNA viruses, polyoma-viruses BK and JC, demonstrated that liver uptake in the mouse was predominantly in LSECs and that LSECs rapidly cleared a large fraction of these virus-like particles.[8]

Lipid clearance—The first article that definitively characterized LSECs as a cell type distinct from Kupffer cells raised the hypothesis that LSEC fenestration functioned as a sieve that permitted filtering of chylomicrons.[9] The sieve is composed of nondiaphragmed fenestrae, 100–150 nm openings that traverse the cytosol of the LSECs. The LSECs also lack an organized basement membrane. Studies have confirmed that LSEC fenestration permits filtering of chylomicron remnants,[10–12] and it has been postulated that the loss of fenestration with aging might account for postprandial hypertriglyceridemia.[13] The role of lipid uptake by LSEC endocytosis has been addressed more recently. Uptake of oxidized low-density lipoprotein (LDL) by macrophages within the atherosclerotic lesion plays a central role in atherosclerosis.[14] Older literature had suggested that Kupffer cells play the major role in uptake of oxidized LDL. A study of LDL with a physiological degree of oxidation demonstrated that LSECs but not Kupffer cells take up the more physiological, mildly oxidized LDL through the stabilin-1 endocytosis receptor.[15] Future studies will need to determine whether stabilin-1 mediated endocytosis changes with aging or with the metabolic syndrome. The latter is of interest given that higher levels of oxidized LDL are associated with the metabolic syndrome,[16] and that oxidized LDL has been proposed as a risk factor for nonalcoholic steatohepatitis.[17] Scavenger receptor type B 1 (SR-B1) mediates selective uptake of high-density lipoprotein (HDL).[18] It was previously assumed that SR-B1 was present on hepatocytes, but careful imaging studies have now reported that SR-B1 is present on LSECs.[19] Future studies will need to determine whether reduced HDL clearance by LSECs might contribute to highly elevated levels of HDL, which may increase cardiovascular risk and all-cause mortality,[20–22] or whether aberrant clearance of HDL by LSECs has implications for ligands that are cleared along with HDL.

Nanoparticles—Nanoparticles, with or without linkage to biological molecules as address tags, are under investigation for numerous biological applications. Given their size, it might be surmised that nanoparticles without address tags might be taken up by the reticuloendothelial cell system. Poly (lactic-co-glycolic acid) (PLGA) is a biodegradable polymer that is widely used because its hydrolysis leads to metabolite monomers, lactic acid, and glycolic acid, which can be effectively eliminated. In mice that had been administered PLGA nanoparticles (average diameter of 271 ± 1.4 nm) in vivo, 98% of Kupffer cells and 89% of LSECs showed uptake but with a higher intensity of uptake by Kupffer cells.[23] Over half the hepatic stellate cells (HSCs) and 7% of hepatocytes also showed uptake. Reports on the size of murine fenestrae vary widely, but ranges from 78 to 144 nm.[24–26] Thus, nanoparticles of the size used in this study are larger than fenestrae, which raises the question of how the particles pass the LSEC barrier to reach HSCs and hepatocytes.

The Immunological Role of LSECs

Clearance of blood borne pathogens and macromolecules—Removal of circulating antigens is a major immunologic function of LSECs. For example, as described above, LSECs play a major role in clearance of some viruses.

Lipopolysaccharide (LPS) is mainly cleared by the liver.[27] Seventy-five percent of LPS clearance is by LSECs and 25% is by Kupffer cells.[28] HDL binds LPS and LSECs also clear this complex,[28] although it remains to be established whether HDL-mediated clearance is the major route of LPS clearance by LSECs. Capillarization occurs in rodents with fatty liver[29] and prior to fibrosis (see section below on LSECs as determinants of hepatic fibrosis). It is not known whether LPS clearance is impaired in capillarization of LSECs, but if it is, then decreased LSEC clearance of LPS might lead to increased Kupffer cell clearance and/or increased systemic endotoxemia after bacterial translocation. Serum LPS levels are elevated in nonalcoholic fatty liver disease,[30] severe alcoholic hepatitis,[31] and cirrhosis[32,33] and capillarization could conceivably contribute to this.

The liver clears most small immunoglobulin G (IgG) immune complexes with only minor participation by the spleen.[34] Within the liver, LSECs clear IgG by receptor-mediated endocytosis through immunoglobulin gamma Fc region receptor II-b2 (FcγRIIb2). This pathway clears blood-borne small IgG immune complexes.[34,35] Over 70% of RIIb in the body is expressed on the liver. Comparing RIIb between LSECs and Kupffer cells, 90% is expressed on LSECs and 10% is expressed on Kupffer cells.[34] Investigators in this field have posited that immune complex disease may occur when LSEC clearance capacity is overwhelmed.[34]

Innate immunity—LSECs express pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns and damage-associated molecular patterns (Table 2). The mannose receptor and stabilins and endocytosis receptors present on LSECs are considered an important part of innate immunity by acting as PRRs.[36,37] Toll-like receptors are membrane-spanning receptors that recognize structurally conserved microbial molecules. Six toll-like receptors have been identified on LSECs.[38–40] Inflammasomes are protein

complexes that induce proteolytic activation of proinflammatory cytokines. Three protein constituents of inflammasomes are expressed by LSECs.[41]

Adaptive immunity—According to the literature, LSECs play critical roles in the development and establishment of hepatic adaptive immunity. LSECs may function as antigen presenting cells (APCs) and as CD4 T lymphocytes recruiters during immune surveillance and liver inflammation.

As noted above, LSECs have high endocytic activity with multiple receptors to take up blood-borne antigens.[3,4] Interestingly, LSECs are less efficient than dendritic cells in FCγR-mediated endocytosis.[42] The antigen presenting function of LSECs has been attributed to the constitutive or inducible expression of several surface markers and costimulatory molecules associated with professional APCs, such as MHC-class I and II, intercellular adhesion molecule (ICAM)-I, vascular cell adhesion protein (VCAM)-I, CD80, and CD86.[43–46]

Cross-presentation of exogenous antigens by LSECs to CD8 T cells does not cause deletion of naive CD8 T cells.[47] It is stringently antigen dependent[47] and results in CD8 T cell tolerance mediated by B7-H1 signaling in vitro and in vivo.[45,48,49] In contrast, LSECs can mediate cross-talk between CD4 T helper and CD8 T cells, in a process that involves concomitant activation of both T cell populations by LSECs, LSEC cross-priming by CD4 T helpers, release of IL-2, and stimulation of T cell receptors (TCRs) and IL-6 trans-signaling.[47,50,51] The result is a rapid upregulation of the effector functions of LSEC-primed CD8 T cells.

LSECs can induce naive CD4 T cells to produce interferon (IFN)-γ, interleukin (IL)-4 and IL-10, but cannot completely differentiate CD4 T cells into Th1 or Th2 phenotypes.[52,53] Conversely, direct contact with LSECs inhibits the Th1 and Th17 functions of CD4 T cells[54] and suppresses the autoreactive CD4+ recent thymic emigrant (RTE) lymphocytes.[55] The LSEC is the most efficient hepatic cell in inducing differentiation of CD4 T naive cells into CD25 + Foxp3+regulatory T cells (Tregs) in the presence and absence of exogenous TGF-β[56] LSECs also induce CD4+ RTEs differentiation into LAG3 + Fox3- type 1 regulatory T (Tr1) cells.[55] The LSEC is reportedly the only nonparenchymal cell that binds latent tumor growth factor (TGF)-β (LAP/TGF-β), which suggests that LSECs can recruit TGF-β from external sources to activate Tregs.[56] The tolerance induced by LSEC-mediated Treg activation has been shown to ameliorate experimental autoimmune encephalomyelitis and autoimmune hepatitis.[57,58]

