Scavenger Receptor Class B Is Required for Hepatitis C Virus Uptake and Cross-Presentation by Human Dendritic Cells

Heidi Barth,1,2* Eva K. Schnober,1,3,4 Christoph Neumann-Haefelin,1 Christine Thumann,1,5 Mirjam B. Zeisel,3,5 Helmut M. Diepolder,1 Zongyi Hu,2 T. Jake Liang,2 Hubert E. Blum,1 Robert Thimme,1 Mélanie Lambotin,3,5 and Thomas F. Baumert1,3,5,7*

Department of Medicine II, University of Freiburg, Freiburg, Germany1; Liver Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland2; Inserm, U748, Strasbourg, France3; Faculty of Biology, University of Freiburg, Freiburg, Germany4; Université Louis Pasteur, Strasbourg, France5; Department of Medicine II, Klinikum Grosshadern, University of Munich, Munich, Germany6; and Service d’Hépatogastroentérologie, Centre Hospitalier Universitaire Strasbourg, Strasbourg, France7

Class B scavenger receptors (SR-Bs) bind lipoproteins and play an important role in lipid metabolism. Most recently, SR-B type I (SR-BI) and its splicing variant SR-BII have been found to mediate bacterial adhesion and cytosolic bacterial invasion in mammalian cells. In this study, we demonstrate that SR-BI is a key host factor required for hepatitis C virus (HCV) uptake and cross-presentation by human dendritic cells (DCs). Whereas monocytes and T and B cells were characterized by very low or undetectable SR-BI expression levels, human DCs demonstrated a high level of cell surface expression of SR-BI similar to that of primary human hepatocytes. Antibodies targeting the extracellular loop of SR-BI efficiently inhibited HCV-like particle binding, uptake, and cross-presentation by human DCs. Moreover, human high-density lipoprotein specifically modulated HCV-like particle binding to DCs, indicating an interplay of HCV with the lipid transfer function of SR-BI in DCs. Finally, we demonstrate that anti-SR-BI antibodies inhibit the uptake of cell culture-derived HCV (HCVcc) in DCs. In conclusion, these findings identify a novel function of SR-BI for viral antigen uptake and recognition and may have an important impact on the design of HCV vaccines and immunotherapeutic approaches aiming at the induction of efficient antiviral immune responses.

Scavenger receptor class B type I (SR-BI) and its splicing variant SR-BII are human high-density lipoprotein (HDL) receptors with an identical extracellular domain. These receptors mediate HDL binding, followed by selective uptake of cholesterol and cholesteryl ester in the liver and steroidogenic tissues (16). Recently, SR-BI and SR-BII have been found to mediate the binding and uptake of a broad range of bacteria into nonphagocytic human epithelial cells overexpressing SR-BI and SR-BII (50, 60), suggesting that SR-Bs may serve as pattern recognition receptors for bacteria. Furthermore, most recent studies have indicated that SR-BI is an important host entry factor for hepatitis C virus (HCV) infection of hepatocytes (25, 31, 69).

HCV is a noncytopathic, hepatotropic member of the Flaviviridae family that causes chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (13). Resolution of HCV infection is associated with a vigorous, long-lasting, HCV-specific CD4+ (helper) and CD8+ (cytotoxic) T-cell response (9, 57), whereas such responses are usually weak or absent in chronic hepatitis C. The priming and expansion of naïve T cells depend on efficient antigen presentation and stimulation by dendritic cells (DCs), which among several unique features have the ability to crossover exogenous antigens to the endogenous pathway to gain access to major histocompatibility complex (MHC) class I-inducing CD8+ T-cell responses. This process, called cross-presentation, results in cytotoxicity against viruses that have restricted tissue tropism (1). DCs express numerous receptors involved in the recognition and endocytosis of a large number of pathogens, as well as self antigens (23) such as FcY-receptors, Toll-like receptors, C-type lectins, and SRs (45, 52). The presence of both positive-strand HCV RNA and its replicative intermediates (negative-strand HCV RNA) in DCs from patients infected with HCV suggests that DCs may be permissive for HCV infection (24, 33, 48). However, the viral load detected in DCs from patients infected with HCV is extremely low compared to the viral load in infected hepatocytes (49).

