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Expression of DC-SIGN and DC-SIGNR on Human Sinusoidal Endothelium

A Role for Capturing Hepatitis C Virus Particles

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Hepatic sinusoidal endothelial cells are unique among endothelial cells in their ability to internalize and process a diverse range of antigens. DC-SIGNR, a type 2 C-type lectin expressed on liver sinusoids, has been shown to bind with high affinity to hepatitis C virus (HCV) E2 glycoprotein. DC-SIGN is a closely related homologue reported to be expressed only on dendritic cells and a subset of macrophages and has similar binding affinity to HCV E2 glycoprotein. These receptors function as adhesion and antigen presentation molecules. We report distinct patterns of DC-SIGNR and DC-SIGN expression in human liver tissue and show for the first time that both C-type lectins are expressed on sinusoidal endothelial cells. We confirmed that these receptors are functional by demonstrating their ability to bind HCV E2 glycoproteins. Although these lectins on primary sinusoidal cells support HCV E2 binding, they are unable to support HCV entry. These data support a model where DC-SIGN and DC-SIGNR on sinusoidal endothelium provide a mechanism for high affinity binding of circulating HCV within the liver sinusoids allowing subsequent transfer of the virus to underlying hepatocytes, in a manner analogous to DC-SIGN presentation of human immunodeficiency virus on dendritic cells. ***(Am J Pathol 2006, 169:200–208; DOI: 10.2353/ajpath.2006.051191)***

Collectins comprise a family of calcium-dependent pattern-recognition lectins that bind oligosaccharide structures on the surface of microorganisms to facilitate clearance through aggregation, complement lysis, and opsonization. Two members of this family, DC-SIGN (CD209) and the related molecule DC-SIGNR (L-SIGN, CD209L), have been extensively studied for their ability to bind a variety of viral pathogens.[1–10] Indeed, these molecules are often referred to as 'viral attachment factors' and can potentiate infectivity of some viruses.[11] DC-SIGN is reported to be expressed on a subset of macrophages and dendritic cells,[12–16] whereas DC-SIGNR is expressed on endothelial cells within the liver sinusoids and lymph nodes.[11,17] DC-SIGN promotes cellular uptake and presentation of antigen and potentiates the interaction of DC-SIGN-expressing cells with leukocytes via an interaction with intercellular adhesion molecule-3.[18] Recent biochemical and structural studies suggest that DC-SIGN and DC-SIGNR have different physiological functions and distinct ligand-binding properties.[19]

Hepatitis C virus (HCV)[3] is an enveloped positive-stranded RNA virus and the sole member of the *Hepacivirus* genus, within the family Flaviviridae. Approximately 170 million individuals are infected worldwide, and the majority are at risk of developing progressive liver disease. Cellular and humoral immune responses are generated during HCV infection, but in the majority of individuals, humoral immune responses are inefficient to effect viral clearance, with ~80% of new infections becoming chronic. The liver is thought to be the primary reservoir supporting HCV replication, although studies on HCV cell entry and tropism have been limited due to

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technical difficulties in propagating infectious HCV in cell culture. However, the recent development of infectious retroviral pseudotypes bearing HCV glycoproteins (HCVpp)[20–22] and the robust replication of HCV strain JFH in cell culture (HCVcc)[8,23–25] have enabled studies on HCV cell entry. HCVpp and HCVcc show a restricted tropism for human liver cell lines, and infection is dependent on CD81 expression.[20–22,24–26] We[6] and others[2,4,5,27] demonstrated that HCVpp can interact with DC-SIGN- and DC-SIGNR-expressing cells; however, the physiological relevance of these virus-lectin interactions for HCV infection of the liver remains unclear.

Hepatic sinusoidal endothelial cells (HSECs) are unique among endothelial cells in their ability to internalize and process a diverse range of antigens.[28] In contrast to most other endothelial cells, HSECs can process and present antigen to naive CD4 T cells and cross prime CD8 T cells. This latter response may lead to antigen-specific tolerance rather than immunity, suggesting the HSECs may contribute to the tolerogenic properties of the liver.[29,30] This is of particular relevance in the setting of HCV infection if one considers its chronic nature and the apparent ineffectiveness of the cellular immune responses. A single report of DC-SIGN expression in brain microvascular endothelial cells[31] supports a model where DC-SIGN may be expressed by HSECs and contribute to their unique antigen-presenting capabilities and regulation of immune responses to pathogens entering the liver.

Here, we report distinct patterns of DC-SIGNR and DC-SIGN expression in human liver tissue and show that both C-type lectins are expressed on HSECs. We confirm that these receptors are functional by demonstrating their ability to bind HCV E2 protein and show that stimulation of isolated HSECs with interleukin-4 (IL-4) increases expression of both DC-SIGN and DC-SIGNR, promoting HCV E2 binding. However, isolated HSECs do not support HCVpp or HCVcc infection, suggesting that expression of these receptors is not sufficient to render these cells permissive for HCV infection. Expression of DC-SIGN on HSECs may allow internalization of antigens, including HCV particles for subsequent processing and presentation to naïve T cells. If these interactions result in ineffective T-cell activation or tolerance, they may contribute to the failed immune response against HCV infection.

Materials and Methods

Tissue Studied

Ethics approval for the study was given by the South Birmingham Local Research Ethics Committee (Queen Elizabeth Hospital, Birmingham, UK) and the University Hospital Birmingham Trust (Queen Elizabeth Hospital). All liver tissue was collected with informed consent. Liver tissue from nondiseased liver was used for immunohistochemical studies. Nondiseased liver was obtained either from patients undergoing hemi-hepatectomy to remove liver metastases or from organ donors in whom the liver tissue was not used for transplantation.

Isolation and Culture of Human HSECs

Liver endothelial cells were isolated from human liver tissue (surplus to surgical requirements) as previously described using a modified collagenase digestion technique.[32] Briefly, nonparenchymal cells were separated by density gradient centrifugation over metrizamide (Sigma-Aldrich, St. Louis, MO), and endothelial cells were isolated from the resultant heterogeneous cell mixture by positive immunomagnetic selection using antibodies against CD31 (M823, 10 μg/ml; Dako, Ely, UK) and Dynabeads conjugated with goat-anti-mouse monoclonal antibody (Dynal Biotech, Wirral, UK) according to the manufacturer's protocol.

Maintenance and Culture of HSECs

Following isolation, HSECs were cultured in complete medium composed of human endothelial basal growth medium (Invitrogen, Carlsbad, CA), 10% AB human serum (HD Supplies, Glasgow, UK), and 10 ng/ml vascular endothelial growth factor and 10 ng/ml hepatocyte growth factor (R&D Systems, Minneapolis, MN). The cells were propagated in collagen-coated culture flasks and maintained at 37°C in a humidified 5% CO_2 incubator until cells were confluent.