Circulating T cells physically interact with LSECs in vivo.[54] For active recruitment, LSECs must be preactivated by IFN-γ and TNF-α[59] Leukocytes can migrate across endothelial borders by either paracellular or transcellular migration. Transendothelial migration of CD8 and CD4 T cells across LSECs uses the transcellular route (i.e., directly penetrates the endothelial cell body),[59,60] which is observed less frequently in human umbilical vein endothelial cells (HUVECs). Surface expression of CD31 (PECAM-1) facilitates transcellular migration in HUVECs.[61] In addition, ICAM-1, VCAM-1, and stabilin-1 also participate in transcellular migration in LSECs.[59,62] LSECs not only produce, but also internalize several chemokines involved in T cell recruitment, such as CXCL12 (stromal cell

derived factor 1 [sdf1]) and CXCL10.[63] Th1 and Th2 lymphocytes depend on CXCL9 and CXCL12 to transmigrate, while CXCL10 favors the transmigration of effector/memory T cells.[63,64] CXCR3 is involved in the transmigration of Tregs and Th17 cells through the hepatic sinusoids under inflammatory conditions.[62,65] In addition to T cells, neutrophils transmigrate across LSEC monolayers, and this process requires LFA-1, Mac-1, CD44, and integrin β2.[66] Adhesion and transmigration of $CD16^+$ monocytes across inflamed hepatic endothelium depends on CX_3CL1 and vascular adhesion protein (VAP)-1.[67] Although the transmigration of CD8 T effector cells through LSECs has been described in liver graft rejection,[60] naive LSECs can induce liver allograft tolerance by suppressing polyclonal expansion of allospecific T cells via Fas ligand (FasL) signaling.[68]

Much of the work described in this section on adaptive immunity comes from two groups of investigators. We present the data they have published, but add two caveats for the readers to be aware of. The cells used were isolated by either CD146 or CD31 immunomagnetic selection; the readers are referred to the section on surface markers used for immunomagnetic selection later in this article to review the limitations of these methods. Many of the studies described were performed in cells after long-term culture (3 to 5 days), and identity and purity of the cells after long-term culture were not reported; concerns about long-term culture have been raised elsewhere[1,4] and are discussed later in this review.

LSEC Interactions with Tumor Metastases

Two C-type lectins, **D**endritic **C**ell-**S**pecific **I**ntercellular adhesion molecule-3-**G**rabbing **N**onintegrin (DC-SIGNR) and liver sinusoidal endothelial cell lectin (LSECtin), are coexpressed on LSECs.[69] Colon cancer cells adhere to LSECtin in vivo and in vitro.[69] In vitro, DC-SIGNR promotes adhesion, migration, and invasion of colon cancer cell lines.[70] In vivo, knocking down DC-SIGNR decreases hepatic metastatic potential of colon cancer and improves survival in mice.[70] Serum-soluble LSECtin and DC-SIGNR are higher in patients with colon cancer with metastases than in those without,[70,71] although the scatter plot shows overlap of values between the two populations. Thus, serum levels may not be a prognostic marker, but therapeutic manipulation of expression of one or both of these lectins on the LSEC surface might be used to reduce colon cancer metastases and perhaps metastases from other cancers.

LSECs as Drivers of Liver Regeneration

It is well established that LSECs are an important driver of liver regeneration,[72–81] at least in part by release of hepatocyte growth factor (HGF), Wnt2, and angiopoietin-2 from LSECs.[72,78,82] However, our laboratory has now accumulated substantial evidence that it is not mature LSECs that drive liver regeneration, but rather that bone marrow (BM)-derived **s**inusoidal endothelial cell **pro**genitor **c**ells or sprocs drive this process. These BM sprocs repair lost or injured LSECs, leading to engraftment of a substantial number of BM sprocs and BM-derived LSECs after injury or partial hepatectomy.[78–81,83] BM sprocs are of the same size and share surface markers with LSECs, and thus sprocs are isolated along with LSECs, which might explain why studies attribute promotion of liver regeneration to mature LSECs. Recruitment of BM sprocs to the liver occurs through signaling by vascular endothelial growth factor (VEGF)-stromal cell derived factor 1 (sdf1).[78,80] Sdf1 is a

chemokine with two receptors, CXCR4 (C-X-C chemokine receptor type 4) and CXCR7. After partial hepatectomy, CXCR4 is expressed on resident LSECs (i.e., not bone marrow derived), whereas CXCR7 is expressed on BM sprocs in the circulation and engrafted in the liver (as well as on resident sprocs). Thus, VEGF-sdf-1 signaling, either after liver injury or partial hepatectomy, recruits CXCR7+ BM sprocs. Ablation of CXCR7+ endothelial cells in the body impairs liver regeneration,[84] consistent with the role of CXCR7+ sprocs in liver regeneration. Irradiation of 40% of the bone marrow impairs liver regeneration by 40%, but infusion of either whole bone marrow or sprocs on day 1 after partial hepatectomy restores hepatocyte proliferation and completely normalizes liver regeneration in the bone marrow-irradiated rats.[81]

It has been reported that the VEGF/VEGFR2 pathway upregulates Id1, a member of the helix-loop-helix transcription family, in LSECs after partial hepatectomy. After partial hepatectomy, HGF and Wnt2 are not upregulated in LSECs from Id1-knockout mice. LSECs from Id1-knockout mice do not drive liver regeneration, and this is restored when LSECs isolated from wild-type mice after partial hepatectomy are transplanted into the Id1-knockout mice.[77] The authors conclude that the VEGF/VEGFR2 pathway drives liver regeneration through an Id1-dependent pathway in LSECs. BM sprocs are the subpopulation of endothelial progenitor cells that become LSECs. Id1 promotes migration and regulates proliferation of endothelial progenitor cells through a Wnt2-dependent mechanism.[85–87] To reconcile the Id1 data described above with our findings, we suggest the following alternate interpretation of the data. The VEGF/VEGFR2 pathway recruits BM sprocs, which have Id1-dependent proliferation. Id1-knockout mice have impaired recruitment of BM sprocs because of impaired migration and proliferation of BM sprocs.[85–87] HGF and Wnt2 are not upregulated in the Id1-knockout mice because the BM sprocs that express HGF and Wnt2 are not recruited after partial hepatectomy. More detailed studies will be needed to determine which interpretation is correct.

As noted above, suppression of BM sprocs impairs liver regeneration. A clinical implication of this is that any disease or iatrogenic intervention that suppresses bone marrow sprocs would impair liver regeneration. Indeed, suppression of bone marrow sprocs is part of the pathophysiology of sinusoidal obstruction syndrome (formerly known as hepatic venoocclusive disease).[83]

LSECs as Determinants of Hepatic Fibrosis

Prior to fibrosis, there is a characteristic change in the LSEC barrier that has been termed capillarization.[88] Ultrastructurally, capillarization is a lack of fenestration and development of a more organized basement membrane. Capillarization precedes fibrosis in alcoholic liver disease in humans,[89,90] in nonalcoholic fatty liver disease in rats,[29] and in experimental cirrhosis in rats.[91] It is not known whether capillarization occurs in biliary fibrosis. In vitro studies with LSECs cocultured with HSCs demonstrated that LSECs from normal liver prevented the activation of HSCs and promoted reversion to quiescence of activated stellate cells.[92] In contrast, when hepatic stellates were cocultured with capillarized LSECs, the LSECs did not prevent HSC activation or promote reversion to quiescence. Thus, these in vitro studies demonstrate that normal LSECs act as a gatekeeper to HSC activation. As

LSECs capillarize prior to fibrosis, the gatekeeper function is lost and this is permissive for HSC activation.

The completely fenestrated LSEC phenotype is maintained by two VEGF-dependent pathways: the VEGF-endogenous nitric oxide synthase (eNOS)-soluble guanylate cyclase (sGC)-cGMP pathway plus an undefined VEGF-dependent, nitric oxide (NO)-independent pathway.[93,94] eNOS activity is impaired in LSECs from cirrhotic liver.[95,96] In cirrhotic rats, restoration of LSEC fenestration can be accelerated by administration of an sGC activator,[93] which restores the VEGF-eNOS-sGC-cGMP pathway downstream of eNOS. Normalization of the LSEC phenotype with the sGC activator promotes stellate cell quiescence and accelerates regression of cirrhosis. In a second approach, when early cirrhosis is induced, followed by administration of an sGC activator concurrent with continued administration of a cirrhosis-inducing toxin, fenestration normalizes, stellate cell quiescence is induced, and progression of cirrhosis is prevented. The effect of the sGC activator is not a direct effect on activated HSCs, which lack the α1β1 heterodimeric sGC required for an effect by the sGC activator.[97] These in vivo studies confirm the in vitro finding that normal LSECs promote HSC quiescence, but add the finding that therapy that induces a normal LSEC phenotype accelerates regression of cirrhosis after cessation of injury and prevents progression despite an ongoing injury.

