The liver sinusoidal endothelial cell: a cell type of controversial and confusing identity

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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 294: G391–G400, 2008. First published December 6, 2007; doi:10.1152/ajpgi.00167.2007.—A look through the literature on liver sinusoidal endothelial cells (LSECs) reveals that there are several conflicts among different authors of what this cell type is and does. Major controversies that will be highlighted in this review include aspects of the physiological role, the characterization, and the protocols of isolation and cultivation of these cells. Many of these conflicts may be ascribed to the fact that the cell was only recently established as a distinct cell type and that researchers from different disciplines tend to define their structure and function differently. This field is in need of a common platform to obtain a sound communication and a unified understanding of how to interpret novel research results. The aim of this review is to encourage scientists not to ignore the fact that there are, indeed, different opinions in the literature on LSECs. We also hope that this review will point out to the reader that some issues that may seem well established regarding our knowledge about the LSECs, in reality, are still unresolved and, indeed, controversial.

REVIEW ARTICLES TYPICALLY put forward unitary views and models as conclusions following a presentation of the updated literature. The present review is different. Due to the many conflicting reports in the field of liver sinusoidal endothelial cells (LSECs), we have, in general, chosen to identify the existing conflicts rather than aiming at establishing unitary views. Some of these conflicts may not appear as obvious in the literature and are rarely discussed openly. It is particularly important that such occult conflicts are brought up in discussion forums.

The study of LSECs is a rather young field; in fact, the first studies on LSECs can be traced back only about 35 years, when Eddie Wisse for the first time presented convincing evidence that the LSEC represents a distinct cell type (85). Wisse showed that open fenestration without a diaphragm or basement membrane is a typical feature of LSECs. He also was the first to report that LSECs contained unusually high amounts of endocytic vesicles and suggested that they were engaged in uptake of protein from blood passing through the sinusoids. The first physiological macromolecule shown to be cleared from the circulation by LSECs was hyaluronan (18, 71). This marked the start of a series of experiments culminating in the notion that LSECs are a specialized type of scavenger endothelium that uses clathrin-mediated endocytosis to clear an array of physiological and foreign macromolecules and colloids from the blood (73). Until about 1990, most of these studies were performed by scientists that had a special interest in the biology of these cells. Most of these researchers were well informed about the young history of this field of research and were open to new ways of classifying LSECs. After about 1990 the general interest in LSECs increased, and the number of scientists that were not raised in the tradition of cells of the reticuloendothelial system (RES) clears waste from the circulation, 1) the hepatic reticuloendothelial system (RES) clears waste from the circulation, 2) the hepatic RES is equal to Kupffer cells (KCs), and 3) the LSEC
represents the cell type that is mainly responsible for the clearance of most colloids and soluble waste macromolecules from the circulation. How can we comprehend these incompatible statements? The answer lies in knowledge about the history of research on RES and certain key concepts dealing with how cells internalize matter.