HCV-like particles (HCV-LPs) generated by self-assembly of the HCV structural proteins core, E1, and E2 in insect cells exhibit antigenic properties similar to those of virions isolated from HCV-infected patients (7) and recombinant infectious virions synthesized in tissue culture (cell culture-derived HCV [HCVcc]) (38, 63, 70). Recently, we have shown that HCV-LPs are efficiently taken up by human monocyte-derived DCs and defined subsets of blood DCs in an envelope- and receptor-mediated manner (5). Following HCV-LP uptake, DCs efficiently activate HCV-specific CD8+ T cells (5), indicating MHC class I presentation of HCV-LP-derived peptides in the absence of viral replication. Thus, HCV-LPs represent a...
unique model system to study the cellular and molecular mechanisms of HCV uptake and cross-presentation. The host entry factors mediating the uptake and cross-presentation of HCV-LPs into DCs are unknown. The identification of these factors would not only help in understanding the molecular mechanism of HCV entry and presentation but also guide the development of therapeutic interventions to modulate the HCV-specific T-cell response.

In this study, we demonstrate that SR-BI plays a crucial role in mediating the first steps of HCV-LP-DC interaction and represents a cell surface receptor for HCV entry into DCs. The involvement of SR-BI in HCV-LP-mediated cross-presentation suggests a functional role for SR-BI in the initiation of HCV-specific immune responses.