It is as yet unreported what the LSEC mediator is that promotes HSC quiescence. We and others had reported that the mediator was NO,[92,98] but we later demonstrated that this was a misinterpretation of the data: Blocking NO production in LSEC–HSC coculture does prevent the LSEC effect on HSC activation, but this is because LSECs capillarize and cannot maintain HSC quiescence rather than a direct effect on the HSC.[93] The transcription factor, Kruppel-Like Factor (klf2) in LSECs is needed for the paracrine, or so-called angiocrine, gatekeeper effect of LSECs, but the target of klf2 is, as yet, unknown.[98,99]

It has been reported that LSECs isolated from a bile duct ligation model actively promoted HSC activation through secretion of EIIIA-fibronectin,[100] but subsequent studies did not find an effect of EIIIA-fibronectin on HSC activation in vitro or in vivo in knockout mice.[101] Thus, capillarization of LSECs is permissive for HSC activation, but it is not clear whether capillarization actively promotes HSC activation.

Knockout of CXCR4+ on endothelial cells in the body reduces fibrosis on day 21 after bile duct ligation.[84] The article that reported this did not determine which endothelial cells expressed CXCR4 in fibrosis, and it is therefore difficult to discern what the possible mechanism of fibrosis reduction might be. Resident LSECs express CXCR4 after partial hepatectomy in normal liver,[78] but it is not known which LSECs express CXCR4 in fibrotic liver. It is controversial to what degree portal fibroblasts contribute to fibrosis after bile duct ligation.[102–104] If portal fibroblasts are important for fibrosis in bile duct ligation, it would be interesting to determine whether there is crosstalk between endothelial cells and portal fibroblasts analogous to the crosstalk between LSECs and HSCs, which endothelial cells are involved (large vessel endothelial cells or LSECs), and whether these endothelial cells are CXCR4 +.

Isolation, Characterization, and Culture of LSEC Isolates: An Ongoing Concern

There has been growing concern worldwide about lack of reproducibility of published data. To address this issue in the United States, the National Institutes of Health (NIH) grant applications now require a discussion of scientific rigor and transparency in study design and methods. In the opinion of these authors, the field of LSEC research would benefit from standardized requirements for characterizing isolated LSECs, as well as greater attention to LSECs maintained in culture.

LSECs are a small fraction of cells within the liver and isolations can easily become contaminated by other cell types, in particular large vessel endothelial cells, lymphatic endothelial cells, HSCs, fibroblasts, dendritic cells, and even natural killer (NK) cells. LSECs have very distinct physiology, so that even contamination by other endothelial cells might alter experimental outcomes. Furthermore, there is heterogeneity in the subpopulations of LSECs and some isolation techniques are unlikely to capture certain subpopulations of the cells. Unlike the greater standardization of isolation techniques for hepatocytes and HSCs, the complexity of isolating LSECs has led to a plethora of techniques. Unfortunately, some of the methods for LSEC isolation that have been developed in the past 15 years have not been completely validated or are controversial.

Some of the LSEC cell lines that have been developed have shown to have fenestration, although the degree of fenestration varies and none have completely normal fenestration (see “Identity” below). Only the SK Hep1 cell line has been shown to take up FITC-FSA (see “Purity” below).[105] Given the heterogeneity of LSECs, experimental outcomes may also be affected by which type of LSEC has been immortalized. Details of the features of human and rodent LSEC cell lines were recently compared and contrasted in tabular form.[2]

The issue of transparency is pertinent to companies that sell human LSECs. Companies that sell human LSECs do not publish their method of isolation, and to the best of our knowledge, there are no published data that demonstrate that these cells show ultrastructural features of LSECs or have been validated properly with a functional assay. Given the pitfalls in isolation and identification of LSECs, this is a breach of transparency. It would be desirable to require that investigators who wish to use these commercially obtained cells either validate the identity and purity of these cells themselves or be able to refer to a publication that has done so. Given the concerns about nonvalidated isolation methods, transparency is also a concern for articles that do not provide details of their method of isolation or that refer to earlier articles that also do not provide details.

The three major criteria to judge the validity of a method of LSEC isolation should be identity, purity, and lack of selection bias. In these authors’ opinion, demonstrating identity and purity of an isolation method is absolutely essential to prove methodological rigor. Lack of selection bias is desirable for many if not most types of studies. For example, it would simply not be accurate to state that LSECs do not express a given protein when a preparation does not contain periportal LSECs.

Identity

Any method to isolate LSECs must be shown to yield cells with well-developed sieve plates, i.e., around 7 to 25 fenestrae per sieve plate on scanning electron microscopy (SEM). This remains the gold standard to demonstrate that at least some of the cells being isolated are LSECs. Although it is possible to scan large number of cells by SEM, this is extremely time intensive. SEM is therefore rarely used to assess purity.

Purity

The gold standard for assessing purity is a functional assay with rapid uptake of an LSEC specific ligand at a low concentration of ligand. In the past, our laboratory assessed purity based on uptake over 2 hours of di-acetylated LDL with exclusion of Kupffer cells based on a peroxidase stain. However, endothelial cells,[106] Kupffer cells,[107,108] dendritic cells,[109] and NK cells[110] take up di-acetylated LDL (di-Ac-LDL) after a lengthy incubation time (hours), so this assay does not demonstrate that liver cells are LSECs. Di-Ac-LDL might be used to distinguish LSECs from other liver cells if used at a low concentration and short incubation time; however, di-Ac-LDL is prone to aggregation,[3] not very stable, and there is no published and validated protocol. In recent years, our laboratory has determined purity based on the percentage of cells able to take up FITC-labeled formaldehyde-treated serum albumin (FITC-FSA), 0.5 μg/mL FITC-FSA in medium over 10 minutes at 37°C, as described by the laboratory of Bard Smedsrod.[3] Other ligands specific for LSECs could also be used to develop a similar functional assay of purity.

Most laboratories that report the purity of their isolations have used immunofluorescence to detect cell surface markers.[111] To the best of our knowledge, no published study has validated that the presence of any given surface marker or combination thereof is specific as a test of LSEC purity. Since CD32b is present on myeloid dendritic cells and lymphatic vessel endothelial hyaluronic acid receptor 1 (LYVE-1) is present on lymphatic endothelial cells, these are not ideal tools to confirm purity. Periportal LSECs lack CD32b and LYVE-1 (see section on “selection bias”), so that LSEC isolates with high purity based on staining for either of these ligands likely lack periportal LSECs. Stabilin-2 would appear to be the most promising candidate for establishment of purity.

Of note, some published studies isolated LSECs by immunomagnetic selection for an epitope and based their determination of purity on that same epitope; although this approach demonstrated that the selection technique worked, it did not prove purity. Until the specificity of immunofluorescence has been validated, uptake of FITC-FSA or another LSEC-specific ligand should be considered the gold standard for demonstrating purity.

Selection Bias

LSEC phenotype varies across the lobule by size, number, and size of fenestration, expression of intracellular and surface markers, expression of lectins and von Willebrand factor, endocytosis, and susceptibility to acetaminophen toxicity.[112–120] Given the marked heterogeneity of LSECs across the lobule, isolation techniques for LSECs would optimally isolate cells without a selection bias for certain subpopulations. It should be concerning that

isolation methods that do not include periportal LSECs would exclude one-third of LSECs with unique characteristics.

A recent immunofluorescence study in human liver demonstrated that periportal LSECs lack CD32, LYVE-1, and ICAM-1,[114] epitopes formerly thought to be present on all LSECs. In our laboratory, studies of LSECs obtained by elutriation found that only 65% of isolated rat LSECs were CD32b (FcγRIIb2) positive and 70% were LYVE-1 positive (unpublished observation). Given that around 30% of LSECs have the ultrastructural features of periportal LSECs,[112] our findings for CD32b and LYVE-1 are quantitatively consistent with the aforementioned immunofluorescence study. Immunomagnetic separation methods that select positively for CD32b or LYVE-1 might not be expected to isolate periportal LSECs, and validation of an LSEC isolation technique based on the presence of these epitopes would not identify the absence of periportal LSECs.

Elutriation will yield around 9 to 10 million LSECs per mouse liver or 80 to 100 million LSECs per rat liver and isolates LSECs from all regions.[112] Until a better method is developed to demonstrate an unbiased selection of LSECs, a similar yield of LSECs from rodents would seem to be a reasonable surrogate marker to rule out selection bias of subpopulations. For methods that cannot obtain a high yield or for LSEC isolation from other species, the next best option would be to demonstrate by scanning electron microscopy that the isolation procedure yields cells with the same variability in the number of fenestrae/sieve plate as is seen in periportal and centrilobular LSECs.

