The Pathogen Receptor Liver and Lymph Node Sinusoidal Endotelial Cell C-Type Lectin Is Expressed in Human Kupffer Cells and Regulated by PU.1

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Human LSECtin (liver and lymph node sinusoidal endothelial cell C-type lectin, CLEC4G) is a C-type lectin encoded within the L-SIGN/DC-SIGN/CD23 gene cluster. LSECtin acts as a pathogen attachment factor for Ebola virus and the SARS coronavirus, and its expression can be induced by interleukin-4 on monocytes and macrophages. Although reported as a liver and lymph node sinusoidal endothelial cell-specific molecule, LSECtin could be detected in the MUTZ-3 dendritic-like cell line at the messenger RNA (mRNA) and protein level, and immunohistochemistry analysis on human liver revealed its presence in Kupffer cells coexpressing the myeloid marker CD68. The expression of LSECtin in myeloid cells was further corroborated through the analysis of the proximal regulatory region of the human LSECtin gene, whose activity was maximal in LSECtin+ myeloid cells, and which contains a highly conserved PU.1-binding site. PU.1 transactivated the LSECtin regulatory region in collaboration with hematopoietic-restricted transcription factors (Myb, RUNX3), and was found to bind constitutively to the LSECtin proximal promoter. Moreover, knockdown of PU.1 through the use of small interfering RNA led to a decrease in LSECtin mRNA levels in THP-1 and monocyte-derived dendritic cells, thus confirming the involvement of PU.1 in the myeloid expression of the lectin.

Conclusion: LSECtin is expressed by liver myeloid cells, and its expression is dependent on the PU.1 transcription factor. (Hepatology 2009;49:287-296.)

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he gene cluster at chromosome 19p13.2 includes the genes encoding for the type II C-type lectins DC-SIGN, L-SIGN, CD23, and LSECtin,1-4 all of which contain a single carbohydrate-recognition domain followed by a stalk domain, a transmembrane region, and a cytoplasmic tail containing various internalization motifs. DC-SIGN, L-SIGN, and LSECtin function as endocytic receptors and mediate binding and internalization of clinically relevant viral, bacterial, and fungal pathogens.5,6 CD23 is expressed on myeloid cells and activated B lymphocytes, where it functions as a low affinity receptor for immunoglobulin E and plays a role in limiting the extent of immunoglobulin E–mediated pathologies.7,8 DC-SIGN is expressed on myeloid dendritic cells (DCs),1,9 alternatively activated in vitro macrophages,10 interstitial DCs,11 a subset of CD14+ peripheral blood DCs,12 and macrophages from various tissues,13-15 whereas L-SIGN is exclusively expressed on endothelial cells of the liver, lymph nodes, and placenta.16,17 Although reported to be exclusively expressed on liver and lymph node sinusoidal endothelial cells,4 LSECtin has been later found to be expressed in ex vivo isolated human peripheral blood and thymic DCs, as well as in DCs and alternatively activated macrophages generated in vitro.5 The carbohydrate specificity of LSECtin has been...
recently determined, and a scavenging function has been proposed because of its ability to recognize glycoproteins with truncated complex and hybrid N-linked glycans terminating in GlcNAcMan.

Kupffer cells constitute more than 50% of resident macrophages in the entire body, account for 15% of all liver cells, and are an integral part of the hepatic sinusoid together with sinusoidal endothelial cells and Ito cells. Kupffer cells exhibit a strong endocytic activity and actively scavenge plasma proteins and potentially hazardous microorganisms from the blood to maintain tissue homeostasis, a function dependent on the large array of scavenger receptors exposed on their cell surface. In fact, Kupffer cells mediate the removal of particulate material from the portal circulation. The presence of LSECtin in myeloid cell subsets prompted us to clarify its cell distribution in liver cells. We report that LSECtin is expressed in human Kupffer cells, where its expression correlates with the presence of the myeloid-restricted CD68 molecule. Moreover, PU.1 binds in vivo to the human LSECtin proximal promoter, and PU.1 protein levels determine the extent of LSECtin messenger RNA (mRNA) expression. Therefore, PU.1 contributes to the myeloid expression of LSECtin, which constitutes a novel addition to the arsenal of scavenging molecules expressed by liver Kupffer cells.