Immunohistochemistry and Dual-Color Co-Immunofluorescence

The following primary antibodies were used: DC-SIGN (MAB161, IgG2b) and DC-SIGNR (MAB162, IgG2b) from R&D Systems, LYVE-1 (8C, IgG1; a gift from David Jackson, University of Oxford), CD68 (EBM11, IgG1; from Abcam, Cambridge, UK), and mannose receptor (MCA2155, IgG1; from Serotec Ltd., Oxford, UK). 5-μm cryostat sections derived from normal liver were fixed in acetone for 10 minutes and stained using a standard alkaline phosphatase anti-alkaline phosphatase technique. Briefly, primary antibody was followed by rabbit anti-mouse monoclonal and mouse monoclonal alkaline phosphatase anti-alkaline phosphatase (Dako). The stain was developed with fast red and naphthol AS-MX phosphate substrate (Sigma-Aldrich). Sections for dual immunofluorescence were prewetted with staining buffer (phosphate-buffered saline containing 10% fetal calf serum and 0.1% sodium azide) for 10 minutes. Slides were incubated with primary antibodies diluted in staining buffer for 1 hour in a humidified chamber. Control sections were incubated with isotype-matched IgG2b or IgG1 (R&D Systems). Sections were stained with goat anti-mouse IgG2b Alexa Fluor (Molecular Probes, Eugene, OR) and goat anti-mouse IgG1 fluorescein isothiocyanate (Serotec). Immunofluorescence was assessed using AxioVision software (Carl Zeiss MicroImaging, Inc., Jena, Germany).

Measurement of Cell-Surface Molecules on HSECs

HSECs were plated at 1×10^5 ml^{-1} and grown to confluence in collagen-coated 96-well flat bottom plates (BD Falcon, Oxford, UK). Cells were left under basal conditions or stimulated with cytokines for 24 hours (10 ng/ml recombinant tumor necrosis factor-α (TNF-α), IL-17, IL-10, or 100 ng/ml recombinant IL-4, all from PeproTech, London, UK). Following stimulation the cell monolayers were fixed in methanol. Nonspecific binding of monoclonal antibody (mAb) was inhibited by preincubation of cells for 1 hour at 37°C with 4% goat serum (Sigma) before the addition of mouse-anti-human mAbs (DC-SIGN: MAB161; DC-SIGNR: MAB162 (5 μg/ml; R&D Systems); E-Selectin: M7105, 1.6 μg/ml; CD31: M0823, 2.25 μg/ml (Dako). The cells were then washed thoroughly before incubation with peroxidase-conjugated goat-anti-mouse secondary Ab (P0447 1/5000; Dako). An enzyme-linked immunosorbent assay was developed using *O*-phenylenediamine substrate (S2045; Dako) according to the manufacturer's instructions, and the enzymatic reaction was stopped by using 0.5 mol/L H_2SO_4 (Fisher Scientific, Leicestershire, UK). Colorimetric analysis was performed by measuring absorbance values at 490 nm using an MRX plate reader (Dynatech Laboratories, Sussex, UK). All treatments were performed in triplicate for each experiment.

Soluble HCV E2 Binding and Blocking Assay

293-T cells were transiently transfected with plasmids expressing HC-J4 E2661 or vector alone (control mock antigen) with Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Tissue culture supernatants containing HC-J4 E2661 were harvested 48 hours after transfection, and the amount of HC-J4 E2 antigen was quantified by enzyme immunoassay, as described previously.[33]

Normal liver sections were preincubated with isotype-matched IgG2b (control block), DC-SIGN, DC-SIGNR (5 μg/ml; both from R&D Systems), or mannan (20 μg/ml; Sigma-Aldrich) for 1 hour, washed and then incubated with mock antigen or HC-J4 E2661 at a saturating concentration in phosphate-buffered saline/1% fetal bovine serum/0.05% sodium azide/1 mmol/L $CaCl_2$ for 1 hour at room temperature, washed, and labeled with rat anti-E2 mAb 9/75, which recognizes the CD81-binding site on E2 and hence fails to interact with E2-CD81 complexes.[33] Detection of binding on normal liver section was as described above using immunofluorescence with goat anti-rat fluorescein isothiocyanate (Serotec). Immunofluorescence was assessed using AxioVision software (Carl Zeiss MicroImaging, Inc.).

Flow Cytometry

HSECs were dissociated from the flask using trypsin (Invitrogen), washed, and preincubated in human immunoglobulins to block Fc receptors. The HCV E2 binding and blocking assay was as described above. The cells were fixed in paraformaldehyde (Sigma-Aldrich), washed, and resuspended in phosphate-buffered saline/10% fetal calf serum before analysis on an Epics XL flow cytometer (Beckman Coulter, Fullerton, CA) using Summit software (DakoCytomation, Glostrup, Denmark).

HCV Pseudoparticle Generation and Infection

HCV pseudoparticles (HCVpp) were generated as previously described.[21] Briefly, 293-T cells were cotransfected with pNL4–3.Luc.R-.E- and a plasmid expressing strain H and HCJ4 E1E2 and vesicular stomatitis virus G protein using Lipofectamine 2000 (Invitrogen). Medium was replaced with Dulbecco's modified Eagle's medium/3% fetal bovine serum after 6 hours. Pseudoparticle-containing supernatants were collected after 48 hours and clarified by low speed centrifugation for 10 minutes. For infection experiments target cells were plated into 96-well plates at 1.2×10^4 per well 24 hours before infection. Pseudoparticle-containing supernatants were diluted in Dulbecco's modified Eagle's medium/3% fetal bovine serum with 4 μg/ml Polybrene with or without the desired lipoprotein species. The mixture was incubated at 37°C for 1 hour before being applied to the target cells for 4 to 6 hours. Pseudoparticles were removed, and the cells were incubated for a further 72 hours. Cells were lysed with 40 μl of Cell Culture Lysis Reagent (Promega, Madison, WI), and infection was measured by quantifying the luciferase reporter gene by the addition of 50 μl of luciferase substrate (Promega) using a Centro LB960 luminometer (Berthold Technologies, Bad Wildbad, Germany).

Generation of J6/JFH Virus Stocks and Culturing of Infected Huh-7.5 Cells with Human HSECs

Huh 7.5 cells were trypsinized, harvested by centrifugation ($500 \times g$, 5 minutes), washed twice, and resuspended in ice-cold phosphate-buffered saline at 1.5×10^7 cells/ml. One microgram of FL-J6/JFH RNA was mixed with 0.4 ml of cells in a 0.2-cm gap cuvette and pulsed using a BTX ElectroSquarePorator (Harvard Apparatus, Hollistion, MA) as described previously.[26] Elec-

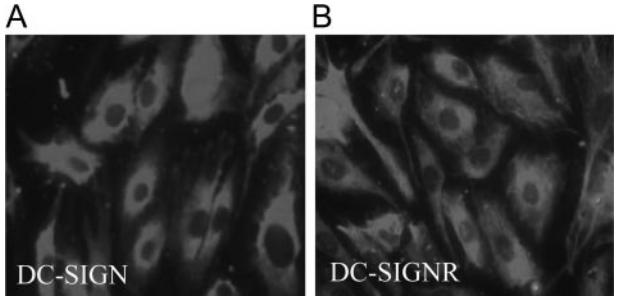

Figure 1. HSECs express both DC-SIGN and DC-SIGNR. Immunofluorescence was used to demonstrate the presence of DC-SIGN on HSEC. **A:** Primary cultures of HSEC-expressed DC-SIGN. **B:** Cells from the same preparation were also DC-SIGNR-positive.