Cell Isolation Approaches

The two most long-standing and best-characterized methods of LSEC isolation are elutriation and Percoll separation with selective adherence. A modified method of elutriation was described by Steffan et al,[82] and an updated, more detailed method was described subsequently.[121] For mouse isolation by Percoll with selective adherence, the best description is on an accessible web site (http://hdl.handle.net/10037/4575, University of Trømso, Norway).[122] The method for rat LSEC isolation by Percoll with selective adherence can be found in a publication available on PubMed,[123] but a more elaborate description of the method has also been published.[124] Detailed protocols for these methods are also posted on the web site of the International Society for Hepatic Sinusoidal Research, but access is for members only.

Protocols that use immunomagnetic separation have lower yields than elutriation or Percoll with selective adherence. This increases the concern that these methods might isolate subpopulations. CD31, CD146, CD32b, CD105, and stabilin-2 have been used as surface antigens for this approach.

CD31—It has been reported that LSECs from normal liver do not express CD31 on their cell surface.[94] On the other hand, vascular and lymphatic endothelial cells in the liver do express CD31 on their cell surface. The majority of endothelial cells isolated by CD31 immunomagnetic selection have decreased fenestration and do not resemble the cells that line most of the sinusoid (see published images[121,125]). There is a short transitional area of LSECs proximal to the origins of central venules where most of the sinusoids are converging

and enter at angles less than 90 degrees to form the central venules. These transitional LSECs are underfenestrated[126] (also Dr Robert S. McCuskey, personal communication) and resemble the published scanning electron microscopy (EM) images of cells isolated by CD31 immunomagnetic separation.[121,125] Given the reports that CD31 is not expressed on the LSEC surface but is present on other endothelial cells in the liver, verification of identity and purity of cells isolated by CD31 immunomagnetic separation is crucial.

CD146—CD146 (muc-18) is not specific for LSECs but is widely expressed on endothelial cells[127,128] and is also present on NK cells.[129] Within the liver, CD146 is expressed on endothelial cells of the hepatic vasculature, vascular pericytes, and HSCs.[114]

CD32b (FcγRIIb2)—One of the first antibodies developed to LSECs was anti-SE-1. SE-1 was subsequently identified to be FcγRIIb2.[130] As noted above, CD32b is not present on periportal LSECs. CD32b is also expressed on myeloid dendritic cells.[131]

CD105 (endoglin)—There are a few publications that have isolated LSECs based on the presence of CD105. There is no consistency in the literature on whether CD105 is expressed only on periportal LSECs and vascular endothelial cells,[132] not on LSECs from normal liver, [133] or on all endothelial cells and HSCs.[134]

Stabilin-2—Immunomagnetic separation with stabilin-2[135] would seem to be a promising approach. LSECs isolated by stabilin-2 are reportedly more than 95% LYVE-1 +.[135] Given that periportal LSECs are LYVE-1 −, it should therefore be determined whether immunomagnetic separation for stabilin-2 isolates periportal LSECs.

ICAM-1—Some laboratories have required further immunomagnetic selection after Percoll with selective adherence to remove Kupffer cells. As noted above, ICAM-1 may be absent in periportal LSECs,[114] and positively selecting for ICAM-1 may therefore not be an optimal strategy.

CD45—Laboratories have improved the purity of their preparations by Percoll separation with selective adherence by negative selection for CD45 to remove Kupffer cells. CD45 is highly expressed on periportal LSECs, has low expression on midlobular LSECs, and is not expressed on centrilobular LSECs.[112] Therefore, negative selection for CD45 may remove periportal and midlobular LSECs.

Culture of LSECs

It is well established in studies of hepatocytes that hepatocytes lose their characteristic phenotype after the first days in standard culture, and long-term hepatocyte culture is generally not acceptable. Similarly, LSECs only maintain fenestration for up to 2 days in culture[94] and endocytotic activity declines rapidly.[1] In contrast to the loss of the characteristic ultra-structural features and functions, light microscopy appearance remains unchanged for several days and cells maintain the ability to take up di-Ac-LDL, albeit with uptake assessed over hours.[136] Thus, assessment by light microscopy and uptake of di-Ac-LDL should not be acceptable surrogates for demonstrating the identity, purity, and functional status of LSECs in long-term culture. Unlike hepatocytes, where the known loss

of phenotype has made it generally unacceptable to conduct studies in long-term culture, studies are published with LSECs maintained in long-term culture. If investigators feel that their method of culture maintains LSEC phenotype for more than 2 days, it would be helpful to document that the cells still have the phenotypic features of LSECs at the end of the experiment. It is also controversial whether or not LSECs proliferate in culture. This may be particularly true for culture with higher concentrations of fetal calf serum, which are reportedly toxic after 48 hours.[136] It would be useful if studies that culture LSECs to confluence or that use passaged LSECs document that the cells present at the end of experimentation are still LSECs and not contaminated by cells that proliferate more rapidly.

Conclusion

LSECs play an important role in liver toxicity, “waste management,” immunology, cancer metastases, liver regeneration, and liver fibrosis. The field has flourished with the influx of productive investigation. However, given the heterogeneity of the methods of isolation and characterization of the populations of cells isolated, continued growth in the field is likely to lead to ever-increasing difficulties in reconciling results from different laboratories. To permit comparison of studies from different laboratories and have results that will stand the test of time, LSEC research would benefit from common standards that form the basis for reviewing research in our major hepatology journals.

References

1. Elvevold K, Smedsrød B, Martinez I. The liver sinusoidal endothelial cell: a cell type of controversial and confusing identity. Am J Physiol Gastrointest Liver Physiol. 2008; 294(02):G391–G400. [PubMed: 18063708]
2. Poisson J, Lemoinne S, Boulanger C, et al. Liver sinusoidal endothelial cells: physiology and role in liver diseases. J Hepatol. 2017; 66(01):212–227. [PubMed: 27423426]
3. Sørensen KK, Simon-Santamaria J, McCuskey RS, Smedsrød B. Liver sinusoidal endothelial cells. Compr Physiol. 2015; 5(04):1751–1774. [PubMed: 26426467]
4. Sørensen KK, McCourt P, Berg T, et al. The scavenger endothelial cell: a new player in homeostasis and immunity. Am J Physiol Regul Integr Comp Physiol. 2012; 303(12):R1217–R1230. [PubMed: 23076875]
5. Breiner KM, Schaller H, Knolle PA. Endothelial cell-mediated uptake of a hepatitis B virus: a new concept of liver targeting of hepatotropic microorganisms. Hepatology. 2001; 34(4 Pt 1):803–808. [PubMed: 11584379]
6. Ganesan LP, Mohanty S, Kim J, Clark KR, Robinson JM, Anderson CL. Rapid and efficient clearance of blood-borne virus by liver sinusoidal endothelium. PLoS Pathog. 2011; 7(09):e1002281. [PubMed: 21980295]
7. Mates JM, Yao Z, Cheplowitz AM, et al. Mouse liver sinusoidal endothelium eliminates hiv-like particles from blood at a rate of 100 million per minute by a second-order kinetic process. Front Immunol. 2017; 8:35. [PubMed: 28167948]
8. Simon-Santamaria J, Rinaldo CH, Kardas P, et al. Efficient uptake of blood-borne BK and JC polyomavirus-like particles in endothelial cells of liver sinusoids and renal vasa recta. PLoS One. 2014; 9(11):e111762. [PubMed: 25375646]
9. Wisse E. An electron microscopic study of the fenestrated endothelial lining of rat liver sinusoids. J Ultrastruct Res. 1970; 31(01):125–150. [PubMed: 5442603]
10. Fraser R, Bosanquet AG, Day WA. Filtration of chylomicrons by the liver may influence cholesterol metabolism and atherosclerosis. Atherosclerosis. 1978; 29(02):113–123. [PubMed: 206268]