Materials and Methods

Cell Culture, Transfection, and Site-Directed Mutagenesis. Monocytes were purified from peripheral blood mononuclear cells via magnetic cell sorting using CD14 microbeads (Miltenyi Biotech, Bergisch Gladbach, Germany), and macrophage-derived dendritic cells (MDDCs) were generated as described. The K562 (chronic myelogenous leukemia) and THP-1 (monocytic leukaemia) cell lines were cultured in Roswell Park Memorial Institute 1640 medium supplemented with 10% fetal bovine serum, 25 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid, and 2 mM glutamine (complete medium), at 37°C in a humidified atmosphere with 5% CO₂. MUTZ-3 cells were kept in culture for 24 hours, and one-fifth of the cells were lysed and underwent western blotting for PU.1 detection. Total RNA was isolated from the remaining nucleofected cells and subjected to real-time PCR for the detection of LSECtin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA. Site-directed mutagenesis was performed on the LSECtin promoter construct pCLEC4G-296Luc using the QuikChange System (Stratagene, La Jolla, CA). For mutation of the PU.1-66 elements, the oligonucleotides were used, and the resulting plasmids were termed pCLEC4G-296/PU.1-99mutS and pCLEC4G-296/PU.1-66mutS. PU.1 expression plasmids for human PU.1, RUNX3, and Myb have been described.

THP-1 cells or MDDCs (2 × 10⁶ cells) were nucleofected with 3 μg of small interfering RNA (siRNA) for PU.1 (sc-36330 PU.1 siRNA gene silencer; Santa Cruz Biotechnology, Santa Cruz, CA) or a control siRNA (sc-37007 Control siRNA-A, Santa Cruz Biotechnology) using the Cell Line Nucleofector kit V for THP-1 and the Human Dendritic Cell Nucleofector kit for MDDCs (Amaza, Cologne, Germany). After nucleofection, cells were kept in culture for 24 hours, and one-fifth of the cells were lysed and underwent western blotting for PU.1 detection. Total RNA was isolated from the remaining nucleofected cells and subjected to real-time PCR for the detection of LSECtin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA. Site-directed mutagenesis was performed on the LSECtin promoter construct pCLEC4G-296Luc using the QuikChange System (Stratagene, La Jolla, CA). For mutation of the PU.1-66 elements, the oligonucleotides were used, and the resulting plasmids were termed pCLEC4G-296/PU.1-99mutS and pCLEC4G-296/PU.1-66mutS. PU.1 expression plasmids for human PU.1, RUNX3, and Myb have been described.

Immunohistochemistry. Immunostaining was performed on formalin-fixed, paraffin-embedded sections.
from normal human livers and lymph nodes showing hyperplasia. Paraffin sections were cut at 4 μm thickness and placed onto positively charged capillary gap microscope slides. Deparaffinization in xylene and hydration through graded alcohols was followed by heat-induced epitope retrieval. The slides were pressure-cooked for 3 minutes in 10 mM buffer citrate (pH 6.0) and then left in the buffer for 20 minutes at room temperature. Preparations were incubated with the distinct antibodies for 25 minutes at room temperature. As a secondary antibody, a biotinylated goat anti-rabbit polyclonal (ChemMate detection Kit, DakoCytomation) was applied, followed by horseradish peroxidase–conjugated streptavidin. Finally, the slides were developed in 3,3’-diaminobenzidine (Chem-Mate Detection Kit, DakoCytomation) and counterstained in hematoxylin. All incubations were performed in a capillary gap principle-based automated immunostainer (TechMate Horizon, DakoCytomation). For each specimen, a negative control was included using preimmune rabbit serum instead of primary antibody. EnVision™ G/2 Doublestain System (Dako) was used for the simultaneous detection of CD68 and LSECtin, following the manufacturer’s recommendations with a mouse monoclonal antibody against CD68 (PG-M1; Dako) and the LSECtin–specific polyclonal antisera ADS1 (against the stalk domain) and ADS4 (against the whole extracellular region). Blockade of endogenous peroxidase and alkaline phosphatase activities was accomplished with 0.5% H₂O₂ and enzymatic inhibitors (Dako). After addition of the anti-CD68 antibody (1/100 dilution) for 30 minutes, tissue was incubated with dextran polymer–conjugated horseradish peroxidase–labeled antisera against murine and rabbit immunoglobulins, and CD68–specific staining detected with 3,3’-diaminobenzidine. After using the Doublestain block reagent (Dako), tissue sections were sequentially incubated with the LSECtin–specific antisera (1/500 dilution for 30 minutes) and dextran polymer–conjugated, alkaline phosphatase–labeled antisera against murine and rabbit immunoglobulins. LSECtin staining was visualized with Permanent Red, and samples were later counterstained in hematoxylin.