Phagocytosis or pinocytosis: a problem of semantics. Although regarded by many as an old-fashioned and ill-defined term, the RES is still a frequently used expression. The RES was for a long time commonly understood as just an alternative way of naming the mononuclear phagocyte system (MPS), or macrophage system. Although recent studies clearly show that a major arm of the RES consists of the LSECs, authors of most textbooks and scientific publications still ignore this fact and continue to bring forward the erroneous understanding that RES and MPS are synonymous terms. As will be shown in the following, the way we today understand terms that were launched more than a century ago plays a central role to explain how this conflict came to be. More than a century ago, Eli Metchnikoff introduced two terms that are still in use and form important parts of the specialized vocabulary of cell biologists: “macrophage” (“cell type that eats a lot”) and “phagocytosis” (“engulfment of material, often associated with macrophages”) (54). Thus it is logical and correct to associate MPS with the specialized process of phagocytosis. However, Metchnikoff did not define phagocytosis the way we do today. The idea of dividing cellular uptake into phagocytosis and pinocytosis was not introduced until 1931 when Lewis launched the concept of pinocytosis to describe the special process internalizing solutes and soluble material (48). Thus Metchnikoff and his contemporaries defined phagocytosis without reference to the physical state of the material to be taken up. This circumstance, combined with the fact that the term RES was also introduced before the idea of pinocytosis appeared and that the universal view that cells of RES generally used phagocytosis to take up material from the circulation, led to the conflict or confusion that was so clearly expressed by Ralph van Furth, who around 1970 advised that there is no need to keep the old-fashioned term RES, since the MPS covers more precisely the structure and function of RES (82). The establishment of the LSEC as a cell type distinct from the KC (85), combined with the discovery during the 1980s that LSECs represent a major scavenger cell system in liver, led Kawai and coworkers (36) to suspect that van Furth’s advice might be misleading and decided to repeat the experimental protocol used a century ago to identify the cell system known today as RES. Using the original protocol of administering the colloidal vital stain lithium carmine (one of the most frequently used test substances employed around a century ago to study the RES) and using modern methodology to identify the cells that accumulated the dye, Kawai et al. were able to show that the cells that most efficiently accumulated vital stain in the liver were the LSECs but not the liver macrophages or KCs. Just as important, present-day research has revealed that the two cell types differ functionally based on their uptake preferences: LSECs do not normally perform phagocytosis but are extremely active in uptake of soluble or colloidal materials, whereas the KCs are geared to uptake of larger particles (see the following section).

Size matters. A study on rat LSECs concluded that the cells are not able to engulf particles greater than 0.23 μm under normal conditions, whereas the KCs take up larger particles (69). However, when the phagocytic function of KCs was impaired, they found that LSECs took up particles larger than 1 μm. Although colloids are too small in size to be taken up by phagocytosis in macrophages, colloidal carbon, probably the most frequently used RES test substance, accumulates primarily in KCs upon intravenous injection. This would appear to contradict the rule that large particles are home to KCs, whereas colloids and soluble macromolecules end up in LSECs. However, Donald reported in 1975 (13) that colloidal carbon rapidly sticks to platelets and activates them to form aggregates. Such free or aggregated platelets, with large amounts of colloidal carbon attached to them, are rapidly taken up by phagocytosis in KCs. Present-day scientists should realize that colloidal carbon interacts very differently than other vital stains with the blood and RES. If Donald’s report on the unusual interaction of colloidal carbon with platelets had been considered by authors on RES, we might have avoided the misconception that colloidal carbon behaves like most other vital stains in its interaction with RES.

It is common to find that present-day studies on LSECs do not take into consideration the special scavenger features of these cells. A recent enzyme replacement therapy report described the use of mannose receptor-deficient mice to study the distribution of acid lipase that was injected intravenously (14). Immunohistochemistry performed on liver sections was, by the use of light microscope, interpreted to mean that the enzyme was taken up foremost in KCs. This conclusion was drawn without any further attempt to distinguish between KCs and LSECs and is in contrast to a number of other studies where other injected lysosomal enzymes were observed to be taken up foremost in LSECs (2, 30, 72). Knowing that acid lipase is a soluble molecule, it is doubtful that it is mainly cleared from the circulation by phagocytosis. Furthermore, the use of plain light/fluorescence microscopy of liver sections following intravenous administration of stained material does not allow a clear distinction among sinusoidal liver cells. It may be found in the literature that staining along the sinusoids is taken to prove uptake in either LSECs or KCs, or both. To illustrate such typical staining patterns we have included a figure showing fluorescence micrographs of liver sections following intravenous injections of a tetramethylrhodamine isothiocyanate (TRITC)-labeled soluble ligand and FITC-labeled particles (Fig. 1). In the example shown in the figure, we have injected soluble TRITC-labeled formaldehyde-treated serum albumin (TRITC-FSA) and FITC-labeled beads (2 μm). Some authors would claim that continuous staining along the sinusoids is taken to prove uptake in either LSECs or KCs. Other authors would argue that the same staining pattern is typical of LSECs. Knowing that FSA and 2-μm particles distinguish LSECs and KCs (32, 60), it is advisable to employ coinjection with fluorescently labeled FSA and particles when the goal is to localize and identify the type(s) of hepatic cells responsible for uptake of certain materials.