11. Cogger VC, Hilmer SN, Sullivan D, Muller M, Fraser R, Le Couteur DG. Hyperlipidemia and surfactants: the liver sieve is a link. Atherosclerosis. 2006; 189(02):273–281. [PubMed: 16458315]
12. Herrnberger L, Hennig R, Kremer W, et al. Formation of fenestrae in murine liver sinusoids depends on plasmalemma vesicle-associated protein and is required for lipoprotein passage. PLoS One. 2014; 9(12):e115005. [PubMed: 25541982]
13. LE Couteur DG, Cogger VC, McCuskey RS, et al. Age-related changes in the liver sinusoidal endothelium: a mechanism for dyslipidemia. Ann N Y Acad Sci. 2007; 1114:79–87. [PubMed: 17804522]
14. Steinberg D, Witztum JL. Oxidized low-density lipoprotein and atherosclerosis. Arterioscler Thromb Vasc Biol. 2010; 30(12):2311–2316. [PubMed: 21084697]
15. Li R, Oteiza A, Sørensen KK, et al. Role of liver sinusoidal endothelial cells and stabilins in elimination of oxidized low-density lipoproteins. Am J Physiol Gastrointest Liver Physiol. 2011; 300(01):G71–G81. [PubMed: 21030611]
16. Hurtado-Roca Y, Bueno H, Fernandez-Ortiz A, et al. Oxidized LDL is associated with metabolic syndrome traits independently of central obesity and insulin resistance. Diabetes. 2017; 66(02): 474–482. [PubMed: 27993926]
17. Houben T, Brandsma E, Walenbergh SM, Hofker MH, Shiri-Sverdlov R. Oxidized LDL at the crossroads of immunity in non-alcoholic steatohepatitis. Biochim Biophys Acta. 2017; 1862(04): 416–429. [PubMed: 27472963]
18. Krieger M. Charting the fate of the “good cholesterol”: identification and characterization of the high-density lipoprotein receptor SR-BI. Annu Rev Biochem. 1999; 68:523–558. [PubMed: 10872459]
19. Ganesan LP, Mates JM, Cheplowitz AM, et al. Scavenger receptor B1, the HDL receptor, is expressed abundantly in liver sinusoidal endothelial cells. Sci Rep. 2016; 6:20646. [PubMed: 26865459]
20. van der Steeg WA, Holme I, Boekholdt SM, et al. High-density lipoprotein cholesterol, high-density lipoprotein particle size, and apolipoprotein A-I: significance for cardiovascular risk: the IDEAL and EPIC-Norfolk studies. J Am Coll Cardiol. 2008; 51(06):634–642. [PubMed: 18261682]
21. Bowe B, Xie Y, Xian H, Balasubramanian S, Zayed MA, Al-Aly Z. High density lipoprotein cholesterol and the risk of all-cause mortality among U.S. veterans. Clin J Am Soc Nephrol. 2016; 11(10):1784–1793. [PubMed: 27515591]
22. März W, Kleber ME, Scharnagl H, et al. HDL cholesterol: reappraisal of its clinical relevance. Clin Res Cardiol. 2017; 106:663–675. [PubMed: 28342064]
23. Park JK, Utsumi T, Seo YE, et al. Cellular distribution of injected PLGA-nanoparticles in the liver. Nanomedicine (Lond). 2016; 12(05):1365–1374.
24. Warren A, Cogger VC, Arias IM, McCuskey RS, Le Couteur DG. Liver sinusoidal endothelial fenestrations in caveolin-1 knockout mice. Microcirculation. 2010; 17(01):32–38. [PubMed: 20141598]
25. Steffan AM, Gendrault JL, Kirn A. Increase in the number of fenestrae in mouse endothelial liver cells by altering the cytoskeleton with cytochalasin B. Hepatology. 1987; 7(06):1230–1238. [PubMed: 3679088]
26. Wisse E, Jacobs F, Topal B, Frederik P, De Geest B. The size of endothelial fenestrae in human liver sinusoids: implications for hepatocyte-directed gene transfer. Gene Ther. 2008; 15(17):1193–1199. [PubMed: 18401434]
27. Mathison JC, Ulevitch RJ. The clearance, tissue distribution, and cellular localization of intravenously injected lipopolysaccharide in rabbits. J Immunol. 1979; 123(05):2133–2143. [PubMed: 489976]
28. Yao Z, Mates JM, Cheplowitz AM, et al. Blood-borne lipopolysaccharide is rapidly eliminated by liver sinusoidal endothelial cells via high-density lipoprotein. J Immunol. 2016; 197(06):2390–2399. [PubMed: 27534554]
29. DeLeve LD, Wang X, Kanel GC, Atkinson RD, McCuskey RS. Prevention of hepatic fibrosis in a murine model of metabolic syndrome with nonalcoholic steatohepatitis. Am J Pathol. 2008; 173(04):993–1001. [PubMed: 18772330]

30. Harte AL, da Silva NF, Creely SJ, et al. Elevated endotoxin levels in non-alcoholic fatty liver disease. J Inflamm (Lond). 2010; 7(01):15. [PubMed: 20353583]
31. Michelena J, Altamirano J, Abraldes JG, et al. Systemic inflammatory response and serum lipopolysaccharide levels predict multiple organ failure and death in alcoholic hepatitis. Hepatology. 2015; 62(03):762–772. [PubMed: 25761863]
32. Bigatello LM, Broitman SA, Fattori L, et al. Endotoxemia, encephalopathy, and mortality in cirrhotic patients. Am J Gastroenterol. 1987; 82(01):11–15. [PubMed: 3799574]
33. Lin RS, Lee FY, Lee SD, et al. Endotoxemia in patients with chronic liver diseases: relationship to severity of liver diseases, presence of esophageal varices, and hyperdynamic circulation. J Hepatol. 1995; 22(02):165–172. [PubMed: 7790704]
34. Ganesan LP, Kim J, Wu Y, et al. FcγRIIb on liver sinusoidal endothelium clears small immune complexes. J Immunol. 2012; 189(10):4981–4988. [PubMed: 23053513]
35. Mousavi SA, Sporstøl M, Fladeby C, Kjeken R, Barois N, Berg T. Receptor-mediated endocytosis of immune complexes in rat liver sinusoidal endothelial cells is mediated by FcgammaRIIb2. Hepatology. 2007; 46(03):871–884. [PubMed: 17680646]
36. Areschoug T, Gordon S. Scavenger receptors: role in innate immunity and microbial pathogenesis. Cell Microbiol. 2009; 11(08):1160–1169. [PubMed: 19388903]
37. Gazi U, Martinez-Pomares L. Influence of the mannose receptor in host immune responses. Immunobiology. 2009; 214(07):554–561. [PubMed: 19162368]
38. Martin-Armas M, Simon-Santamaria J, Pettersen I, Moens U, Smedsrød B, Sveinbjørnsson B. Toll-like receptor 9 (TLR9) is present in murine liver sinusoidal endothelial cells (LSECs) and mediates the effect of CpG-oligonucleotides. J Hepatol. 2006; 44(05):939–946. [PubMed: 16458386]
39. Uhrig A, Banafsche R, Kremer M, et al. Development and functional consequences of LPS tolerance in sinusoidal endothelial cells of the liver. J Leukoc Biol. 2005; 77(05):626–633. [PubMed: 15860798]
40. Wu J, Meng Z, Jiang M, et al. Toll-like receptor-induced innate immune responses in non-parenchymal liver cells are cell type-specific. Immunology. 2010; 129(03):363–374. [PubMed: 19922426]
41. Boaru SG, Borkham-Kamphorst E, Tihaa L, Haas U, Weiskirchen R. Expression analysis of inflammasomes in experimental models of inflammatory and fibrotic liver disease. J Inflamm (Lond). 2012; 9(01):49. [PubMed: 23192004]
42. Schurich A, Berg M, Stabenow D, et al. Dynamic regulation of CD8 T cell tolerance induction by liver sinusoidal endothelial cells. J Immunol. 2010; 184(08):4107–4114. [PubMed: 20212092]
43. Rubinstein D, Roska AK, Lipsky PE. Liver sinusoidal lining cells express class II major histocompatibility antigens but are poor stimulators of fresh allogeneic T lymphocytes. J Immunol. 1986; 137(06):1803–1810. [PubMed: 3489042]
44. Lohse AW, Knolle PA, Bilo K, et al. Antigen-presenting function and B7 expression of murine sinusoidal endothelial cells and Kupffer cells. Gastroenterology. 1996; 110(04):1175–1181. [PubMed: 8613007]
45. Limmer A, Ohl J, Kurts C, et al. Efficient presentation of exogenous antigen by liver endothelial cells to CD8+T cells results in antigen-specific T-cell tolerance. Nat Med. 2000; 6(12):1348–1354. [PubMed: 11100119]
46. Lalor PF, Edwards S, McNab G, Salmi M, Jalkanen S, Adams DH. Vascular adhesion protein-1 mediates adhesion and transmigration of lymphocytes on human hepatic endothelial cells. J Immunol. 2002; 169(02):983–992. [PubMed: 12097405]
47. Diehl L, Schurich A, Grochtmann R, Hegenbarth S, Chen L, Knolle PA. Tolerogenic maturation of liver sinusoidal endothelial cells promotes B7-homolog 1-dependent CD8+ T cell tolerance. Hepatology. 2008; 47(01):296–305. [PubMed: 17975811]
48. Limmer A, Ohl J, Wingender G, et al. Cross-presentation of oral antigens by liver sinusoidal endothelial cells leads to CD8 T cell tolerance. Eur J Immunol. 2005; 35(10):2970–2981. [PubMed: 16163670]
49. Berg M, Wingender G, Djandji D, et al. Cross-presentation of antigens from apoptotic tumor cells by liver sinusoidal endothelial cells leads to tumor-specific CD8+ T cell tolerance. Eur J Immunol. 2006; 36(11):2960–2970. [PubMed: 17039564]