**Chromatin Immunoprecipitation.** Chromatin immunoprecipitation assays were performed using the Chromatin Immunoprecipitation Assay Kit (Upstate Biotechnology, Charlotteville, VA) as described. For PCR detection of the LSECtin promoter, the oligonucleotides 5’- CCAAGCTTGGTACTAGCAACAGAACAGAGAG-3’ and 5’-GGGTATTCCCCCAGTCGACGCCACCCAGTC-3’ were used, which together amplify a 312-bp and 263-bp fragment spanning from -296/-247 to +16. Immunoprecipitating antibodies included affinity-purified rabbit polyclonal antibody against human PU.1 (sc-352; Santa Cruz Biotechnology) and purified polyclonal rabbit immunoglobulin G as a control.

**Quantitative Real-Time Reverse-Transcription PCR.** Oligonucleotides for LSECtin and GAPDH genes were designed according to the Roche software for quantitative real-time PCR. Total RNA from MDDCs and THP-1 cells was extracted using the RNAeasy kit (Qiagen) and retrotranscribed and amplified using the Universal Human Probe Roche library (Roche Diagnostics). Assays were made in triplicate, and the results were normalized according to the expression levels of GAPDH. Results were processed with the BioRad IQ5 2.0 software and were expressed relative to the mRNA level of control or untreated samples (relative mRNA level).

**Results**

**Expression of LSECtin in the Human MUTZ-3 Myeloid Cell Line.** We have reported the expression of LSECtin in human monocyte-derived macrophages and DCs. To further extend these findings, the presence of the lectin was analyzed in the human CD34+ acute myeloid leukemia cell line MUTZ-3, which exhibits the capability to differentiate toward DCs. As shown in Fig. 1A, LSECtin mRNA was barely detectable in MUTZ-3 cells grown in the continuous presence of granulocyte-macrophage colony-stimulating factor, but was greatly increased upon IL-4–mediated dendritic differentiation. Quantitative reverse-transcription PCR (RT-PCR) revealed that the level of LSECtin mRNA increased more than 100 times after addition of IL-4 (Fig. 1B). Moreover, LSECtin protein was also detected in IL-4–treated MUTZ-3 cells (Fig. 1C). Therefore, LSECtin is expressed in human myeloid cells with the capability to acquire a DC-like phenotype.

**Cellular Distribution of LSECtin in Liver Cells.** Because LSECtin was originally reported as a liver and lymph node sinusoidal-specific molecule, we decided to determine the identity of LSECtin-expressing cells within hepatic tissue. The LSECtin–specific ADS1 and ADS4 antisera stained cells with a dendritic-like appearance within liver sinusoids, a morphology consistent with Kupffer cells (Fig. 2). Parallel analysis with myeloid cell–specific and endothelial cell–specific markers revealed that anti-CD31 exclusively stained sinusoidal endothelial cells, whereas anti-CD68 and anti-PU.1 antibodies only marked intrasinusoidal Kupffer cells (Fig. 2). Comparison of the staining patterns revealed that LSECtin–specific antisera recognized Kupffer cells as well as other cells lining the hepatic sinusoids (Fig. 2), suggesting that LSECtin can be found in both Kupffer cells and other cells in...
the sinusoid wall. The presence of LSECtin on CD68+ macrophages was also suggested upon analysis of human lymph node sections, where LSECtin staining differed from that of anti-CD31 and anti–factor VIII (endotheli-

um-specific) and resembled the tissue staining pattern yielded by a factor XIII–specific antibody (macrophage/DC-specific) (Fig. 3A). Moreover, LSECtin-positive cells were preferentially observed in lymph node areas enriched in CD68+ macrophages (Fig. 3B-C). Double-labeling experiments confirmed the presence of LSECtin in CD68+ cells (Fig. 4), indicating the presence of LSECtin in Kupffer cells. Further support for the presence of LSECtin in Kupffer cells was obtained via RT-PCR on RNA isolated from human hepatocytes, sinusoidal endothelial cells, Ito cells, and Kupffer cells. LSECtin mRNA could be exclusively amplified from Kupffer RNA, which showed considerably higher levels of both CD68 and PU.1 transcripts than the other cell types (Fig. 4B). Therefore, immunohistochemistry on human liver sections and RT-PCR on isolated hepatic cell types revealed that LSECtin is preferentially expressed by myeloid CD68+ PU.1+ Kupffer cells in the human liver.