MATERIALS AND METHODS

Recombinant proteins, antibodies, and cells. HCV-LPs were synthesized in Sf9 insect cells as previously described (65). The HCV-LP E2 concentration was determined as previously described (15). Mouse anti-E2 monoclonal antibodies (McAb) (16A6 and AP33), mouse anti-core McAb (C1 and C2), mouse anti-E1 (1C4), and chimpanzee anti-E2 McAb (49F3) have been previously described (5, 65). Polyclonal antibodies against the extracellular loop of SR-BI were raised by genetic immunization of BALB/c mouse or Wistar rats with a plasmid expressing the full-length human SR-BI cDNA. The SR-BI plasmid (pcDNA CLA-1) was kindly provided by T. Huby (Inserm, Dyslipoproteinemia and Atherosclerosis Research Unit, Hôpital de la Pitié, Paris, France) (40). Preimmune serum was collected from mice and rats before immunization. Immunoglobulin G (IgG) from preimmune and anti-SR-BI-positive sera were purified by using a MabTrap kit (Amersham Biosciences, Freiburg, Germany) according to the manufacturer’s instructions, as described previously (69). Anti-human CD36 (FA6-152) McAb was obtained from Immunotech (Marseille, France), anti-human LOX-1 (24C11) McAb from HyCult Biotechnology (Uden, The Netherlands), anti-human CD81 (J518) from BD Pharmingen (San Jose, CA), and rabbit anti-SR-BI (NB 400-104 and NB 400-102) polyclonal sera from Novus Biologicals (Littleton, CO). Fucoidan, poly(C), lactacystin, and lipopolysaccharide (LPS; Escherichia coli 026:B6) were obtained from Sigma-Aldrich (St. Louis, MO). HDL and low-density lipoprotein (LDL) were isolated from plasma of healthy individuals by ultracentrifugation and dialyzed against phosphate-buffered saline (PBS). Lipoprotein cholesterol concentrations were determined as previously described (47). Oxidized LDL was generously provided by O. Quevedo (Department of Medicine, University of California, San Diego, CA). LDL oxidation was induced with 5 × 10⁵ virus particles/100 μl or about 50,000 viral particles per cell (according to Yu et al. [88]) or with insect cell control preparations (derived from insect cells infected with a recombinant baculovirus containing the cDNA for β-glucuronidase [GUS]) (65) for 1 h at 4°C, and cell-bound HCV-LPs were detected by using mouse (AP33) or chimpanzee anti-E2 McAb (49F3) and FACS as described previously (5). To assess the inhibition of HCV-LP binding by antibodies directed against SRs or CD81, cells were preincubated with anti-SR-BI (1:10 or 1:20 dilution), anti-CD36, anti-CD81, control IgG (50 μg/ml each), preimmune serum (1:10 or 1:20 dilution), or anti-SR-BI IgG and control IgG purified from serum (100 μg/ml each) in PBS for 1 h at 4°C. Then, HCV-LPs were added for 1 h at 4°C. The cellular binding of HCV-LPs was quantified by FACS using chimpanzee anti-E2 (49F3) or mouse anti-E2 (AP33) McAb and PE-conjugated anti-human or anti-mouse IgG antibody. To study whether cellular HCV-LP binding was affected by SR-BRs lipids, human serum, or lipoproteins, HCV-LPs were preincubated with fucoidan, poly(C), HDL, LDL, and oxidized LDL at different concentrations for 1 h at room temperature. Then, HCV-LP-ligand complexes were added to the cells for 1 h at 4°C and cell-bound HCV-LPs were detected as described above. To analyze the uptake of HCV-LPs, DCs were incubated with HCV-LPs or GUS for 3 h at 37°C, and internalized particles were stained using an antiserum against the entire core (C1 and C2), human anti-E1 antibody (1C4), mouse anti-E2 (AP33), or chimpanzee anti-E2 antibody (49F3). To assess the inhibition of HCV-LP uptake, HCV-LPs were preincubated for 1 h at 37°C with mouse anti-E2 antibody (AP33) or mouse control IgG (each 100 μg/ml) in PBS. Then, HCV-LP-antibody complexes were added to DCs and incubated for 3 h at 37°C. The uptake of HCV-LPs by DCs was determined by immunofluorescence and confocal laser scanning microscopy (LSM). HCV-LP cross-presentation. To study the role of SRs in HCV-LP cross-presentation, we analyzed HCV-LP-mediated antigen cross-presentation using HCV core-specific CD8⁺ T cells. Peripheral HCV core-specific CD8⁺ T cells (recognizing an epitope in the HCV core protein comprising amino acids 36 to 53) were generated from a patient chronically infected with HCV, as described previously (5). After preincubation of autologous DCs with anti-SR-BI serum, immunomagnetic (1:10 dilution), anti-CD81, control IgG (50 μg/ml each), or SR ligand fucoidan and poly(C) (1 μg/ml each) for 1 h, HCV-LPs (corresponding to an E2 concentration of 2.5 μg/ml) or insect cell control preparations were added to the DCs for 1 h at 37°C. Then, cells were extensively washed to remove unbound HCV-LPs and cultured for 4 h at 37°C, allowing efficient uptake and antigen processing. During the final 16 h, CD40 ligand (1 μg/ml) was added to the culture medium as a maturation stimulus. Then, the cells were harvested, washed and cocultured with HCV core-specific CD8⁺ T cells at a ratio of 1:2. After 5 h of incubation, intracellular gamma interferon (IFN-γ) staining of core-specific CD8⁺ T cells was performed as recently described (58). To study the mechanisms of HCV-LP antigen processing, DCs were preincubated for 1 h at 37°C in the absence or presence of increasing concentrations of lactacystin (0 to 50 μM), a highly specific proteasome inhibitor. After preincubation, HCV-LPs were added to the wells in the continuous presence of inhibitor for 4 h at 37°C and cross-presentation was analyzed as described above. Approval of the studies was obtained from the Freiburg University Hospital institutional review board. Informed consent was obtained according to the Declaration of Helsinki.

Uptake of HCVcc into DCs. HCVcc were generated as previously reported (65). To obtain high-titer and purified HCVcc, the culture medium of JFH1-infected Huh7.5.1 cells was concentrated and subjected to iodixanol density gradient ultracentrifugation. Then, the gradient fractions were collected and analyzed for HCV RNA and infectivity titers as recently described (32). To study HCVcc uptake into DCs, DCs (1 × 10⁵ cells/100 μl) were incubated with HCVcc (5 × 10⁵ to 1 × 10⁶ copies/ml, corresponding to an infectivity titer of 1 × 10⁶ focus-forming units/ml) for 2 h at 4°C, followed by a temperature shift to 37°C for 2 h. Following the incubation at 37°C, the DCs were washed, fixed, and permeabilized. HCVcc uptake was detected by using mouse monoclonal anti-E2 antibody (AP33) and the protocol described above for HCV-LPs. For studies in which cytoplasmic structures, cells were incubated with an anti-human actin antibody to assess the uptake of HCVcc without SR-BI IgG, DCs were preincubated for 1 h at 37°C with purified rat anti-SR-BI IgG or rat control IgG (250 μg/ml). Then, HCVcc were added as described above and the uptake of polyclonal anti-SR-BI serum, CHO cells were transfected with pcDNA3 (control vector) or pcDNA-SR-BI by using liposome-mediated gene transfer (Lipofectamine; Invitrogen, Karlsruhe, Germany) according to the manufacturer’s instructions (2, 54). The CHO cells were then incubated with polyclonal anti-SR-BI serum or preimmune serum and analyzed for SR-BI expression by flow cytometry as described above.