50. Böttcher JP, Schanz O, Garbers C, et al. IL-6 trans-signaling-dependent rapid development of cytotoxic CD8+ T cell function. Cell Reports. 2014; 8(05):1318–1327. [PubMed: 25199826]
51. Wittlich M, Dudek M, Böttcher JP, et al. Liver sinusoidal endothelial cell cross-priming is supported by CD4 T cell-derived IL-2. J Hepatol. 2017; 66(05):978–986. [PubMed: 28025060]
52. Knolle PA, Uhrig A, Hegenbarth S, et al. IL-10 down-regulates T cell activation by antigen-presenting liver sinusoidal endothelial cells through decreased antigen uptake via the mannose receptor and lowered surface expression of accessory molecules. Clin Exp Immunol. 1998; 114(03):427–433. [PubMed: 9844054]
53. Knolle PA, Germann T, Treichel U, et al. Endotoxin down-regulates T cell activation by antigen-presenting liver sinusoidal endothelial cells. J Immunol. 1999; 162(03):1401–1407. [PubMed: 9973395]
54. Carambia A, Frenzel C, Bruns OT, et al. Inhibition of inflammatory CD4 T cell activity by murine liver sinusoidal endothelial cells. J Hepatol. 2013; 58(01):112–118. [PubMed: 22989568]
55. Xu X, Jin R, Li M, et al. Liver sinusoidal endothelial cells induce tolerance of autoreactive CD4+ recent thymic emigrants. Sci Rep. 2016; 6:19861. [PubMed: 26794038]
56. Carambia A, Freund B, Schwinge D, et al. TGF-β-dependent induction of $CD4^{+}CD25^{+}Foxp3^{+}$ Tregs by liver sinusoidal endothelial cells. J Hepatol. 2014; 61(03):594–599. [PubMed: 24798620]
57. Carambia A, Freund B, Schwinge D, et al. Nanoparticle-based autoantigen delivery to Treg-inducing liver sinusoidal endothelial cells enables control of autoimmunity in mice. J Hepatol. 2015; 62(06):1349–1356. [PubMed: 25617499]
58. Kruse N, Neumann K, Schrage A, et al. Priming of CD4+ T cells by liver sinusoidal endothelial cells induces CD25low forkhead box protein 3- regulatory T cells suppressing autoimmune hepatitis. Hepatology. 2009; 50(06):1904–1913. [PubMed: 19787806]
59. Patten DA, Wilson GK, Bailey D, et al. Human liver sinusoidal endothelial cells promote intracellular crawling of lymphocytes during recruitment: A new step in migration. Hepatology. 2017; 65(01):294–309. [PubMed: 27770554]
60. Kariya T, Ueta H, Xu XD, et al. Direct evidence for activated CD8+ T cell transmigration across portal vein endothelial cells in liver graft rejection. J Gastroenterol. 2016; 51(10):985–998. [PubMed: 26891909]
61. Mamdouh Z, Mikhailov A, Muller WA. Transcellular migration of leukocytes is mediated by the endothelial lateral border recycling compartment. J Exp Med. 2009; 206(12):2795–2808. [PubMed: 19887395]
62. Oo YH, Weston CJ, Lalor PF, et al. Distinct roles for CCR4 and CXCR3 in the recruitment and positioning of regulatory T cells in the inflamed human liver. J Immunol. 2010; 184(06):2886–2898. [PubMed: 20164417]
63. Neumann K, Erben U, Kruse N, et al. Chemokine Transfer by liver sinusoidal endothelial cells contributes to the recruitment of CD4+ T cells into the murine liver. PLoS One. 2015; 10(06):e0123867. [PubMed: 26052942]
64. Schrage A, Wechsung K, Neumann K, et al. Enhanced T cell transmigration across the murine liver sinusoidal endothelium is mediated by transcytosis and surface presentation of chemokines. Hepatology. 2008; 48(04):1262–1272. [PubMed: 18697212]
65. Oo YH, Banz V, Kavanagh D, et al. CXCR3-dependent recruitment and CCR6-mediated positioning of Th-17 cells in the inflamed liver. J Hepatol. 2012; 57(05):1044–1051. [PubMed: 22796894]
66. Yang H, Li N, Du Y, et al. Neutrophil adhesion and crawling dynamics on liver sinusoidal endothelial cells under shear flow. Exp Cell Res. 2017; 351(01):91–99. [PubMed: 28077302]
67. Aspinall AI, Curbishley SM, Lalor PF, et al. CX(3)CR1 and vascular adhesion protein-1-dependent recruitment of CD16(+) monocytes across human liver sinusoidal endothelium. Hepatology. 2010; 51(06):2030–2039. [PubMed: 20512991]
68. Onoe T, Ohdan H, Tokita D, et al. Liver sinusoidal endothelial cells tolerize T cells across MHC barriers in mice. J Immunol. 2005; 175(01):139–146. [PubMed: 15972640]
69. Liu WL, Tang L, Zhang G, et al. Characterization of a novel C-type lectin-like gene, LSECtin - Demonstration of carbohydrate binding and expression in sinusoidal endothelial cells of liver and lymph node. J Biol Chem. 2004; 279(18):18748–18758. [PubMed: 14711836]