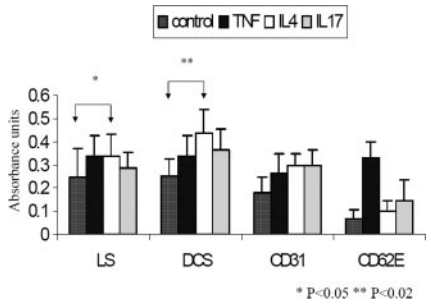

Figure 2. Treatment with IL-4 increases the expression of DC-SIGNR and DC-SIGN on primary HSEC. HSECs were stimulated with IL-4 (100 ng ml^{-1}), TNF-α (10 ng ml^{-1}), or IL-17 (10 ng ml^{-1}) for 24 hours before assessing expression of DC-SIGNR (LS), DC-SIGN (DCS), CD31, and E-Selectin (CD62E) by enzyme-linked immunosorbent assay. An equivalent volume of complete media was added to the control wells. Data represent mean ± SE of four experiments using different HSEC isolates. IL-4 induced a significant increase in DC-SIGNR and DC-SIGN expression ($P < 0.05$ and $P < 0.02$; using Student's *t*-test). * $P < 0.05$; ** $P < 0.02$.

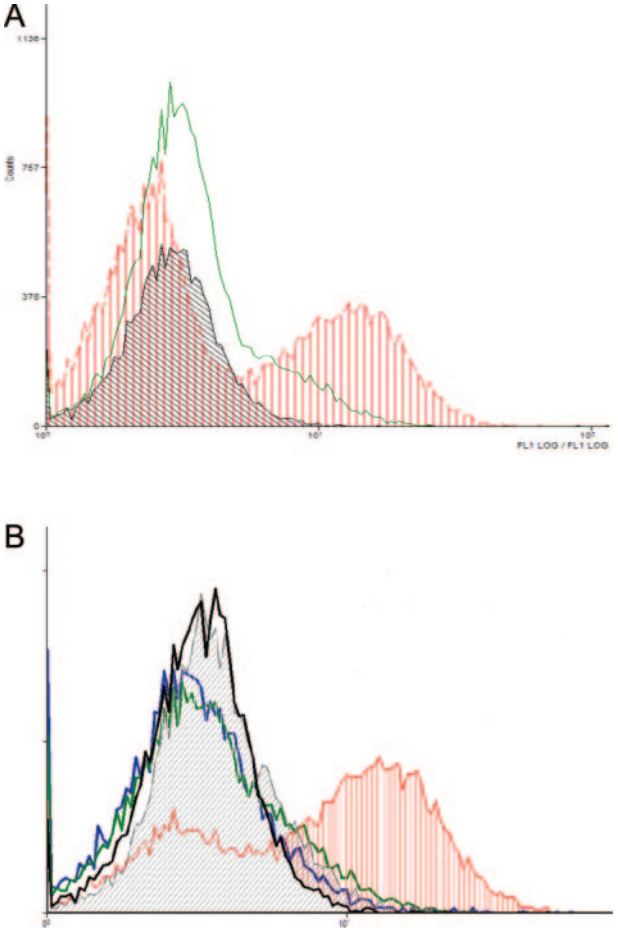

Figure 3. DC-SIGNR and DC-SIGN on HSECs can bind HCV E2. Binding assays were performed using a strain of HCV HCJ4 with minimal interaction with CD81 when expressed as a truncated E2 species and flow cytometric detection with an antibody raised against E2, which fails to detect E2-CD81 complexes. **A:** Flow cytometric analysis of HCV E2 binding following treatment with IL-4 (100 ng ml^{-1} for 24 hours) confirmed that IL-4 significantly increased the expression of both C-type lectins, which led to an increased HCV E2 interaction with the stimulated HSECs (**red**) compared to unstimulated HSECs (**green**) and control mock protein in **black**. **B:** HCV E2 bound to HSECs (**red**), and this interaction was inhibited by prior incubation of the cells with mAbs specific for DC-SIGN (**gray**) and DC-SIGNR (green line) alone or in combination (**blue**). Control binding with mock protein is in **black**.

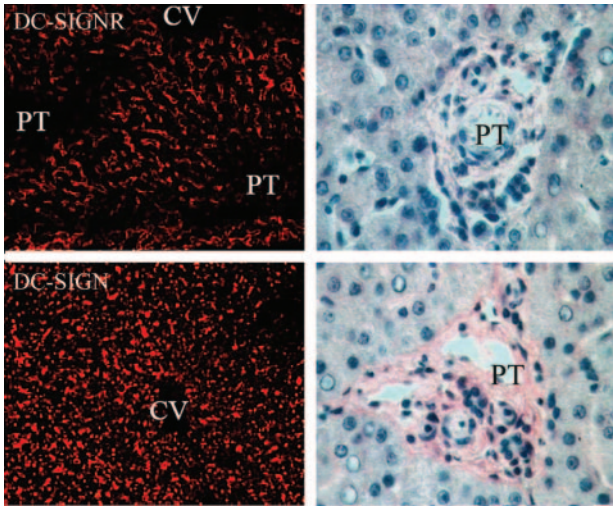

Figure 4. Distribution of DC-SIGNR and DC-SIGN expression in liver tissue. The **left panel** is a representative immunofluorescent staining (original magnification, ×10) showing DC-SIGNR is not expressed on portal tracts (PT) as shown with alkaline phosphatase anti-alkaline phosphatase staining on the **right panel** (original magnification, ×20). DC-SIGN, however, does not have a patchy distribution and is expressed around portal tracts. CV, central vein.

troporated cells were allowed to recover for 10 minutes at room temperature before the addition of complete media and plated into a 150-mm diameter cell culture dish. After 72 hours, virus containing supernatant was harvested and viral stocks stored at −80°C.

Naïve Huh-7.5 cells were infected with J6/JFH at a multiplicity of infection of 0.01, and the cells were maintained in culture for 3 days. The frequency of HCV-infected cells in the culture was investigated by staining cells for the expression of NS5A as previously described.[26] The persistently infected Huh-7.5 culture was mixed 1:4 with primary HSECs, cultured for 48 hours, and stained for HCV NS5A expression.[26]

Results

Expression and Cytokine Regulation of DC-SIGN and DC-SIGNR on HSECs

HSECs were isolated from human liver tissue, propagated in cell culture, and shown to internalize acetylated low density lipoprotein as previously described.[32] Monoclonal antibodies specific for DC-SIGN and DC-SIGNR bound the cultured cells (Figure 1, A and B). Appropriate isotype-matched controls were negative in staining (not shown). DC-SIGN expression during monocyte differentiation is dependent on the cytokine microenvironment.[34,35] To determine whether cytokines similarly regulate DC-SIGN and DC-SIGNR expression in HSECs, the cells were treated with various cytokines for 24 hours, and lectin expression was measured in a quantitative cell-based immunoassay. IL-4 significantly increased expression of DC-SIGN ($P < 0.02$) and DC-SIGNR ($P < 0.05$), TNF-α and IL-17 had a small but nonsignificant effect (Figure 2), whereas, IFN-γ and IL-12 had no detectable effect (data not shown). CD31, an endothelial

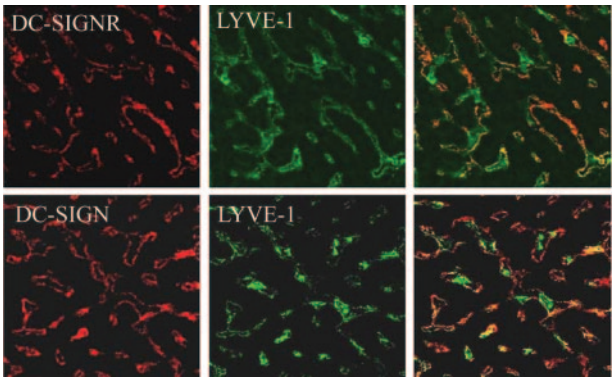

Figure 5. DC-SIGN and DC-SIGNR colocalize with LYVE-1 staining on sinusoidal endothelium. Because LYVE-1 expression in the liver is restricted to sinusoidal endothelium, we used LYVE-1 staining to confirm the cellular localization of DC-SIGN staining. DC-SIGNR and DC-SIGN are stained red and LYVE-1 green. Colocalization is demonstrated in merged images by yellow staining confirming that DC-SIGN and DC-SIGNR are expressed on sinusoidal endothelial cells (original magnification, ×20).