SR expression. For the analysis of SR expression, cells (1 × 10⁵ cells/100 μl) were incubated with antibodies directed against SR-BI, LOX-1, CD36, anticalcineurin antibodies (control IgG), or preimmune serum (control serum). Subsequently, cells were incubated with PE-conjugated anti-mouse IgG and analyzed by fluorescence-activated cell sorter (FACS) as described recently (5). For the FACS analysis of SR-BI and SR-BIL cells, cells were permeabilized with 0.05 to 0.1% saponin prior to incubation with rabbit polyclonal anti-SR-BI/I ser and allophycocyanin-conjugated anti-rabbit IgG. To demonstrate the specificity of
HCVcc by DCs was determined by immunofluorescence and confocal LSM analysis as described above. The HCVcc uptake was quantified by counting the average number of cells with positive staining for HCV E2 protein per total cells \( (n/H) \) in the presence or absence of anti-SR-BI IgG or control IgG.

**RESULTS**

High level of expression of SR-Bs on human DCs. SR-BI is a type III transmembrane protein that crosses the membrane twice to form a heavily glycosylated extracellular loop with two short intracellular tails. SR-BI and its isoform SR-BII are identical except for the region encoding the C-terminal cytoplasmic domain, suggesting that alternative splicing of a single transcript yields two distinct mRNAs (64). Using commercially available rabbit polyclonal anti-SR-BI and SR-BII antibodies directed against the C-terminal cytoplasmic tail of SR-BI and SR-BII, we demonstrated that DCs are characterized by high levels of expression of SR-BI and SR-BII on their cell surface (Fig. 1A).

Since antibodies targeting the C-terminal cytoplasmic tail of SR-BI or SR-BII do not interfere with SR-B–ligand interaction, we generated a polyclonal anti-SR-BI antibody directed against the extracellular loop of SR-BI by genetic immunization with a plasmid carrying the full-length human SR-BI cDNA. To demonstrate that the resulting anti-SR-BI antibodies specifically interact with human SR-BI, we studied the binding of anti-SR-BI to CHO cells expressing human SR-BI on their cell surface. As shown in Fig. 1B, human SR-BI-transfected CHO cells specifically interacted with anti-SR-BI antibodies. By contrast, there was no interaction between CHO cells transfected with control vector and anti-SR-BI serum or CHO cells transfected with SR-BI cDNA and preimmune serum (Fig. 1B). In addition, polyclonal anti-SR-BI se-
rum, but not preimmune serum, bound to the cell surface of human monocyte-derived DCs, suggesting a specific binding to SR-BI expressed on the DC surface (Fig. 1C). Taken together, these findings demonstrate that polyclonal anti-SR-BI directed against epitopes of the SR-BI extracellular loop specifically recognizes human SR-BI expressed on the surface of DCs.

Next, we analyzed the expression profiles of other SRs on the surface of DCs and other cell types. As shown in Fig. 2, DCs expressed high levels of SR-BI, which were comparable to the levels of SR-BI expressed on human HepG2 hepatoma cells. By comparison, cell-surface SR-BI expression on monocytes and T and B cells was weak or absent (Fig. 2). CD36, another member of the class B family, was highly expressed both on monocytes and on DCs and human HepG2 hepatoma cells (Fig. 2). The expression of LOX-1, a member of the class E family, was very weak or absent on DCs (Fig. 2).