LSECs and uptake of virus. The majority of studies on interaction of virus with LSECs deal with transfection and infectivity (4, 65, 76). Very few studies, if any, focus on the general clearance of blood-borne virus particles in these cells. Studies on uptake of phage particles and distribution of virus used in gene therapy all show that the liver has a very high capacity to remove these particles from the circulation (6, 65,
In fact, it has been noted as a problem in gene therapy that virus carrying the therapeutic gene material must be administered in huge doses to overcome hepatic clearance, allowing virus to reach their target organs. This problem is not often mentioned in the literature but nevertheless represents an important obstacle in virus-based gene therapy. On this basis, it is surprising that very few studies have been carried out to study the mechanism of elimination of virus in liver. Interestingly, a study in 1969 concluded that injected phage particles were eliminated very efficiently by “phagocytic uptake” in the KC (28). It should be noted here that virus represents colloidal particles, most of them <100 nm, that are too small to be taken up by phagocytosis. However, it is frequently seen in the literature that “virus is phagocytosed by macrophages.” Clearly, studies should be carried out to specifically address the different roles of LSECs and KCs in the elimination of virus in general. By regarding uptake of virus in LSECs as part of the innate immune system, it is conceivable that LSECs may function as a sink for elimination and degradation of virus rather than representing the gate of infection. In fact, it is highly likely that “silent” elimination of virus is a major task of LSECs, whereas the more dramatic scenarios leading to infection may represent exceptions. However, since viral infection is easily detected and much studied, whereas silent elimination is not, the literature gives a false picture: silent elimination of virus in these cells may be a far more important issue than interaction with those few types of virus that may result in infection. For instance, many viruses are highly mannosylated (91), which makes them a likely ligand for the mannose receptors (a typical pattern-recognition receptor) present on these cells.

Discrepancies in Identification of LSECs

Membrane markers. During the 1970s and 80s, some laboratories reported the existence of membrane molecules that specifically stained endothelia of numerous organs, including both large vessels and capillaries (26, 33, 59, 81, 84). Since then, these markers have been frequently used by many laboratories to characterize endothelial cells in different tissues and species. The expression of many of these marker molecules by LSECs remains controversial (Table 1). Experiments in vitro have revealed discrepancies in the expression of CD31, von Willebrand factor (vWF), CD106, and immunomarkers such as major histocompatibility complex (MHC) class II, CD4, CD40, CD80, and CD86 in different species. Expression of vWF has been generally reported on human liver sections, whereas in cultured human LSECs the expression of this marker has been variably detected. This shows that there are differences between in vivo and in vitro observations in addition to differences between species. Another controversial LSEC marker is CD31. Electron microscopic studies in rat showed that CD31 is located intracellularly shortly after establishing cultures but later, after defenestration, CD31 is expressed on the surface as in conventional endothelial cells (10). Thus immunohistochemistry gives positive staining for CD31 in LSECs in liver sections, whereas freshly isolated LSECs do not stain for CD31 unless the cells are permeabilized before staining. The absence or presence of surface markers in LSECs is of major importance in immunomagnetic isolation of the cells. This issue will be discussed in greater detail in the section dealing with isolation and cultivation. Of note, certain treatments of animals may induce the expression of markers that are not normally present on LSECs. For instance, injection of colloidal carbon have been observed to induce the expression of vWF in LSECs.

Fig. 1. Fluorescence micrographs of a rat liver section following double intravenous administration of 0.5 mg tetramethylrhodamine isothiocyanate (TRITC)-labeled formaldehyde-treated serum albumin (TRITC-FSA), a substance known to be taken up by liver sinusoidal endothelial cells (LSECs) (32), and 5 × 10⁸ FITC-labeled beads (2-µm diameter), known to be taken up exclusively in Kupffer cells (KCs) (60). Both probes can be observed to have accumulated along the sinusoids. By superimposing A and B, it can be seen that the beads have accumulated in sites that do not coincide with the presence of TRITC-FSA (C).