70. Na H, Liu X, Li X, et al. Novel roles of DC-SIGNR in colon cancer cell adhesion, migration, invasion, and liver metastasis. J Hematol Oncol. 2017; 10(01):28. [PubMed: 28109307]
71. Zuo Y, Ren S, Wang M, et al. Novel roles of liver sinusoidal endothelial cell lectin in colon carcinoma cell adhesion, migration and in-vivo metastasis to the liver. Gut. 2013; 62(08):1169–1178. [PubMed: 22637699]
72. Maher JJ. Cell-specific expression of hepatocyte growth factor in liver. Upregulation in sinusoidal endothelial cells after carbon tetrachloride. J Clin Invest. 1993; 91(05):2244–2252. [PubMed: 7683700]
73. Nanji AA, Tahan SR, Wei Y, Sadrzadeh SM. Hepatic sinusoidal endothelial cell G1/S arrest correlates with severity of alcoholic liver injury in the rat. Gastroenterology. 1994; 107(03):818–823. [PubMed: 7915700]
74. Taniguchi E, Sakisaka S, Matsuo K, Tanikawa K, Sata M. Expression and role of vascular endothelial growth factor in liver regeneration after partial hepatectomy in rats. J Histochem Cytochem. 2001; 49(01):121–130. [PubMed: 11118484]
75. LeCouter J, Moritz DR, Li B, et al. Angiogenesis-independent endothelial protection of liver: role of VEGFR-1. Science. 2003; 299(5608):890–893. [PubMed: 12574630]
76. Greene AK, Wiener S, Puder M, et al. Endothelial-directed hepatic regeneration after partial hepatectomy. Ann Surg. 2003; 237(04):530–535. [PubMed: 12677150]
77. Ding BS, Nolan DJ, Butler JM, et al. Inductive angiocrine signals from sinusoidal endothelium are required for liver regeneration. Nature. 2010; 468(7321):310–315. [PubMed: 21068842]
78. DeLeve LD, Wang X, Wang L. VEGF-sdf1 recruitment of CXCR7+ bone marrow progenitors of liver sinusoidal endothelial cells promotes rat liver regeneration. Am J Physiol Gastrointest Liver Physiol. 2016; 310(09):G739–G746. [PubMed: 26939868]
79. DeLeve LD. Liver sinusoidal endothelial cells and liver regeneration. J Clin Invest. 2013; 123(05): 1861–1866. [PubMed: 23635783]
80. Wang L, Wang X, Wang L, et al. Hepatic vascular endothelial growth factor regulates recruitment of rat liver sinusoidal endothelial cell progenitor cells. Gastroenterology. 2012; 143(06):1555–1563e2. [PubMed: 22902870]
81. Wang L, Wang X, Xie G, Wang L, Hill CK, DeLeve LD. Liver sinusoidal endothelial cell progenitor cells promote liver regeneration in rats. J Clin Invest. 2012; 122(04):1567–1573. [PubMed: 22406533]
82. Steffan AM, Gendrault JL, McCuskey RS, McCuskey PA, Kirn A. Phagocytosis, an unrecognized property of murine endothelial liver cells. Hepatology. 1986; 6:830–836. [PubMed: 3758936]
83. Harb R, Xie G, Lutzko C, et al. Bone marrow progenitor cells repair rat hepatic sinusoidal endothelial cells after liver injury. Gastroenterology. 2009; 137(02):704–712. [PubMed: 19447108]
84. Ding BS, Cao Z, Lis R, et al. Divergent angiocrine signals from vascular niche balance liver regeneration and fibrosis. Nature. 2014; 505(7481):97–102. [PubMed: 24256728]
85. Yu Y, Liang Y, Liu X, et al. Id1 modulates endothelial progenitor cells function through relieving the E2-2-mediated repression of FGFR1 and VEGFR2 in vitro. Mol Cell Biochem. 2016; 411(1–2):289–298. [PubMed: 26476925]
86. Yu Y, Liang Y, Yin C, et al. Inhibitor of DNA-binding 1 promotes endothelial progenitor cell proliferation and migration by suppressing E2-2 through the helix-loop-helix domain. Int J Mol Med. 2016; 38(05):1549–1557. [PubMed: 27635429]
87. Xia X, Yu Y, Zhang L, Ma Y, Wang H. Inhibitor of DNA binding 1 regulates cell cycle progression of endothelial progenitor cells through induction of Wnt2 expression. Mol Med Rep. 2016; 14(03): 2016–2024. [PubMed: 27432753]
88. Schaffner F, Poper H. Capillarization of hepatic sinusoids in man. Gastroenterology. 1963; 44:239–242. [PubMed: 13976646]
89. Horn, T., Christoffersen, P. Sinusoidal endothelial cells: a SEM investigation of the fenestration in normal and diseased human liver. In: Bioulac Sage, P., Balabaud, C., editors. Sinusoids in Human Liver: Health and Disease. Rijswijk, The Netherlands: The Kupffer Cell Foundation; 1988.
90. Horn T, Christoffersen P, Henriksen JH. Alcoholic liver injury: defenestration in noncirrhotic livers–a scanning electron microscopic study. Hepatology. 1987; 7(01):77–82. [PubMed: 3542781]

91. Fraser, R., Rogers, GWT., Bowler, LM., Day, WA., Dobbs, BR. Defenestration and vitamin A status in a rat model of cirrhosis. In: Wisse, E.Knook, DL., McCuskey, RS., editors. Cells of the hepatic sinusoid. Vol. 3. Leiden: Kupffer Cell Foundation; 1991. p. 195-198.
92. Deleve LD, Wang X, Guo Y. Sinusoidal endothelial cells prevent rat stellate cell activation and promote reversion to quiescence. Hepatology. 2008; 48(03):920–930. [PubMed: 18613151]
93. Xie G, Wang X, Wang L, et al. Role of differentiation of liver sinusoidal endothelial cells in progression and regression of hepatic fibrosis in rats. Gastroenterology. 2012; 142(04):918–927e6. [PubMed: 22178212]
94. DeLeve LD, Wang X, Hu L, McCuskey MK, McCuskey RS. Rat liver sinusoidal endothelial cell phenotype is maintained by paracrine and autocrine regulation. Am J Physiol Gastrointest Liver Physiol. 2004; 287(04):G757–G763. [PubMed: 15191879]
95. Rockey DC, Chung JJ. Reduced nitric oxide production by endothelial cells in cirrhotic rat liver: endothelial dysfunction in portal hypertension. Gastroenterology. 1998; 114(02):344–351. [PubMed: 9453496]
96. Shah V, Toruner M, Haddad F, et al. Impaired endothelial nitric oxide synthase activity associated with enhanced caveolin binding in experimental cirrhosis in the rat. Gastroenterology. 1999; 117(05):1222–1228. [PubMed: 10535886]
97. Failli P, DeFRANCO RM, Caligiuri A, et al. Nitrovasodilators inhibit platelet-derived growth factor-induced proliferation and migration of activated human hepatic stellate cells. Gastroenterology. 2000; 119(02):479–492. [PubMed: 10930383]
98. Marrone G, Russo L, Rosado E, et al. The transcription factor KLF2 mediates hepatic endothelial protection and paracrine endothelial-stellate cell deactivation induced by statins. J Hepatol. 2013; 58(01):98–103. [PubMed: 22989565]
99. Marrone G, Maeso-Díaz R, García-Cardena G, et al. KLF2 exerts antifibrotic and vasoprotective effects in cirrhotic rat livers: behind the molecular mechanisms of statins. Gut. 2015; 64(09):1434–1443. [PubMed: 25500203]
100. Jarnagin WR, Rockey DC, Koteliansky VE, Wang SS, Bissell DM. Expression of variant fibronectins in wound healing: cellular source and biological activity of the EIIIA segment in rat hepatic fibrogenesis. J Cell Biol. 1994; 127(6 Pt 2):2037–2048. [PubMed: 7806580]
101. Olsen AL, Sackey BK, Marcinkiewicz C, Boettiger D, Wells RG. Fibronectin extra domain-A promotes hepatic stellate cell motility but not differentiation into myofibroblasts. Gastroenterology. 2012; 142(04):928–937e3. [PubMed: 22202457]
102. Koyama Y, Wang P, Liang S, et al. Mesothelin/mucin 16 signaling in activated portal fibroblasts regulates cholestatic liver fibrosis. J Clin Invest. 2017; 127(04):1254–1270. [PubMed: 28287406]
103. Lua I, Li Y, Zagory JA, et al. Characterization of hepatic stellate cells, portal fibroblasts, and mesothelial cells in normal and fibrotic livers. J Hepatol. 2016; 64(05):1137–1146. [PubMed: 26806818]
104. Mederacke I, Hsu CC, Troeger JS, et al. Fate tracing reveals hepatic stellate cells as dominant contributors to liver fibrosis independent of its aetiology. Nat Commun. 2013; 4:2823. [PubMed: 24264436]
105. Cogger VC, Arias IM, Warren A, et al. The response of fenestrations, actin, and caveolin-1 to vascular endothelial growth factor in SK Hep1 cells. Am J Physiol Gastrointest Liver Physiol. 2008; 295(01):G137–G145. [PubMed: 18497335]
106. Voyta JC, Via DP, Butterfield CE, Zetter BR. Identification and isolation of endothelial cells based on their increased uptake of acetylated-low density lipoprotein. J Cell Biol. 1984; 99(06): 2034–2040. [PubMed: 6501412]
107. Van Berkel TJ, De Rijke YB, Kruijt JK. Different fate in vivo of oxidatively modified low density lipoprotein and acetylated low density lipoprotein in rats. Recognition by various scavenger receptors on Kupffer and endothelial liver cells. J Biol Chem. 1991; 266(04):2282–2289. [PubMed: 1989982]
108. Kamps JA, Kruijt JK, Kuiper J, van Berkel TJ. Characterization of the interaction of acetylated LDL and oxidatively modified LDL with human liver parenchymal and Kupffer cells in culture. Arterioscler Thromb. 1992; 12(09):1079–1087. [PubMed: 1525123]