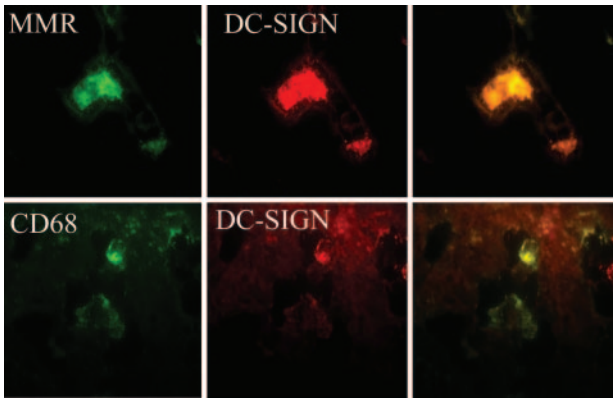

Figure 6. DC-SIGN is detected on Kupffer cells in liver tissue. Immunofluorescent staining of mannose receptor and CD68 (green) was used to detect Kupffer cells, and DC-SIGN stained red. Colocalization of the two receptors is seen as yellow staining confirming that Kupffer cells express DC-SIGN in the liver (original magnification, ×40).

marker, and E-Selectin were included as positive controls.

Expression of Functionally Active DC-SIGN and DC-SIGNR on HSECs

To determine whether these lectins are able to interact with high mannose-containing glycoproteins we tested their ability to bind a truncated version of HCV E2, previously reported to interact with DC-SIGN and DC-SIGNR.[6] Since HCV E2 interacts with the viral co-receptor CD81,[20–22,36] which is also expressed on HSECs (data not shown), we selected a strain of HCV HCJ4 that shows minimal interaction with CD81 when expressed as a truncated E2 species and detected cell-bound E2 with a mAb that fails to detect E2 when complexed with CD81.[22,33] The IL-4-stimulated expression of DC-SIGN and DC-SIGNR induced a concomitant increase in HCV E2 binding, further supporting the cytokine regulation of these functionally active C-type lectins on HSECs (Figure 3A). HCV E2 binding to IL-4-stimulated HSECs could be inhibited by prior incubation of the cells with mAbs specific for either DC-SIGN or DC-SIGNR. Preincubation with combinations of both DC-SIGN and DC-SIGNR mAbs did not alter the inhibition of HCV E2 binding seen with the single mAb alone (Figure 3B). The finding of complete block with each antibody is surprising. One possible interpretation derives from the observation that these lectins exist as tetramers at the cell surface.[37,38] Our finding that primary HSECs express both receptors may support a model for hetero-oligomer formation between the two related lectins, which may explain our observations with antibody blocking HCV E2 to these primary cell types. We have shown previously that the mAbs used in this study bind specifically to 293-T cells transduced to express the appropriate receptors.[39]

DC-SIGN and DC-SIGNR Expression in Liver Tissue

To evaluate the distribution of these lectins in normal human liver we examined the ability of DC-SIGN- and DC-SIGNR-specific mAbs to react with cells within tissue sections by immunohistochemistry. The staining pattern of the two receptors differed: DC-SIGNR was only expressed within the liver sinusoids, whereas DC-SIGN was detected on portal tracts in addition to the hepatic sinusoidal endothelium (Figure 4). DC-SIGN and DC-SIGNR expression by endothelial cells in the sinusoids was confirmed by dual staining with anti-LYVE-1 antibody, which selectively stains sinusoidal endothelial cells in human liver (Figure 5).[40] DC-SIGN expression on Kupffer cells was demonstrated by dual staining with the macrophage mannose receptor-positive cells and CD68 (Figure 6). There appear to be differences in the intensity of DC-SIGN-R staining across the sinusoidal bed with a relatively weaker staining in the periportal sinusoids. This may reflect differences in the local microenvironment, a conclusion that is supported by the observations that other proteins, including adhesion molecules, are differentially expressed across hepatic sinusoids. Appropriate isotype-matched controls were negative in staining (data not shown).

To assess whether DC-SIGN and DC-SIGNR expressed in normal liver are able to interact with HCV E2, intact liver sections were incubated with truncated E2 from HC-J4 E2661 and bound antigen visualized with rat anti-E2 antibody 9/75, which recognizes the CD81-binding site on E2 and hence fails to interact with E2-CD81 complexes. E2 bound diffusely and in focal areas within the sinusoids, and binding could be inhibited by mannan, a sugar molecule reported to interact with the carbohydrate recognition sites of C-type lectins (Figure 7, A and B). To confirm the identity of cells that bound HCV E2, sections were counterstained with mAbs specific for macrophage mannose receptor, CD68, and DC-SIGNR (Figure 8, A and B). These data confirm that HCV E2 interacts with both Kupffer and sinusoidal endothelial

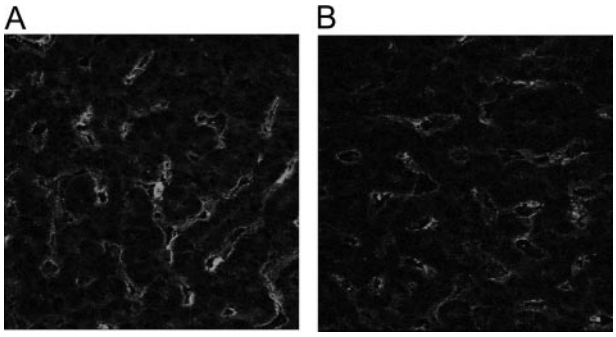

Figure 7. HCV E2 glycoprotein binding to sinusoids in normal liver tissue can be blocked by mannan. **A:** HCV E2 glycoprotein binding on normal liver tissue is seen on sinusoids and plump cells with Kupffer cell morphology. **B:** The HCV E2 binding can be inhibited by preincubating with mannan (20 μg/ml) for 1 hour before the addition of HCV E2 glycoprotein resulting in the attenuation of both the sinusoidal and Kupffer cell staining. Original magnification, ×20.

cells within the liver, consistent with the pattern of DC-SIGN and DC-SIGNR expression. To confirm that HCV E2 binding was dependent on DC-SIGN and DC-SIGNR expression, liver sections were preincubated with mAbs specific for DC-SIGN and DC-SIGNR and evaluated for their ability to bind HCV E2. Pretreatment of liver sections with a control isotype-matched IgG2b mouse immunoglobulin had no effect on E2 binding (Figure 9A). The mAb specific for DC-SIGN inhibited E2 binding to both Kupffer and sinusoidal cells (Figure 9B), whereas the