86, 90). In fact, it has been noted as a problem in gene therapy that virus carrying the therapeutic gene material must be administered in huge doses to overcome hepatic clearance, allowing virus to reach their target organs. This problem is not
in vivo (37). Variation in the expression of most of these molecules by LSECs has been observed during the development of certain liver diseases including chronic inflammatory disorders, fibrosis, viral infection, or tumor development (19, 23–25, 79, 80, 83, 87, 88). Normal aging is associated with sinusoidal capillarization (5, 9, 31, 47), leading to changes in the expression of marker molecules along the sinusoidal endothelium. This fact is not often taken into consideration in studies of human liver that frequently involve samples from individuals of different age. The presence of markers normally associated with cells of the immune system will be discussed later.

### Fenestration

A characteristic feature of LSECs is the presence of numerous transcytoplasmic canals arranged in sieve plates called “fenestrae” (Fig. 2, A and C). Fenestration is generally considered to be a reliable marker of LSECs, making them clearly distinguishable from all other types of liver cells including endothelial cells from larger vessels (3). However, preparation of cells for scanning electron microscopy might easily result in the formation of holes in the cell surface (Fig. 2 B) that sometimes are wrongly interpreted as fenestration. Such nonfenestral transcytoplasmic holes are often observed in long-time-cultured LSECs. This observation, along with the well documented (yet by many authors ignored) fact that

**Table 1. Phenotypic markers used to identify liver sinusoidal endothelial cells on liver slices and on cultured cells**

<table>
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<tr>
<th>Liver Slices</th>
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<td>Human (81)</td>
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<td>Rat (57)</td>
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<td>Rat, 0–15 days, 0, 1 and 10% serum (29)</td>
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<tr>
<td>20% human serum (34)</td>
<td>+ + + + + +</td>
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<td>20% human serum (8)</td>
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<td>20% human serum (40)</td>
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<td>10% serum not reported (62)</td>
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Intensity of immunochemical staining: absent, 0; +, low; + +, medium; + + +, high. AcLDL, acetylated low-density lipoprotein; vWF, von Willebrand factor; MHC, major histocompatibility complex; HLA, human leukocyte antigen; L-SIGN, liver/lymph node-specific ICAM-3-grabbing nonintegrin. *Performed both on liver slices and on cultured cells; †Intensity of immunochemical staining only + and/or –; ‡RT-PCR; §flow cytometry.

**Fig. 2.** Scanning electron micrographs of fenestration on LSECs. A and B are cultured on fibronectin-coated glass slides, whereas C shows the liver sinusoids with numerous fenestrae. Freshly isolated cells (A) show a well-defined and organized appearance of fenestrae in sieve plates (circle), whereas after 1 day in culture (B), the cells are largely defenestrated, and only sporadic fenestrae in sieve plates are observed (circle). Transcytoplasmic holes are observed (arrows), but these structures are most likely artefacts resulting from the preparation of the specimens and should be cautiously interpreted. N = nucleus; bars are 2 μm.
fenestration in LSECs of most species studied is rapidly lost upon in vitro culturing (see also Fig. 2), may lead some authors to misjudge mere transcytoplasmic holes for true fenestration. Additionally, a considerable variation in the number, size, and localization of fenestration is observed among different species and also during the development of some liver disorders or during aging (27, 47, 51, 56). Moreover, fenestration can be induced in vascular endothelial cells upon stimulation with VEGF or hepatocyte-conditioned medium (17, 20, 43, 89). In conclusion, the LSEC-specific feature of fenestration must be used with some caution because it is a highly dynamic marker that is sensitive to the protocol of preparation for electron microscopy.