109. Katz SC, Pillarisetty VG, Bleier JI, Shah AB, DeMatteo RP. Liver sinusoidal endothelial cells are insufficient to activate T cells. J Immunol. 2004; 173(01):230–235. [PubMed: 15210779]
110. De Sanctis JB, Blanca I, Bianco NE. Expression of different lipoprotein receptors in natural killer cells and their effect on natural killer proliferative and cytotoxic activity. Immunology. 1995; 86(03):399–407. [PubMed: 8550077]
111. Meyer J, Gonelle-Gispert C, Morel P, Bühler L. Methods for isolation and purification of murine liver sinusoidal endothelial cells: a systematic review. PLoS One. 2016; 11(03):e0151945. [PubMed: 26992171]
112. Xie G, Wang L, Wang X, Wang L, DeLeve LD. Isolation of periportal, midlobular, and centrilobular rat liver sinusoidal endothelial cells enables study of zonated drug toxicity. Am J Physiol Gastrointest Liver Physiol. 2010; 299(05):G1204–G1210. [PubMed: 20813915]
113. Barberá-Guillem E, Rocha M, Alvarez A, Vidal-Vanaclocha F. Differences in the lectin-binding patterns of the periportal and perivenous endothelial domains in the liver sinusoids. Hepatology. 1991; 14(01):131–139. [PubMed: 1906044]
114. Strauss O, Phillips A, Ruggiero K, Bartlett A, Dunbar PR. Immunofluorescence identifies distinct subsets of endothelial cells in the human liver. Sci Rep. 2017; 7:44356. [PubMed: 28287163]
115. Asumendi A, Alvarez A, Martinez I, Smedsrød B, Vidal-Vanaclocha F. Hepatic sinusoidal endothelium heterogeneity with respect to mannose receptor activity is interleukin-1 dependent. Hepatology. 1996; 23(06):1521–1529. [PubMed: 8675173]
116. Rocha, M., Lentini, A., Asumendi, A., et al. Macrophage Mannose Receptor. Leiden, The Netherlands: The Kupffer Cell Foundation; 1993. In situ and in vitro correlation between mannose receptor expression and fenestration pattern in endothelial cells selected from different zones of liver lobule.
117. Scoazec JY, Racine L, Couvelard A, Flejou JF, Feldmann G. Endothelial cell heterogeneity in the normal human liver acinus: in situ immunohistochemical demonstration. Liver. 1994; 14(03): 113–123. [PubMed: 8078390]
118. Vidal-Vanaclocha F, Barbera-Guillem E. Fenestration patterns in endothelial cells of rat liver sinusoids. J Ultrastruct Res. 1985; 90:115–123. [PubMed: 4068137]
119. Wisse E, De Zanger RB, Charels K, Van Der Smissen P, McCuskey RS. The liver sieve: considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of Disse. Hepatology. 1985; 5(04):683–692. [PubMed: 3926620]
120. Wack KE, Ross MA, Zegarra V, Sysko LR, Watkins SC, Stolz DB. Sinusoidal ultrastructure evaluated during the revascularization of regenerating rat liver. Hepatology. 2001; 33(02):363–378. [PubMed: 11172338]
121. DeLeve LD, Wang X, McCuskey MK, McCuskey RS. Rat liver endothelial cells isolated by anti-CD31 immunomagnetic separation lack fenestrae and sieve plates. Am J Physiol Gastrointest Liver Physiol. 2006; 291(06):G1187–G1189. [PubMed: 16782698]
122. [Accessed November 20, 2017] Protocol for the preparation of mouse liver Kupffer cells and liver sinusoidal endothelial cells. Available at: http://hdl.handle.net/10037/4575
123. Smedsrød B, Pertoft H. Preparation of pure hepatocytes and reticuloendothelial cells in high yield from a single rat liver by means of Percoll centrifugation and selective adherence. J Leukoc Biol. 1985; 38(02):213–230. [PubMed: 2993459]
124. Pertoft, H., Smedsrod, B. Separation and characterization of liver cells. In: Pretlow, TG., II, Pretlow, TP., editors. Cell Separation: Methods and Selected Applications. Cambridge, MA: Academic Press; 1987. p. 1-24.
125. Huebert RC, Jagavelu K, Liebl AF, et al. Immortalized liver endothelial cells: a cell culture model for studies of motility and angiogenesis. Lab Invest. 2010; 90(12):1770–1781. [PubMed: 20644520]
126. Vonnahme, FJ. The Human Liver- A Scanning Electron Microscopic Atlas. Basel: Karger; 1993.
127. Bardin N, Francès V, Lesaule G, Horschowski N, George F, Sampol J. Identification of the S-Endo 1 endothelial-associated antigen. Biochem Biophys Res Commun. 1996; 218(01):210–216. [PubMed: 8573133]

128. Schrage A, Loddenkemper C, Erben U, et al. Murine CD146 is widely expressed on endothelial cells and is recognized by the monoclonal antibody ME-9F1. Histochem Cell Biol. 2008; 129(04):441–451. [PubMed: 18214516]

129. Despoix N, Walzer T, Jouve N, et al. Mouse CD146/MCAM is a marker of natural killer cell maturation. Eur J Immunol. 2008; 38(10):2855–2864. [PubMed: 18958886]

130. March S, Hui EE, Underhill GH, Khetani S, Bhatia SN. Microenvironmental regulation of the sinusoidal endothelial cell phenotype in vitro. Hepatology. 2009; 50(03):920–928. [PubMed: 19585615]

131. Su K, Yang H, Li X, et al. Expression profile of FcgammaRIIb on leukocytes and its dysregulation in systemic lupus erythematosus. J Immunol. 2007; 178(05):3272–3280. [PubMed: 17312177]

132. Minhajat R, Mori D, Yamasaki F, Sugita Y, Satoh T, Tokunaga O. Organ-specific endoglin (CD105) expression in the angiogenesis of human cancers. Pathol Int. 2006; 56(12):717–723. [PubMed: 17096728]

133. Theuerkauf I, Zhou H, Fischer HP. Immunohistochemical patterns of human liver sinusoids under different conditions of pathologic perfusion. Virchows Arch. 2001; 438(05):498–504. [PubMed: 11407479]

134. Lalor PF, Lai WK, Curbishley SM, Shetty S, Adams DH. Human hepatic sinusoidal endothelial cells can be distinguished by expression of phenotypic markers related to their specialised functions in vivo. World J Gastroenterol. 2006; 12(34):5429–5439. [PubMed: 17006978]

135. Klein D, Demory A, Peyre F, et al. Wnt2 acts as a cell type-specific, autocrine growth factor in rat hepatic sinusoidal endothelial cells cross-stimulating the VEGF pathway. Hepatology. 2008; 47(03):1018–1031. [PubMed: 18302287]

136. Krause P, Markus PM, Schwartz P, et al. Hepatocyte-supported serum-free culture of rat liver sinusoidal endothelial cells. J Hepatol. 2000; 32(05):718–726. [PubMed: 10845657]

Table 1

Main concepts and learning points

• LSCECs play an important role in clearance of blood borne waste, immunology, liver regeneration, and liver fibrosis
• After liver injury or partial hepatectomy, hepatic VEGF and downstream sdf1 recruit and engraft CXCR7+ bone marrow-derived progenitors of sinusoidal endothelial cells (bone marrow sprocs) that replace injured and lost LSECs. BM sprocs rather than mature LSECs are drivers of liver regeneration
• In normal liver, LSECs act as gatekeepers that prevent hepatic stellate cell activation. Prior to fibrosis, LSECs capillarize and lose the ability to prevent stellate cell activation. Capillarization is therefore permissive for stellate cell activation
• The authors advocate that methods that isolate liver sinusoidal endothelial cells should validate the identity of isolated cells based on ultrastructure and purity based on evidence of high-affinity endocytosis of specific ligands. Most studies of LSECs likely require an isolation method that obtains all populations of liver sinusoidal endothelial cells

Abbreviations: BM, bone marrow; LSCECs, liver sinusoidal endothelial cells; VEGF, vascular endothelial growth factor

Table 2

Pattern recognition receptors and components of the inflammasome expressed by LSECs

Endocytosis receptors[36,37]
Stabilins
Mannose receptor
Toll-like receptors[38–40]
TLR2
TLR3
TLR4
TLR6/2
TLR8
TLR9
Inflammasomes[41]
NLRP-1
NLRP-3
AIM2

Abbreviations: AIM2, absent in melanoma 2; LSCECs, liver sinusoidal endothelial cells; NLRP, nod-like receptor protein; TLR, toll-like receptor.