**SR-BI and HDL mediate binding of HCV-LPs to DCs.** Recent evidence suggests that the function of SR-BI and SR-BII is not linked only to lipoprotein metabolism. SR-BI and SR-BII overexpressed in nonphagocytotic human epithelial cells have been shown to mediate the binding and uptake of live, as well as dead, gram-negative and gram-positive bacteria, suggesting a conserved role for SR-BI and SR-BII in pattern recognition and host defense (50, 60). Intracellular signaling pathways activated by pattern recognition receptors have been shown to dictate the maturation profile of DCs. To study whether SR-BI is involved in DC maturation, we purified IgG from anti-SR-BI serum and assessed the influence of the anti-SR-BI antibody on the DC maturation state. As shown in Fig. 3A, purified anti-SR-BI IgG interacted strongly with SR-BI on the surface of immature DCs. Then, immature DCs were exposed to anti-SR-BI IgG for 16 h and the activation of immature DCs was measured by flow cytometric analysis of HLA-DR, CD80, CD86, and CD83 cell surface expression (dark lines). Histograms corresponding to background expression of the respective cell surface molecules in unexposed DCs are shown as gray lines. A result representative of three independent experiments using immature DCs from three different donors is shown. FL2-H, fluorescence 2-height.

**FIG. 3.** Binding of anti-SR-BI IgG and DC activation. (A) Cell surface expression of SR-BI detected by purified anti-SR-BI IgG. Cells were incubated with purified anti-SR-BI IgG or purified preimmune control IgG (CTRL IgG) and subsequently stained with PE-conjugated anti-rat IgG. Cells stained with the secondary antibody alone served as negative controls (gray-shaded curve [NC]). (B) Anti-SR-BI IgG and DC activation by anti-SR-BI IgG. Immature DCs were exposed to purified anti-SR-BI IgG, purified CTRL IgG (50 μg/ml each), or LPS (10 μg/ml). After 16 h, DC activation by purified anti-SR-BI IgG, CTRL IgG, or LPS was assessed by flow cytometric analysis of HLA-DR, CD80, CD86, and CD83 cell surface expression (dark lines). Histograms corresponding to background expression of the respective cell surface molecules in unexposed DCs are shown as gray lines. A result representative of three independent experiments using immature DCs from three different donors is shown. FL2-H, fluorescence 2-height.
of anti-SR-BI IgG to cell surface SR-BI may be not sufficient to induce DC maturation. However, we cannot exclude the possibility that SR-BI is capable of modulating intercellular signals originated from other maturation-inducing factors, as shown for DC-SIGN (12).

At an immature stage, DCs are characterized by their high ability to capture antigens. We have previously shown that immature DCs bind and rapidly internalize HCV-LPs in a concentration-dependent manner. To study whether cell-surface SR-BI expression correlates with the ability of DCs to capture viral antigens, we determined SR-BI expression and HCV-LP binding during differentiation of monocytes into DCs (B). Monocyte-derived DCs were harvested at different time points during culture in cytokine-conditioned medium. Then, monocytes and DCs were analyzed for SR-BI expression and HCV-LP binding. Expression of SR-BI was determined by flow cytometry using anti-SR-BI polyclonal serum as described in the legend for panel C. HCV-LP binding to DCs was determined by flow cytometry using a monoclonal anti-HCV E2 antibody and PE-conjugated anti-mouse IgG. Data are shown as mean net fluorescence intensity (Δ MFI) of a representative experiment.

![Graph A](image1)

**FIG. 4.** SR-BI expression correlates with HCV-LP binding during DC differentiation. Analysis of SR-BI cell surface expression (A) and HCV-LP binding during differentiation of monocytes into DCs (B). Monocyte-derived DCs were harvested at different time points during culture in cytokine-conditioned medium. Then, monocytes and DCs were analyzed for SR-BI expression and HCV-LP binding. Expression of SR-BI was determined by flow cytometry using anti-SR-BI polyclonal serum as described in the legend for panel C. HCV-LP binding to DCs was determined by flow cytometry using a monoclonal anti-HCV E2 antibody and PE-conjugated anti-mouse IgG. Data are shown as mean net fluorescence intensity (Δ MFI) of a representative experiment.