**Uptake of latex particles.** Uptake of microbeads has been used as a functional marker for identification of LSECs (21, 75). During some abnormal situations in which the hepatic scavenger system is either saturated or KCs are selectively depleted, LSECs may develop a limited phagocytic capacity, a phenomenon that is also observed during special in vitro conditions. The observation that LSECs under certain abnormal conditions may be induced to carry out limited phagocytosis is not surprising, since most cells, even the nonphagocytic hepatocyte, may become phagocytic (38).

Under normal conditions LSECs employ only receptor-mediated endocytosis to eliminate soluble or colloidal substances smaller than 0.23 μm (69). Under any circumstance, it would seem useless to employ phagocytosis as a functional marker of LSECs, given the fact that they are located next to the KCs, the body’s largest population of macrophages.

**Uptake of soluble macromolecules.** A unique feature of LSECs is their high capacity to eliminate colloids and soluble waste macromolecules from the circulation. Although this function can be used as a reliable marker to identify LSECs, it is important to be aware of the fact that every cell in the body is in principle able to perform endocytosis. However, the unsurpassed speed and capacity of endocytosis in LSECs set these cells apart from all other cell types. By challenging LSECs for a limited period (<20 min) (16) and with low amounts of ligand (<10 μg/ml), only this cell type will endocytose detectable amounts of the ligand. Of note, it is not uncommon to see in the literature that LSECs claim to be functionally identified by endocytosis by using ligand concentrations in the order of mg/ml and incubating periods of several hours. Clearly, under such conditions many cell types will accumulate detectable levels of ligand, which will make it difficult to distinguish LSECs. It should be noted that the characteristic high-activity receptor-mediated endocytosis is rapidly lost in cultured LSECs. This phenomenon is particularly prominent in rodents (Fig. 4).

Endocytosis of acetylated low-density lipoprotein (AcLDL) has been used by several authors as a specific functional marker for LSECs (29). AcLDL is cleared by the scavenger receptors on LSECs, but scavenger receptors are also expressed on KCs and a number of other cell types; also endocytosis of AcLDL has frequently been used as a functional marker of several types of extrahepatic endothelial cells. Therefore, AcLDL is not a specific endocytic marker for LSECs. However, other ligands have been reported to be taken up exclusively by receptor-mediated endocytosis in LSECs, such as denatured α-collagen chains (16, 70). If LSECs are to be identified by virtue of their specific endocytic ability, the ligands chosen should be of the type that is taken up preferentially or exclusively by LSECs.

**New markers related to specific LSEC functions.** A new member of the scavenger receptor family that is expressed exclusively on the surface of LSECs is stabilin-2 (22), also called the liver hyaluronan receptor. Lymphatic vessel endothelial hyaluronan receptor (LYVE-1) is another member of the scavenger receptor family that is constitutively expressed on LSECs and absent on other hepatic cells and conventional endothelium (58). Recent literature has identified two new lectins expressed on LSECs. Liver/lymph node-specific ICAM-3-grabbing nonintegrin (L-SIGN) (1, 44, 74) is strongly and constitutively expressed on LSECs and lymph node endothelium but not on other hepatic cells or other endothelia. Liver and lymph node sinusoidal endothelial cell C-type lectin (LSECtin) has been shown to have the same cellular distribution and similar binding capacities as L-SIGN (12, 49). These new marker molecules claimed to be exclusively expressed on LSECs were described only very recently, and since the literature on these markers is still rather limited, the use of these new marker molecules to identify LSECs should be carried out with some caution.