**Involvement of PU.1 in the Myeloid Expression of LSECtin.** The DC-SIGN lectin is expressed in human myeloid cells, and its expression is regulated by PU.1,26 a member of the Ets family of transcription factors. The expression of LSECtin in myeloid cells5 and the similar pattern of staining yielded by anti-LSECtin and anti–PU.1 antibodies on human liver tissue (Fig. 2) suggest that PU.1 could participate in the restricted expression of LSECtin. This hypothesis was supported by the presence of three potential Ets-binding sequences (TTCCCTTC-CTTCC) at position −66 within the LSECtin gene proximal regulatory region (Fig. 5A). In fact, alignment of the

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**Fig. 1.** Expression of LSECtin in the MUTZ-3 cell line. (A) Detection of LSECtin, DC-SIGN, and GAPDH mRNA in untreated and IL-4–treated MUTZ-3 via conventional RT-PCR. The results of control experiments without RNA (H2O) or without reverse-transcriptase (CNT RT) are shown in each case. (B) Relative levels of LSECtin mRNA in untreated (−) and IL-4–treated MUTZ-3 cells via quantitative RT-PCR, after normalization for the levels of 18S RNA. Determination was performed in triplicate, and the mean and standard deviation is shown. (C) Detection of DC-SIGN and LSECtin protein expression in untreated (−) and IL-4–treated MUTZ-3 cells via western blotting, using a polyclonal antiserum against their corresponding neck regions. For control purposes, the expression of both lectins in MDDCs and untransfected or LSECtin-transfected COS-7 is shown.

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**Fig. 2.** LSECtin expression in human liver. Immunolocalization of LSECtin, PU.1, CD68, and CD31 on formalin-fixed, paraffin-embedded human liver tissue sections. LSECtin was detected with rabbit polyclonal antisera against its extracellular region (ADS4). The upper right panel shows the staining yielded by a preimmune rabbit antiserum. Arrowheads indicate the position of LSECtin- or CD68-positive cells in their respective panels.
corresponding proximal regulatory regions of the human, murine, and rat LSECtin genes evidenced that this putative Ets-binding sequence is highly conserved (Fig. 5A). Evaluation of the activity of the LSECtin gene proximal regulatory region revealed that three distinct constructs exhibit promoter activity well above the promoterless pXP2 plasmid (Fig. 5B). More importantly, the activity of the three constructs was significantly higher in cells of myeloid origin and with the ability to express LSECtin (THP-1) than in erythroleukemic (K562) and T lymphoid cells (Jurkat), which do not express LSECtin (Fig. 5B). Therefore, the proximal regulatory region of the LSECtin promoter preferentially functions within a myeloid context, providing a molecular explanation for the presence of the lectin in normal and leukemic myeloid cells. The relevance of the −66 putative Ets-binding element in LSECtin promoter activity was evaluated after mutation of the three putative Ets cognate sequences in
the context of the pCLEC4G−296Luc construct. As shown in Fig. 5C, pCLEC4G−296/−66MUTLuc construct exhibited significantly lower activity in the THP-1 myeloid cell line than the wild-type pCLEC4G−296Luc construct (P < 10^{-4}), while mutation of the putative Ets-binding site at −99 did not affect LSECtin proximal promoter activity (Fig. 5C). These results indicate that the preferential activity of the LSECtin gene regulatory region in myeloid cells is partly dependent on the integrity of the sequence around −66, which includes three potential binding sites for Ets family members.