![Graph B](image2)

manner (Fig. 5A and B). By contrast, preincubation of DCs with anti-CD36 did not affect HCV-LP binding (Fig. 5C). To confirm that the inhibition of HCV-LP binding was indeed mediated by anti-SR-BI antibodies, we assessed HCV-LP binding to DCs in the presence of purified IgG from both anti-SR-BI serum and control serum. Purified anti-SR-BI IgG inhibited HCV-LP binding in a manner similar to anti-SR-BI serum; the inhibition of HCV-LP binding in the presence of anti-SR-BI IgG (100 μg/ml) was 64% compared to the inhibition of its binding to DCs in the presence of control IgG (100 μg/ml). These data indicate that SR-BI plays a crucial role in mediating the binding of HCV particles to DCs. A key role of SR-BI for HCV binding to DCs is supported by two further observations: (i) HCV-LP binding to monocytes is weak despite a high level of expression of CD36 (Fig. 2 and 4B) and (ii) only cells transfected with the human SR-BI, but not CD36, resulted in recombinant HCV envelope glycoprotein E2 binding (54).

The ability of transfected or retrovirally delivered synthetic small interfering RNAs to block the expression of specific transcripts has proved useful for the analysis of gene function in mammalian cells. However, since the efficient and sustained delivery of small interfering RNA into DCs was not easily achievable (data not shown), we assessed the role of SR-BI for HCV antigen recognition by the ability of SR-BI ligands to block HCV-LP binding. Like most SRs, SR-BI recognizes a wide range of ligands, including polyanionic molecules, native HDL, LDL, and very-low-density lipoprotein, as well as various chemically modified HDL and LDL species. Distinct ligand-binding sites for HDL and LDL have been reported to exist on SR-BI (26), indicating distinct modes of binding and perhaps distinct binding sites for the various SR-BI ligands. Recently, HDL and human serum have been shown to enhance HCV pseudoparticle (HCVpp) and HCVcc infectivity (6, 19, 34, 43, 61), whereas oxidized LDL inhibited HCVpp and HCVcc infectivity of human hepatoma cells (62). To study whether similar mechanisms operate in DCs, we analyzed HCV-LP binding to DCs in the presence of lipoproteins. As shown in Fig. 5D, oxidized LDL reduced HCV-LP binding to DCs to about 40%, while native LDL had no effect. By contrast, HDL enhanced HCV-LP binding to DCs by about four-fold (Fig. 6A), similar to human serum (Fig. 6B). The mechanism by which lipoproteins modulate HCV infectivity is still unclear. It is possible that distinct lipoproteins induce conformational changes of the HCV particles and/or that the lipoprotein–SR-BI interaction may modulate virus binding and entry. Interestingly, the presence of fucoidan, a nonspecific inhibitor that blocks lipoprotein uptake by class A and B SRs (29) and has been previously shown to interfere with the binding and uptake of mycobacteria in monocyte-derived macrophages (71), reduced HCV-LP binding to DCs by up to 90% (Fig. 5D). Furthermore, in the presence of anti-SR-BI, the HDL-mediated enhancement of HCV-LP binding to DCs was reversed (Fig. 6C). Taken together, these data suggest that SR-BI is involved in HCV binding to DCs.

**SR-BI is a host entry factor for HCV-LP uptake into DCs.** Since SR-BI has been shown to represent a cellular cofactor for HCV infection in human hepatoma cells (25, 31, 69), we analyzed the role of SR-BI in HCV-LP uptake by DCs by immunofluorescence and confocal LSM. First, to demonstrate
that intact HCV-LPs are taken up by DCs, we used LSM to visualize the HCV core protein, as well as the two envelope glycoproteins E1 and E2, inside these cells (Fig. 7A). By double staining core and E1 or core and E2, we showed that these proteins colocalized both on the cell surface of DCs in binding experiments and inside DCs when HCV-LPs were allowed to enter DCs at 37°C (Fig. 7B). These findings demonstrate that, indeed, particular structures containing the HCV structural proteins are internalized. To study whether HCV-LP uptake is mediated by envelope glycoprotein E2, HCV-LPs were preincubated with an antibody directed against envelope glycoprotein E2 prior to incubation with DCs. As shown in Fig. 7C and D, preincubation of HCV-LPs with anti-E2 antibodies significantly inhibited HCV-LP uptake. These data demonstrate that the uptake of HCV-LPs by DCs is mediated at least in part by E2-cell surface protein interactions.