**Controversies Associated With Isolation and Cultivation of LSECs**

**Isolation.** Detailed studies on LSECs require reliable methods for cell isolation and cultivation. The first paper describing immunoisolation of LSECs was published in 2002 (77). By using the LSEC-specific antibody SE-1, 98% pure rat LSECs were reported. Unfortunately this monoclonal antibody has so far not been commercially available. In a series of experiments where LSECs were isolated by counterflow elutriation, Knolle and coworkers (41, 42) reported that LSECs function as antigen-presenting cells (APC). However, by using immunomagnetic beads to remove CD45+ KCs and CD11b+ dendritic cells (DC) to obtain highly purified LSECs, Katz et al. (35) found no evidence of antigen-presenting function of the highly purified LSECs and speculated that the contradictory results might be explained by the possibility that Knolle and coworkers used LSEC preparations that were contaminated by other cell types. Recently, the isolation methods used by both Katz and coworkers and Knolle and coworkers were questioned by Onoe et al. (62), who employed the membrane marker CD105+ for positive selection of LSECs by using MACS. Onoe et al. used positive selection to obtain their cells, whereas Katz and coworkers obtained their LSECs by negative selection. However, CD105 is also expressed on liver stellate cells and myofibroblasts (55), and it has been advised to exercise caution when using this marker to identify or purify LSECs (46). Another endothelial cell marker, CD31, has been used for immunomagnetic isolation of human LSECs (45). However, in rat, the selection of CD31-positive cells yielded a population of LSECs with fewer fenestrae than LSECs isolated by elutriation (11). The latter study concluded that “cells isolated by anti-CD31 and immunomagnetic sorting lacked the hallmark features of LSEC.” Although all isolation methods mentioned above may yield high relative proportions of LSECs, it is likely that the degree of purity of LSECs and the main contaminating cell types vary from one method to the other.
**Cultivation.** Cultivation procedures may represent the chief source of conflict in the studies on LSECs today. Some laboratories culture LSECs under conditions that allow cell proliferation and subsequent splitting and passaging before the cells are used in experiments several days or even weeks after the isolation (34, 64). This frequently used practice is associated with conflicts that can be sorted in two “schools,” 1) the one claiming that LSEC features can only be studied in short-term (true primary) culture and 2) the one that frequently makes use of long-term cultures (including use of cell lines). Although these schools rarely, if ever, discuss openly their disagreements, it appears important to point out this conflict. To illustrate this, we show some of the contradictory statements that are often seen in the literature related to time of cultivation of LSECs: 1) LSEC cultures can be propagated by trypsin splitting and subcultivation (21, 34, 64); 2) studies on LSECs can be performed using cell lines originating from native LSECs (53, 64); 3) important features of LSECs, such as scavenger function and fenestration, are severely decreased or disappear completely in LSECs that are cultivated for more than 1–2 days (15, 43, 61); 4) serum (5–20%), which is normally supplemented in long-term cultures of cells (and often also in short-term primary cultures), has been noted to act toxic to dividing LSECs (43, 61); 5) LSECs proliferate only very slowly or not at all in culture (15, 43).

A fact that is often underscored is that many phenotypic features of LSECs change gradually when the cells are placed in a culture dish, and most importantly, many of the signature functions of LSECs are also rapidly lost during in vitro culture (15, 43) (see also Figs. 2 and 4). The drastic change in environment associated with the transfer of the LSECs from the intact liver to culture conditions is clearly responsible for many of these alterations. The use of light microscopy to determine the quality of a LSEC culture is at best semioptimal. In Fig. 3B, the 3-day-old cells appear very similar to the fresh cells in Fig. 3A. However, ultrastructural studies by scanning electron microscopy reveal a clear loss of fenestrae already after 1 day (Fig. 2B). In addition, the very high endocytic activity is lost after some days in culture (Fig. 4). Given that serum is lethal to dividing LSECs (43), the cultures propagated in the presence of serum will gradually change from being composed of mainly LSECs to a stromal-like culture (Fig. 3C). This puts forward the following questions: should serum be used to cultivate LSECs? How soon after seeding of freshly isolated LSECs should experiments be carried out on these cells? Do the results obtained with long-term cultivated LSECs reflect the function of these cells in the intact liver? How is the term “primary culture of LSECs” defined? Is there a possibility that cells taken to represent proliferating LSECs are instead contaminating non sinusoidal or nonendothelial cells? If some authors claim that LSECs do not proliferate in vitro, then how come other authors use terms like “subcultivation of LSECs” and “LSECs grown to confluence”? Do LSECs from different species differ in their proliferative potential? How come some authors use LSEC cell lines when other authors claim that cell lines that originate from primary LSEC cultures have lost their original scavenger activity and fenestration? Finally, how many specialized differentiated LSEC characteristics can be lost before the cells loose their status as LSECs?