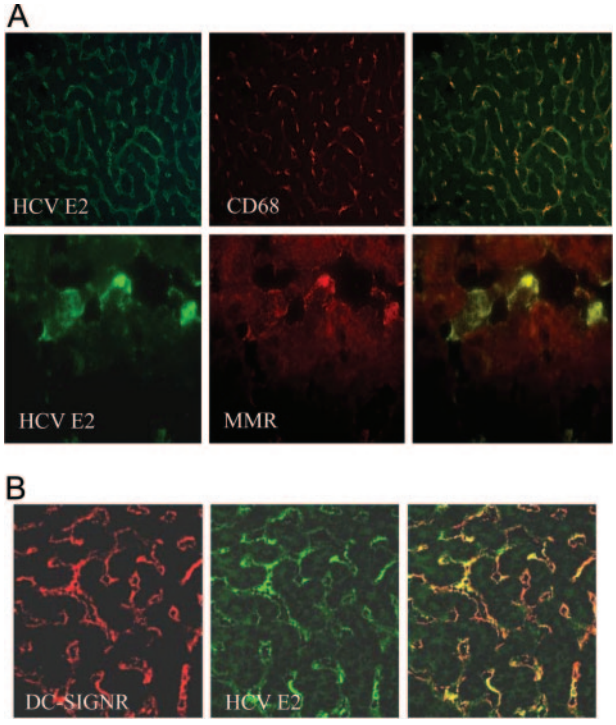

Figure 8. HCV E2 binds to Kupffer cells and sinusoidal endothelial cells in liver tissue. **A:** Kupffer cells labeled with macrophage mannose receptor (original magnification ×40, **lower panel**) and CD68 (original magnification ×20, **upper panel**) in red are seen clearly in the sinusoidal space, and HCV E2 (green) is shown binding to these cells. Colocalization of macrophage mannose receptor and CD68 with HCV E2 is demonstrated by the yellow staining in merged images. **B:** HCV E2 (green) also binds to DC-SIGNR-expressing sinusoidal endothelium (red) as confirmed by colocalization (yellow). Original magnification, ×20.

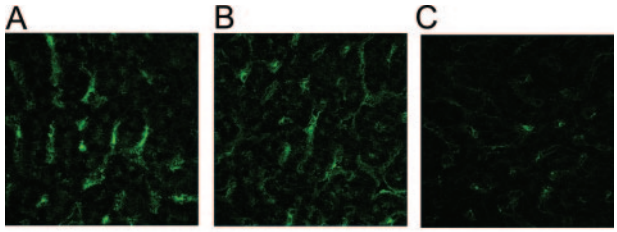

Figure 9. HCV E2 binds to normal sinusoidal endothelium and Kupffer cells via DC-SIGNR and DC-SIGN. **A:** Normal liver sections were preincubated with an isotype IgG2b control antibody an hour before labeling with HCV E2 glycoprotein (green). The staining pattern reveals sinusoidal and Kupffer cell distribution. **B:** When preincubated with DC-SIGN mAb (5 μg/ml) the binding of HCV E2 to sinusoidal and Kupffer cells was attenuated. **C:** Preincubation with DC-SIGNR (5 μg ml^{-1}) only attenuated binding to sinusoidal endothelium. Original magnification, ×20.

mAb specific for DC-SIGNR only attenuated E2 binding to sinusoidal cells (Figure 9C). However, we also used E2 derived from HCV strain H77, which binds with high affinity to CD81. To distinguish E2-H77 interacting with CD81 and the lectins DC-SIGN(R), we used two anti-E2 mAbs that could discriminate between E2 bound to CD81. These experiments allowed us to confirm the results we obtained with E2-HCJ4 with an independent strain of E2 (data not shown). These data conclusively show DC-SIGN and DC-SIGNR expressed in normal liver are able to interact with HCV E2.

HSECs Do Not Support HCV Infection

As a model for studying HCV interaction with DC-SIGN/DC-SIGNR in the liver, several investigators have expressed DC-SIGN and DC-SIGNR in heterologous cell systems and studied HCVpp interactions.[4–6,35] HCVpp fails to infect cells engineered to express DC-SIGN and DC-SIGNR; however, infectivity can be transferred to permissive hepatoma cells,[41,42] suggesting that these lectin molecules may act as 'transfer receptors' within the liver, enabling endothelial cells to 'trap and concentrate' HCV within the sinusoids and to transfer virus to the permissive underlying hepatocytes. However, the assumption that DC-SIGN/DC-SIGNR expressed in heterologous cells accurately mimics primary HSEC lectin-dependent interactions with HCV is undermined by a recent report[43] that DC-SIGN interaction with human immunodeficiency virus is dependent on the cellular context. To address this issue we purified HSECs from three donor livers, confirmed CD81, DC-SIGN, and DC-SIGNR expression, and evaluated their ability to support HCVpp entry. Virus entry and uncoating is quantified by luciferase expression in the target cell under the control of the retroviral promoter and is directly proportional to the frequency of infected cells.[21] Pseudotypic particles with no envelope glycoproteins were used to control for nonspecific particle uptake and viruses expressing vesicular stomatitis virus G served as a control for retroviral RNA transcription and translation of the luciferase reporter gene within the primary cells. HCVpp bearing strain HCJ4 and H77 glycoproteins infected Hep3B hepatoma cells and primary human hepatocytes but failed to infect HSECs (Figure 10). Vesicular stomatitis virus pseudoparticle infected all

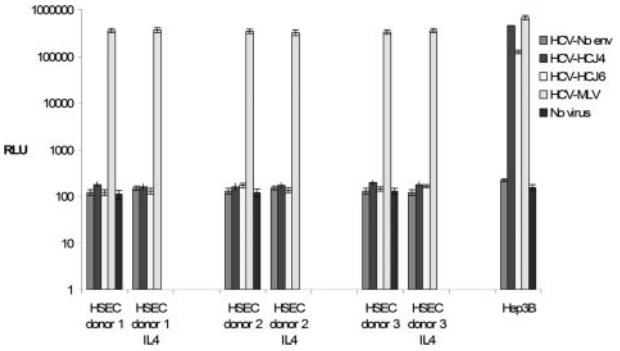

Figure 10. Sinusoidal endothelium does not support HCVpp viral infection. Because HSECs can support binding of HCV glycoproteins, we went on to determine whether they can also support viral infection. HSECs were prepared from three different livers, and all infections were preformed in triplicates. HCVpp bearing strain HCJ4 and H77 glycoproteins infected Hep3B hepatoma cells but failed to infect HSECs. Preincubating the HSECs with IL-4 (100 ng/ml) for 24 hours did not alter the results. HCV-MLV and HCV-No env served as positive and negative control experiments, respectively. Successful infection was measured by quantifying the luciferase reporter gene and expressed as relative light units (RLU).

target cells with different efficiencies, and particles with no envelope glycoproteins gave background signals comparable to uninfected cells. Treatment of HSECs with IL-4 24 hours before infection did not alter the results. To establish whether J6/JFH HCVcc could infect HSECs, we cocultured J6/JFH-infected Huh-7.5 cells with primary HSECs. After 48 hours, HCV infection in the isolated and cocultured cells was determined by immunostaining for HCV NS5A antigen. Viral antigen was only detected in the Huh-7.5 cells (Figure 11), consistent with the HSECs cells failing to support HCVpp infection. These data confirm that sinusoidal endothelial cells are not permissive for HCV infection.