Next, we studied SR-BI expression by immunofluorescence using anti-SR-BI antibody, as described in the Fig. 1 legend. As shown in Fig. 8A, LSM of DCs incubated with anti-SR-BI demonstrated the expression of SR-BI on the DC surface. Next, to study HCV-LP binding, DCs were preincubated with anti-SR-BI prior to the addition of HCV-LPs. As shown in Fig. 8B, the incubation of DCs with HCV-LPs at 4°C in the presence of preimmune serum resulted in the detection of HCV-LPs exclusively on the cell surface, consistent with HCV-LP binding to the DC surface. The incubation of DCs with HCV-
LPs at 37°C following preincubation of DCs with preimmune serum resulted in the translocation of E2 immunoreactivity into the cell, which is consistent with HCV-LP entry (Fig. 8C). By contrast, the binding and uptake of HCV-LPs into DCs were markedly inhibited by anti-SR-BI (Fig. 8B and C), indicating that SR-BI is required for HCV-LP binding and uptake into DCs. Of note, the anti-SR-BI serum used, as well as purified anti-SR-BI IgG, have been shown to specifically inhibit HCVcc infection of human hepatoma cells (69), suggesting that the uptake of HCV-LPs into DCs, as well as HCVcc infection of hepatoma cells, may be mediated by similar SR-BI–HCV envelope interactions.

SR-BI-mediated HCV-LP uptake results in trafficking of viral antigens to the MHC class I pathway. Bacterial uptake by nonphagocytic human epithelial cells overexpressing SR-BI has been shown to colocalize with cytosolic polyubiquitins and proteasome (60). Moreover, macrophages from SR-BI-knockout mice showed a reduced cytosolic bacterial accumulation (60), suggesting that SR-BI mediates bacterial recognition and processing through a proteasome-dependent mechanism. Since the uptake of HCV-LPs leads to an efficient processing and presentation of HCV-LP-derived peptides on MHC class I molecules (5), we studied HCV-LP cross-presentation in the presence of anti-SR-BI and SR-BI ligands. DCs were preincubated with anti-SR-BI or preimmune serum prior to the addition of HCV-LPs. Then, HCV-LP-pulsed DCs were matured overnight with CD40L. After being washed, the DCs were cocultured with HCV core-specific CD8\(^+\) T cells. As shown in Fig. 9A and B, anti-SR-BI serum markedly inhibited the IFN-\(\gamma\) production of HCV core-specific CD8\(^+\) T cells in comparison to the results for preimmune serum. A similar inhibition of the IFN-\(\gamma\) production of HCV core-specific CD8\(^+\) T cells was observed when DCs were incubated with the SR-BI ligand fucoidan (Fig. 9B). By contrast, preincubation of DCs with control ligand poly(C) did not affect HCV-LP cross-presentation (Fig. 9B). These findings indicate that SR-BI may target viral antigens into the cytosol, where the viral antigens gain access to the MHC class I presentation pathway.

Recently, HCVcc infection of human hepatoma cells has been shown to depend on cholesterol and the cooperation between SR-BI and CD81 (31). CD81 belongs to the tetraspanin family. These proteins associate with partner proteins and facilitate their lateral positioning in the membrane, which in turn affects the association with molecules involved in intracellular signaling (37). Even though CD81 is highly expressed on the DC surface (Fig. 2) (41), HCV-LP binding (Fig. 5C) and cross-presentation (Fig. 9B) were not inhibited by anti-CD81. Furthermore, T cells with a high level of expression of CD81 and no expression of SR-BI (Fig. 2) did not bind HCV-LPs (5). These data suggest that, in contrast to SR-BI, CD81 appears not to play a key role for HCV uptake and presentation in DCs in our model system.