These conflicts exist due the lack of unified criteria to characterize LSEC. It is therefore advisable that workers in this field openly discuss these issues.

Fig. 3. Effect of serum on primary rat LSECs cultured on fibronectin-coated dishes. LSECs were cultivated for 12 h (A), and then 3 days in a special serum-free medium (B) (16), or 3 days in RPMI 1640 medium containing 10% fetal calf serum (C). The morphology of the cells in the serum-free medium (B) is very similar to the cells at early phases of culture (A). However, incubation in the presence of serum for longer periods (C) had dramatic negative effects on the viability of LSECs, also favoring the growth of contaminating fibroblast-like cells (arrows) and/or other types of endothelial cells (arrowheads).

The Confusing Role of LSECs in Immunity

The liver is enriched in macrophages (KCs), natural killer (NK) cells, and NKT cells, which have traditionally been regarded as key cellular components of the innate immune system. However, the LSEC also promotes active antigen uptake via its Fc-γ receptor and pattern recognition receptors such as the mannose receptor and scavenger receptor. For this reason alone, these cells must be considered as an important part of the innate immune system. In recent years the LSECs have also been implicated as inducers of tolerance in the liver. In a series of experiments, Knolle and coworkers reported that LSECs express molecules that promote antigen presentation, including MHC class I and II and the costimulatory molecules CD40, CD80, and CD86, in addition to their endocytic scav-
LSECs induced negligible proliferation of CD4+ T cells. Katz et al. found that LSECs were poor stimulators of allogeneic T cells. Furthermore, in the absence of exogenous costimulation, their function of the LSEC, they found that DCs captured AcLDL to a similar extent in vivo. Consistent with their phenotype, their uptake of AcLDL has been reported to be a specific function of LSECs. LSECs demonstrated a high capacity for internalization and degradation of radioactively labeled FSA, a ligand for the scavenger receptor. This specific function is gradually lost with time in culture and disappears faster in LSEC cultures from small vertebrates like rodents (A) than larger vertebrates like pigs (B).

Fig. 4. Progressive loss of endocytic capacity by LSECs in culture. Freshly isolated LSECs show high capacity for internalization and degradation of soluble antigens such as food antigens or self proteins. The liver DCs were thought to take up antigen in the liver as well and then migrate to local lymph nodes, mediating immune tolerance in the lymphoid compartment. This view of LSECs as functional APC was seriously challenged by Katz et al. (35), who employed new efficient separation techniques to obtain highly purified LSEC cultures. They found that, unlike DCs, LSECs had low or absent expression of MHC class II, CD86, and CD11c. However, they found that LSECs demonstrated a high capacity for antigen uptake in vitro and in vivo, and although uptake of AcLDL has been reported to be a specific function of the LSEC, they found that DCs captured AcLDL to a similar extent in vivo. Consistent with their phenotype, LSECs were poor stimulators of allogeneic T cells. Furthermore, in the absence of exogenous costimulation, Katz et al. found that LSECs induced negligible proliferation of CD4+ or CD8+ T cells. They concluded that LSECs alone are insufficient to activate naive T cells. A year later, a study by Onoe et al. (62), contradictory to the results of Katz et al. but in agreement with Knolle and coworkers, concluded that primary LSECs from mice do, indeed, express MHC class II and CD86 but not CD11c. The contradictory findings were believed to occur due to the separation technique used by Katz et al. that, according to Onoe, was unsatisfactory (as mentioned in section III above). However, selection of CD105+ cells as used by Onoe may not result in pure LSECs as indicated by others (46) because this marker is also expressed on liver stellate cells (55). It is also very important to note that the length of cultivation and presence of serum were different in the studies reported by Knolle, Katz, and Onoe groups. These parameters have been shown previously to seriously affect the outcome of LSEC studies.