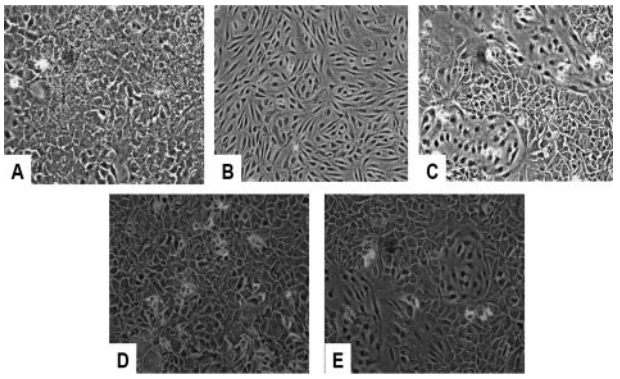

Figure 11. Infection of Huh-7.5 cells, but not primary HSECs, with J6/JFH HCVcc in coculture. Huh-7.5 cells (**A**) and primary HSECs (**B**) have contrasting and distinctive morphologies in culture. To assess whether primary HSECs can be infected with J6/JFH, HSECs were cultured with persistently infected Huh-7.5 cells. The cells were mixed at an Huh-7.5:HSEC ratio of 1:4 and seeded at 200,000 cells per well in collagen coated 6-well plates. The following day the coculture had a distinct appearance in which HSECs and Huh-7.5 cells formed discrete foci (**C**). A control population of J6/JFH-infected Huh-7.5 cells was seeded at the same density in the absence of HSECs (**D**). Cocultures were maintained for 48 hours, and J6/JFH infection was monitored by staining for HCV NS5A antigen. Viral antigen was detected in the persistently infected Huh-7.5 cells (**D**) and within the coculture (**E**). Viral antigen was only detected in the Huh-7.5 cells and not within the HSECs, consistent with primary HSECs failing to support HCVpp infection (Figure 10).

Discussion

Our findings demonstrate that human HSECs express DC-SIGNR and DC-SIGN *in vivo* and that both receptors can interact with HCV E2. Expression of DC-SIGN by HSECs provides further evidence for the ability of HSECs to process and present microbial antigens. We show that primary HSECs are unable to support HCVpp and HCVcc entry. These data are consistent with a model where DC-SIGN and DC-SIGNR on sinusoidal endothelium provide a mechanism for high affinity binding of circulating HCV within the liver sinusoids allowing transfer of the virus to underlying hepatocytes, in a manner analogous to dendritic cell DC-SIGN presenting HIV to T lymphocytes.[41] Viral capture at the cell surface can be rate limiting for infection,[11,44,45] suggesting that expression of both DC-SIGN and DC-SIGNR on HSECs may enhance the rate and efficiency of virus infection of hepatocytes expressing the coreceptors, CD81 and scavenger receptor BI.[11,46]

DC-SIGN is able to recognize highly mannosylated glycoproteins at the surface of a broad range of pathogens, including viruses, bacteria, fungi, and parasites.[47,48] For at least some of these agents, this interaction appears to be an important component of the infection process, as demonstrated for human immunodeficiency virus and Ebola virus, making DC-SIGN an attractive target for drug design.[47,49,50] Glycodendritic polymers that block the binding of pathogen glycoproteins to DC-SIGN inhibit DC-SIGN-mediated infection in an Ebola-pseudotyped viral model.[47,49] In addition, these lectins may provide a viral escape mechanism as uptake of HCV by DC-SIGN and DC-SIGNR has been reported to target nonlysosomal compartments in immature DCs, whereas Lewis X antigen, another ligand of DC-SIGN, was internalized to lysosomes.[51] Thus, DC-SIGN and DC-SIGNR on HSECs may not only act to capture HCV from blood but may also allow HSECs to act as reservoirs for HCV, allowing the virus to avoid detection and to be transmitted to the underlying hepatocyte, the primary target for HCV.

The detection of functional DC-SIGN on HSECs is further evidence of the unique antigen-capturing and -scavenging properties of these cells. HSECs can act as organ-resident, nonmyeloid antigen processing cells by cross-presenting soluble exogenous antigen to CD8+ T cells using similar mechanisms of antigen processing and presentation as dendritic cells. However, the outcome of cross-presentation by HSECs is CD8+ T cell tolerance, and major histocompatibility class II-restricted antigen presentation by HSECs to naive $CD4^+$ T cells leads to differentiation into IL-4/IL-10-expressing Th2 cells.[30,52] The presence of DC-SIGN on HSECs may allow these cells to capture and endocytose a range of microbial pathogens for subsequent presentation to the immune system. Furthermore, DC-SIGN can also act as an adhesion receptor to support interactions with intercellular adhesion molecule-3 on T cells during antigen presentation and on dendritic cells during transendothelial migration, extending the potential role of HSECs in immune responses.

We found that IL-4 but not IL-12, interferon-γ, TNF-α, or IL-17 increased expression of DC-SIGN on HSECs *in vitro*. IL-4 has been shown to increase expression of DC-SIGN on monocytes and THP-1 cells.[34,35] In monocytes IL-4 treatment favors differentiation into dendritic cells rather than macrophages,[53,54] and evidence suggests that IL-4 is the key cytokine for DC-SIGN acquisition during monocyte-derived dendritic cell differentiation. Our findings provide evidence that DC-SIGN expression is IL-4-dependent in endothelial cells as well as myeloid cells, although we did not see suppression of DC-SIGN by TNF-α treatment as reported with macrophages.[34,35] The lack of response to IL-12 and interferon-γ suggests that induction of DC-SIGN on HSECs will be promoted by Th2 rather than Th1 cytokine responses consistent with the requirement for vigorous Th1 responses to clear viral infection.

In conclusion, this report is the first description of DC-SIGN expression on sinusoidal endothelium and provides evidence of the unique antigen-processing and -scavenging properties of these cells. The distinct distribution of DC-SIGN and DC-SIGNR in the liver and the ability of both lectins to bind HCV E2 *in vivo* suggest a complementary role in trapping HCV within the hepatic sinusoids. The up-regulation of DC-SIGN and DC-SIGNR by IL-4 suggests that the local cytokine microenvironment may affect HCV cell attachment and infectivity.