Next, because PU.1 is a myeloid-restricted Ets transcription factor, we evaluated its ability to modulate the function of the LSECtin proximal promoter. In agreement with its preferential activity in myeloid cells, both Myb and RUNX3 transactivated the LSECtin regulatory region in a nonhematopoietic cellular context (Fig. 6A). More importantly, PU.1 overexpression enhanced the LSECtin promoter activity in the presence of RUNX3 (Fig. 6A), although it reduced the transactivation ability of c-Myb (Fig. 6A). Therefore, PU.1 positively modulates the LSECtin promoter activity in the presence of transcription factors which, like RUNX3, are preferentially expressed in hematopoietic cells. The positive regulatory action of PU.1 on the LSECtin promoter was mainly exerted via the −66 Ets element, because its mutation reduced RUNX3/PU.1 transactivation by more than 50% (Fig. 6B). Considering the above results, we decided to determine whether the LSECtin regulatory region was actually occupied by PU.1 in vivo. To that end, genomic DNA from LSECtin MDDCs was subjected to chromatin immunoprecipitation with an anti-PU.1 polyclonal antiserum. The LSECtin promoter was readily amplified in the anti-PU.1–precipitated DNA, whereas no amplification was detected in the DNA brought down by a control antibody or in the absence of antibody (Fig. 6C). The presence of two distinct bands derived from the LSECtin promoter (Fig. 6C) is explained by the presence
of a direct repeat within the LSECtin proximal regulatory region (Fig. 5A). Therefore, LSECtin expression correlates with that of the PU.1 transcription factor, which binds to and enhances the activity of the LSECtin proximal regulatory region in LSECtin-expressing cells of myeloid origin.

Finally, the influence of PU.1 on LSECtin mRNA expression level was assessed by a knockdown approach on LSECtin-expressing cells. Nucleofection of a PU.1–specific siRNA in THP-1 cells, which reduced PU.1 levels by more than 50% (Fig. 7A), led to down-modulation of the LSECtin mRNA levels as determined via quantitative RT-PCR (Fig. 7A). Moreover, nucleofection of the PU.1–specific siRNA also reduced the steady-state level of LSECtin mRNA in MDDCs (Fig. 7B). Therefore, decreasing PU.1 expression had a direct impact on the LSECtin RNA levels in both cell types, thus confirming the involvement of PU.1 in LSECtin gene expression.

Discussion

We herein provide evidence that the LSECtin pathogen-attachment lectin, originally described as a liver/lymph node sinusoidal-specific molecule, is expressed in cells of myeloid origin within the liver, and that the PU.1 transcription factor contributes to its restricted expression. The myeloid expression of LSECtin allows the definition of a chromosome 19 cluster of lectin-encoding genes (CD23, DC-SIGN, and LSECtin) which mediate antigen capture for subsequent presentation during immune responses. The presence of DC-SIGN and LSECtin (this study) on human Kupffer cells indicates that at least two members of this gene cluster are actively involved in scavenging and antigen capture by liver myeloid cells, and might therefore contribute to the establishment of the peripheral tolerance. As in the case of DEC-205 and DC-SIGN, the generation of LSEC-
A tin-specific reagent will be a very useful tool to evaluate its potential role as a tolerance-promoting capturing receptor. On the other hand, the pattern of expression of DC-SIGN and LSECtin, together with the presence of DC-SIGNR on liver sinusoidal endothelial cells, supports for a role of this family of C-type lectins in the scavenging function of the liver. Such a function can be inferred from a large list of pathogenic and endogenous ligands of DC-SIGN and by the restricted sugar specificity of LSECtin. Kupffer cells constitute more than 80% of the tissue macrophages present in the body and are the first macrophage population exposed to material derived from the gastrointestinal tract. The removal of bacteria-derived products and microbial debris by Kupffer cells is mediated through a large array of scavenging molecules on their cell surface, and LSECtin could be an additional molecule engaged in their clearance function. The ability of LSECtin to interact with virally encoded molecules and glycoproteins with truncated complex and hybrid N-linked glycans suggests its role as a scavenging molecule. Moreover, biochemical studies using recombinant LSECtin indicates its ability to specifically interact with serum proteins (data not shown). On the other hand, and by analogy with DC-SIGN, LSECtin could be also implicated in cell–cell adhesion. If so, LSECtin might participate in either attachment of Kupffer cells to liver sinusoidal cells or in Kupffer cell interactions with Ito cells, a process that has proven relevant during liver tissue injury and repair.