Recently it was reported that LSECs endocytose splenocytes injected via the portal vein. The LSECs that endocytosed the splenocytes showed enhanced expression of MHC class II molecules and CD80 (78). Of note, the notion that LSECs are capable of endocytosing/phagocytosing splenocytes is in contrast to the general concept that the cells under normal conditions do not take up material exceeding ~0.23 μm (69). The finding that neutrophils are removed by KCs and not by LSECs in agreement with this notion (68). On this basis, it is difficult to explain LSEC phagocytosis of splenocytes.

The conflicts associated with the expression of MHC class II in LSECs is further underlined by a number of earlier studies that failed to demonstrate this molecule on rat or human LSECs (36, 50, 66) (see also Table 1). Obviously, further studies are needed to elucidate the role of LSECs in the immune system.

Conclusions

The study of LSECs is still a young discipline. Different laboratories have different opinions about the identity of LSECs, and therefore design methods and interpret results differently. Some of the conflicting views can be readily identified and discussed in a scientific way. But other conflicting issues are based on the fact that some authors do not even appreciate those issues of conflict that other authors identify as highly conflicting.

Role in blood clearance. A large body of evidence strongly suggests that the two major scavenger cell types of liver are KCs, which take up particulate matter, and LSECs that clear soluble macromolecules and colloids of size <0.23 μm. However, most authors of textbooks and scientific publications still ignore this and maintain the old notion that “hepatic RES = KCs.” A meaningful further development of this field is difficult unless the correct notion (the hepatic RES = LSECs + KCs) is generally accepted.

Identification. Some markers used to identify LSECs are also expressed on KCs, stellate cells, or endothelial cells from different vascular beds and are therefore inadequate as LSEC-specific markers. In addition, phenotypic and functional variations are observed in LSECs from diseased livers, as well as in healthy livers of different age groups. Characterization of LSECs based on their unique scavenger function seems to be a reliable approach; however, caution should be taken for choosing the adequate experimental settings.

Isolation and cultivation. Some authors use long-term cultures and cell lines and subcultivate LSECs, whereas other authors claim that LSECs lose their unique native characteristics upon cultivation for more than 1–2 days and differ too much from native LSECs. Some authors cultivate LSECs in medium with serum, whereas others claim that serum is toxic to the cells. Clearly, the results of in vitro experiments depend on, to a large extent, the isolation and cultivation procedures used by the different authors.

Role in immunity. Some authors claim that LSECs carry MHC class II molecules and present antigens to generate hepatic immune tolerance. Other authors have not found evidence that LSECs express MHC class II or present antigens.
These conflicts are largely based on different views on isolation and cultivation procedures. The present review was written to remind scientists dealing with LSECs about the important controversies that still exist regarding these cells. Both old scholars and new scientists entering the field should consider that some issues that may seem well established regarding our knowledge about the LSECs are in reality still unresolved and controversial.

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REFERENCES


41. Lewis WHR.

42. Meurer SK, Tihaa L, Lahme B, Gressner AM, Weiskirchen R.


44. Mouta Carreira C, Nasser SM, di Tomaso E, Padera TP, Boucher Y, Lewis WHR, McKeating JA, Adams DH.


46. 50.


75. AJP-Gastrointest Liver Physiol • VOL 294 • FEBRUARY 2008 • www.ajpgi.org

76. Table 1. Characteristics of AJP-Gastrointest Liver Physiol • VOL 294 • FEBRUARY 2008 • www.ajpgi.org


79. Downloaded from http://ajpgi.physiology.org/ on June 23, 2017

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85. Wisse E. An ultrastructural characterization of the endothelial cell in the rat liver sinusoid under normal and various experimental conditions, as a contribution to the distinction between endothelial and Kupffer cells. *J Ultrastruct Res* 38: 528–562, 1972.