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References

1. Alvarez CP, Lasala F, Carrillo J, Muniz O, Corbi AL, Delgado R: C-type lectins DC-SIGN and L-SIGN mediate cellular entry by Ebola virus in cis and in trans. J Virol 2002, 76:6841–6844
2. Gardner JP, Durso RJ, Arrigale RR, Donovan GP, Maddon PJ, Dragic T, Olson WC: L-SIGN (CD 209L) is a liver-specific capture receptor for hepatitis C virus. Proc Natl Acad Sci USA 2003, 100:4498–4503
3. Klimstra WB, Nangle EM, Smith MS, Yurochko AD, Ryman KD: DC-SIGN and L-SIGN can act as attachment receptors for alphaviruses and distinguish between mosquito cell- and mammalian cell-derived viruses. J Virol 2003, 77:12022–12032
4. Lozach PY, Amara A, Bartosch B, Virelizier JL, Arenzana-Seisdedos F, Cosset FL, Altmeyer R: C-type lectins L-SIGN and DC-SIGN capture and transmit infectious hepatitis C virus pseudotype particles. J Biol Chem 2004, 279:32035–32045
5. Lozach PY, Lortat-Jacob H, de Lacroix d, Staropoli I, Foung S, Amara A, Houles C, Fieschi F, Schwartz O, Virelizier JL, Arenzana-Seisdedos F, Altmeyer R: DC-SIGN and L-SIGN are high affinity binding receptors for hepatitis C virus glycoprotein E2. J Biol Chem 2003, 278:20358–20366
6. Pohlmann S, Zhang J, Baribaud F, Chen Z, Leslie GJ, Lin G, Granelli-Piperno A, Doms RW, Rice CM, McKeating JA: Hepatitis C virus glycoproteins interact with DC-SIGN and DC-SIGNR. J Virol 2003, 77:4070–4080
7. Tassaneetrithep B, Burgess TH, Granelli-Piperno A, Trumpfheller C, Finke J, Sun W, Eller MA, Pattanapanyasat K, Sarasombath S, Birx DL, Steinman RM, Schlesinger S, Marovich MA: DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. J Exp Med 2003, 197:823–829
8. Halary F, Amara A, Lortat-Jacob H, Messerle M, Delaunay T, Houles C, Fieschi F, Arenzana-Seisdedos F, Moreau JF, Dechanet-Merville J: Human cytomegalovirus binding to DC-SIGN is required for dendritic cell infection and target cell trans-infection. Immunity 2002, 17:653–664
9. Marzi A, Gramberg T, Simmons G, Moller P, Rennekamp AJ, Krumbiegel M, Geier M, Eisemann J, Turza N, Saunier B, Steinkasserer A, Becker S, Bates P, Hofmann H, Pohlmann S: DC-SIGN and DC-SIGNR interact with the glycoprotein of Marburg virus and the S protein of severe acute respiratory syndrome coronavirus. J Virol 2004, 78:12090–12095
10. Yang ZY, Huang Y, Ganesh L, Leung K, Kong WP, Schwartz O, Subbarao K, Nabel GJ: pH-dependent entry of severe acute respiratory syndrome coronavirus is mediated by the spike glycoprotein and enhanced by dendritic cell transfer through DC-SIGN. J Virol 2004, 78:5642–5650
11. Bashirova AA, Geijtenbeek TB, van Duijnhoven GC, van Vliet SJ, Eilering JB, Martin MP, Wu L, Martin TD, Viebig N, Knolle PA, Kewal-Ramani VN, van Kooyk Y, Carrington M: A dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN)-related protein is highly expressed on human liver sinusoidal endothelial cells and promotes HIV-1 infection. J Exp Med 2001, 193:671–678
12. Krutzik SR, Tan B, Li H, Ochoa MT, Liu PT, Sharfstein SE, Graeber TG, Sieling PA, Liu YJ, Rea TH, Bloom BR, Modlin RL: TLR activation triggers the rapid differentiation of monocytes into macrophages and dendritic cells. Nat Med 2005, 11:653–660
13. McCully ML, Chau TA, Luke P, Blake PG, Madrenas J: Characterization of human peritoneal dendritic cell precursors and their involvement in peritonitis. Clin Exp Immunol 2005, 139:513–525
14. Jameson B, Baribaud F, Pohlmann S, Ghavimi D, Mortari F, Doms RW, Iwasaki A: Expression of DC-SIGN by dendritic cells of intestinal and genital mucosae in humans and rhesus macaques. J Virol 2002, 76:1866–1875
15. Turville SG, Cameron PU, Handley A, Lin G, Pohlmann S, Doms RW, Cunningham AL: Diversity of receptors binding HIV on dendritic cell subsets. Nat Immunol 2002, 3:975–983
16. Soilleux EJ, Morris LS, Leslie G, Chehimi J, Luo Q, Levroney E, Trowsdale J, Montaner LJ, Doms RW, Weissman D, Coleman N, Lee B: Constitutive and induced expression of DC-SIGN on dendritic cell and macrophage subpopulations in situ and in vitro. J Leukoc Biol 2002, 71:445–457
17. Pohlmann S, Soilleux EJ, Baribaud F, Leslie GJ, Morris LS, Trowsdale J, Lee B, Coleman N, Doms RW: DC-SIGNR, a DC-SIGN homologue expressed in endothelial cells, binds to human and simian immunodeficiency viruses and activates infection in trans. Proc Natl Acad Sci USA 2001, 98:2670–2675
18. Engering A, Geijtenbeek TB, van Vliet SJ, Wijers M, van Liempt E, Demaurex N, Lanzavecchia A, Fransen J, Figdor CG, Piguet V, van Kooyk Y: The dendritic cell-specific adhesion receptor DC-SIGN internalizes antigen for presentation to T cells. J Immunol 2002, 168:2118–2126
19. Guo Y, Feinberg H, Conroy E, Mitchell DA, Alvarez R, Blixt O, Taylor ME, Weis WI, Drickamer K: Structural basis for distinct ligand-binding and targeting properties of the receptors DC-SIGN and DC-SIGNR. Nat Struct Mol Biol 2004, 11:591–598
20. Bartosch B, Vitelli A, Granier C, Goujon C, Dubuisson J, Pascale S, Scarselli E, Cortese R, Nicosia A, Cosset FL: Cell entry of hepatitis C virus requires a set of co-receptors that include the CD81 tetraspanin and the SR-B1 scavenger receptor. J Biol Chem 2003, 278:41624–41630
21. Hsu M, Zhang J, Flint M, Logvinoff C, Cheng-Mayer C, Rice CM, McKeating JA: Hepatitis C virus glycoproteins mediate pH-dependent cell entry of pseudotyped retroviral particles. Proc Natl Acad Sci USA 2003, 100:7271–7276
22. Zhang J, Randall G, Higginbottom A, Monk P, Rice CM, McKeating JA: CD81 is required for hepatitis C virus glycoprotein-mediated viral infection. J Virol 2004, 78:1448–1455
23. Lindenbach BD, Rice CM: Unravelling hepatitis C virus replication from genome to function. Nature 2005, 436:933–938
24. Zhong J, Gastaminza P, Cheng G, Kapadia S, Kato T, Burton DR, Wieland SF, Uprichard SL, Wakita T, Chisari FV: Robust hepatitis C virus infection in vitro. Proc Natl Acad Sci USA 2005, 102:9294–9299
25. Wakita T, Pietschmann T, Kato T, Date T, Miyamoto M, Zhao Z, Murthy