The data in the present manuscript may help resolving the issue of the cellular distribution of LSECtin in liver tissue. The immunochemical colocalization of CD68 and LSECtin in Kupffer cells is compatible with the PU.1 dependency of the LSECtin expression, since PU.1 is expressed by Kupffer cells, which are characterized by the expression of the PU.1-dependent CD68 gene. However, immunohistochemistry also revealed the existence of LSECtin-expressing cells that were devoid of CD68. Therefore, and based on data from previous reports, it is possible that LSECtin can be expressed by myeloid (Kupffer) and nonmyeloid (sinusoidal endothelial) cells. A similar conclusion has been reached for a number of scavenger and lectin receptors expressed on human lymph node sinuses. Along this line, the mannose receptor, whose expression in myeloid cells is PU.1-dependent, is expressed on both liver sinusoidal endothelial cells and Kupffer cells, and its absence from myeloid cells appears to trigger a compensatory enhancement of its expression on liver sinusoidal cells. Therefore, the expression of LSECtin on two distinct liver cell types might be indicative that its sugar-binding specificity and internalization capability contributes to both arms of the innate response: scavenging and antigen-presentation.

Regarding the regulation of LSECtin expression, the inducibility of CD23, DC-SIGN, and LSECtin by IL-4 suggests the presence of a common mechanism for their expression in myeloid cells. We have previously demonstrated that the activity of the DC-SIGN promoter is regulated by the myeloid-specific transcription factor PU.1, whose level of expression is critically dependent on the presence of IL-4. The present study demonstrates that PU.1 is also involved in the expression of LSECtin, since (1) PU.1 is constitutively bound to the LSECtin proximal promoter in vivo; (2) PU.1 potentiates the activity of the LSECtin regulatory region; and (3) PU.1 down-modulation translates into diminished levels of LSECtin mRNA in leukemic and primary LSECtin+ cells. Although induced by IL-4, the participation of PU.1 in the expression of CD23 has not been reported.
but it is tempting to hypothesize that PU.1 might also regulate CD23 expression, especially considering their overlapping patterns of expression. If this is the case, PU.1 would become an essential component for the expression of the three antigen-capturing and pathogen-attachment lectins encoded within the chromosome 19p13.2 gene cluster.

However, although PU.1 appears as a requirement for DC-SIGN and LSECtin expression, it is not sufficient for their expression, because several PU.1-expressing myeloid cell types are devoid of both lectins (e.g., neutrophils). It is therefore possible that a threshold level of PU.1 is required for both lectins to be expressed, and that IL-4 might induce their expression primarily through an increase in PU.1 expression. Alternatively, IL-4 might activate a signaling pathway/transcription factor that ultimately synergizes with PU.1 for the induction of both lectins. An obvious potential candidate would be STAT6, which functionally collaborates with PU.1, appears to bind to the CD23 gene regulatory region, and whose transcriptional activity is induced by IL-4.44 However, no obvious STAT6-binding sequences are located within the LSECtin proximal regulatory region, and STAT6 does not influence the activity of the DC-SIGN promoter in vitro in the presence of three elements with STAT6-binding ability. A second potential factor that might participate in the IL-4 inducibility of LSECtin expression is peroxisome proliferator-activated receptor gamma (PPARγ), whose activity and expression is controlled by IL-4.45 In fact, PPARγ expression is currently considered as a hallmark for the alternative activation of macrophages,46,47 which results in the induction of both LSECtin5 and DC-SIGN.10 However, the synthetic PPARγ ligand GW7845, or the PPAR antagonist inhibitor GW9662, did not modify the basal or inducible expression of either LSECtin or DC-SIGN (data not shown), thus arguing against a role for PPARγ in the control of the expression of both lectins.

In conclusion, we report that the pathogen attachment C-type lectin receptor LSECtin is expressed in Kupffer cells, and that its expression is controlled by the PU.1 transcription factor. Unlike DC-SIGN or DC-SIGNR, an obvious murine orthologue of LSECtin gene (Clec4g) has been mapped between the DC-SIGN-related cd209a and cd23 murine genes. Given its recognition specificity and internalization capability, LSECtin constitutes an additional scavenger molecule present on liver myeloid cells whose specific range of endogenous and pathogenic ligands needs to be determined. The conservation of the LSECtin gene in other mammals suggests that it might play a relevant and nonredundant function and will allow the generation of animal models in which both issues can be addressed.

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