K, Habermann A, Krausslich HG, Mizokami M, Bartenschlager R, Liang TJ: Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. Nat Med 2005, 11:791–796
26. Lindenbach BD, Evans MJ, Syder AJ, Wolk B, Tellinghuisen TL, Liu CC, Maruyama T, Hynes RO, Burton DR, McKeating JA, Rice CM: Complete replication of hepatitis C virus in cell culture. Science 2005, 309:623–626
27. Cormier EG, Tsamis F, Kajumo F, Durso RJ, Gardner JP, Dragic T: CD81 is an entry coreceptor for hepatitis C virus. Proc Natl Acad Sci USA 2004, 101:7270–7274
28. Seternes T, Sorensen K, Smedsrod B: Scavenger endothelial cells of vertebrates: a nonperipheral leukocyte system for high-capacity elimination of waste macromolecules. Proc Natl Acad Sci USA 2002, 99:7594–7597
29. Crispe IN: Hepatic T cells and liver tolerance. Nat Rev Immunol 2003, 3:51–62
30. Knolle PA, Schmitt E, Jin S, Germann T, Duchmann R, Hegenbarth S, Gerken G, Lohse AW: Induction of cytokine production in naive CD4(+) T cells by antigen-presenting murine liver sinusoidal endothelial cells but failure to induce differentiation toward Th1 cells. Gastroenterology 1999, 116:1428–1440
31. Mukhtar M, Harley S, Chen P, BouHamdan M, Patel C, Acheampong E, Pomerantz RJ: Primary isolated human brain microvascular endothelial cells express diverse HIV/SIV-associated chemokine coreceptors and DC-SIGN and L-SIGN. Virology 2002, 297:78–88
32. 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, 169:983–992
33. Flint M, Maidens C, Loomis-Price LD, Shotton C, Dubuisson J, Monk P, Higginbottom A, Levy S, McKeating JA: Characterization of hepatitis C virus E2 glycoprotein interaction with a putative cellular receptor, CD81. J Virol 1999, 73:6235–6244
34. Puig-Kroger A, Serrano-Gomez D, Caparros E, Dominguez-Soto A, Relloso M, Colmenares M, Martinez-Munoz L, Longo N, Sanchez-Sanchez N, Rincon M, Rivas L, Sanchez-Mateos P, Fernandez-Ruiz E, Corbi AL: Regulated expression of the pathogen receptor dendritic cell-specific intercellular adhesion molecule 3 (ICAM-3)-grabbing nonintegrin in THP-1 human leukemic cells, monocytes, and macrophages. J Biol Chem 2004, 279:25680–25688
35. Relloso M, Puig-Kroger A, Pello OM, Rodriguez-Fernandez JL, de la RG, Longo N, Navarro J, Munoz-Fernandez MA, Sanchez-Mateos P, Corbi AL: DC-SIGN (CD209) expression is IL-4 dependent and is negatively regulated by IFN, TGF-beta, and anti-inflammatory agents. J Immunol 2002, 168:2634–2643
36. Pileri P, Uematsu Y, Campagnoli S, Galli G, Falugi F, Petracca R, Weiner AJ, Houghton M, Rosa D, Grandi G, Abrignani S: Binding of hepatitis C virus to CD81. Science 1998, 282:938–941
37. Feinberg H, Guo Y, Mitchell DA, Drickamer K, Weis WI: Extended neck regions stabilize tetramers of the receptors DC-SIGN and DC-SIGNR. J Biol Chem 2005, 280:1327–1335
38. Mitchell DA, Fadden AJ, Drickamer K: A novel mechanism of carbohydrate recognition by the C-type lectins DC-SIGN and DC-SIGNR. Subunit organization and binding to multivalent ligands. J Biol Chem 2001, 276:28939–28945
39. Pohlmann S, Leslie GJ, Edwards TG, Macfarlan T, Reeves JD, Hiebenthal-Millow K, Kirchhoff F, Baribaud F, Doms RW: DC-SIGN interactions with human immunodeficiency virus: virus binding and transfer are dissociable functions. J Virol 2001, 75:10523–10526
40. Grant AJ, Goddard S, Ahmed-Choudhury J, Reynolds G, Jackson DG, Briskin M, Wu L, Hubscher SG, Adams DH: Hepatic expression of secondary lymphoid chemokine (CCL21) promotes the development of portal-associated lymphoid tissue in chronic inflammatory liver disease. Am J Pathol 2002, 160:1445–1455
41. Cormier EG, Durso RJ, Tsamis F, Boussemart L, Manix C, Olson WC, Gardner JP, Dragic T: L-SIGN (CD209L) and DC-SIGN (CD209) mediate transinfection of liver cells by hepatitis C virus. Proc Natl Acad Sci USA 2004, 101:14067–14072
42. Lozach PY, Amara A, Bartosch B, Virelizier JL, Arenzana-Seisdedos F, Cosset FL, Altmeyer R: C-type lectins L-SIGN and DC-SIGN capture and transmit infectious hepatitis C virus pseudotype particles. J Biol Chem 2004, 279:32035–32045
43. Trumpfheller C, Park CG, Finke J, Steinman RM, Granelli-Piperno A: Cell type-dependent retention and transmission of HIV-1 by DC-SIGN. Int Immunol 2003, 15:289–298
44. Lee B, Leslie G, Soilleux E, O'Doherty U, Baik S, Levroney E, Flummerfelt K, Swiggard W, Coleman N, Malim M, Doms RW: cis Expression of DC-SIGN allows for more efficient entry of human and simian immunodeficiency viruses via CD4 and a coreceptor. J Virol 2001, 75:12028–12038
45. Pohlmann S, Baribaud F, Lee B, Leslie GJ, Sanchez MD, Hiebenthal-Millow K, Munch J, Kirchhoff F, Doms RW: DC-SIGN interactions with human immunodeficiency virus type 1 and 2 and simian immunodeficiency virus. J Virol 2001, 75:4664–4672
46. Geijtenbeek TB, Kwon DS, Torensma R, van Vliet SJ, van Duijnhoven GC, Middel J, Cornelissen IL, Nottet HS, Kewal Ramani VN, Littman DR, Figdor CG, van Kooyk Y: DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. Cell 2000, 100:587–597
47. Appelmelk BJ, Van D, I, van Vliet SJ, Vandenbroucke-Grauls CM, Geijtenbeek TB, van Kooyk Y: Cutting edge: carbohydrate profiling identifies new pathogens that interact with dendritic cell-specific ICAM-3-grabbing nonintegrin on dendritic cells. J Immunol 2003, 170:1635–1639
48. Maeda N, Nigou J, Herrmann JL, Jackson M, Amara A, Lagrange PH, Puzo G, Gicquel B, Neyrolles O: The cell surface receptor DC-SIGN discriminates between Mycobacterium species through selective recognition of the mannose caps on lipoarabinomannan. J Biol Chem 2003, 278:5513–5516
49. Lasala F, Arce E, Otero JR, Rojo J, Delgado R: Mannosyl glycodendritic structure inhibits DC-SIGN-mediated Ebola virus infection in cis and in trans. Antimicrob Agents Chemother 2003, 47:3970–3972
50. Simmons G, Reeves JD, Grogan CC, Vandenberghe LH, Baribaud F, Whitbeck JC, Burke E, Buchmeier MJ, Soilleux EJ, Riley JL, Doms RW, Bates P, Pohlmann S: DC-SIGN and DC-SIGNR bind ebola glycoproteins and enhance infection of macrophages and endothelial cells. Virology 2003, 305:115–123
51. Ludwig IS, Lekkerkerker AN, Depla E, Bosman F, Musters RJ, Depraetere S, van Kooyk Y, Geijtenbeek TB: Hepatitis C virus targets DC-SIGN and L-SIGN to escape lysosomal degradation. J Virol 2004, 78:8322–8332
52. Limmer A, Ohl J, Kurts C, Ljunggren HG, Reiss Y, Groettrup M, Momburg F, Arnold B, Knolle PA: Efficient presentation of exogenous antigen by liver endothelial cells to CD8+ T cells results in antigen-specific T-cell tolerance. Nat Med 2000, 6:1348–1354
53. Ebner S, Hofer S, Nguyen VA, Furhapter C, Herold M, Fritsch P, Heufler C, Romani N: A novel role for IL-3: human monocytes cultured in the presence of IL-3 and IL-4 differentiate into dendritic cells that produce less IL-12 and shift Th cell responses toward a Th2 cytokine pattern. J Immunol 2002, 168:6199–6207
54. Romani N, Gruner S, Brang D, Kampgen E, Lenz A, Trockenbacher B, Konwalinka G, Fritsch PO, Steinman RM, Schuler G: Proliferating dendritic cell progenitors in human blood. J Exp Med 1994, 180:83–93