SR-BI is a host entry factor for tissue culture-derived HCV uptake into DCs. Aiming to study whether SR-BI mediates the uptake of HCVcc, we analyzed the uptake of iodixanol gradient-purified HCVcc into DCs in the presence of purified anti-SR-BI IgG or control IgG. HCVcc uptake into DCs was analyzed by anti-E2-specific immunofluorescence and LSM. First, to demonstrate that HCVcc are taken up by DCs, we used LSM to visualize the HCVcc envelope protein E2 inside the

FIG. 6. HCV-LP binding to human DCs is enhanced by HDL. (A) Enhancement of HCV-LP binding to DCs by HDL. HCV-LPs were preincubated for 1 h at room temperature with different concentrations of HDL (diamonds) and LDL (triangles). After the addition of HCV-LP-lipoprotein complexes to the DCs, HCV-LP binding was determined as described in the Fig. 4 legend for panel A. Data are shown as percent HCV-LP binding (means ± standard deviations of the results from three experiments) in the presence of lipoproteins compared to HCV-LP binding in the presence of PBS (100%). (B) Enhancement of HCV-LP binding in the presence of lipoproteins present in human serum. HCV-LPs were preincubated with human serum from a healthy individual at the concentrations indicated and then added to DCs at 4°C, allowing HCV-LP binding. (C) HDL-mediated enhancement of HCV-LP binding is reversed by anti-SR-BI antibodies. HCV-LPs were incubated with HDL (10 µg cholesterol/ml or 50 µg cholesterol/ml) for 1 h at room temperature. Following the addition of HCV-LP-lipoprotein complexes to DCs incubated with anti-SR-BI or control, HCV-LP binding was determined using mouse anti-E2 MAb (AP33) as described above. Data are shown as percent HCV-LP binding (means ± standard deviations of the results from three independent experiments) relative to HCV-LP binding in the absence of ligands (100%).
FIG. 7. HCV-LP uptake into DCs is mediated by envelope glycoprotein E2. (A) HCV-LP uptake by DCs. DCs were incubated with HCV-LPs or insect cell control preparations (GUS) and triple stained for actin (green); viral protein core, E1, or E2 (red); and nucleus (DAPI [4',6'-diamidino-2-phenylindole], in blue). Arrows indicate viral protein staining. (B) HCV-LPs internalized in DCs. DCs incubated with HCV-LPs were triple stained for nucleus (DAPI, in blue), core (green), and E1 or E2 (red). Overlay of images shows colocalization of core/E1 or core/E2 (right panel). (C) HCV-LP uptake by DCs is mediated by envelope glycoprotein E2. HCV-LPs were preincubated (1 h at 37°C) with anti-E2 antibody (AP33; 50 μg/ml) or control IgG (50 μg/ml) before incubation with DCs. HCV-LP-anti-E2 complexes were then added to DCs and incubated at 37°C for 3 h. Following fixation, DCs were triple stained for actin (green), E2 (red), and nucleus (DAPI, in blue). (D) Quantitation of HCV-LP uptake in the presence and absence of anti-E2 antibody. HCV-LP uptake by DCs in the presence of anti-E2 MAb or control IgG is shown as percentage of cells with positive intracellular HCV-LP E2 staining relative to the total number of cells. The means ± standard deviations of the results from three independent experiments are shown. Statistical analysis was performed by Student’s t test.
cells. As shown in Fig. 10A, HCVcc envelope glycoprotein E2 colocalizes with the cytoplasm of DCs following an incubation step of DCs with HCVcc at 37°C. Interestingly, only about 8 to 15% of DCs incubated with HCVcc stained positive for HCV E2 protein. In contrast, no internalization of HCVcc E2 protein was observed when DCs were incubated with HCVcc at 4°C (data not shown). These findings demonstrate that HCVcc-derived envelope glycoprotein E2 is internalized into DCs in a temperature-dependent manner. To study whether HCVcc uptake is mediated by SR-BI, DCs were preincubated with purified anti-SR-BI IgG or control IgG. As shown in Fig. 10, purified anti-SR-BI IgG markedly and significantly inhibited HCVcc uptake into DCs, whereas purified control IgG had no effect. These data demonstrate that the uptake of HCVcc by DCs is mediated at least in part by SR-BI and that SR-BI most likely represents a host entry factor for the uptake of infectious HCV into DCs.

DISCUSSION

In this study, we assessed the functional role of SR-BI for the uptake and cross-presentation of HCV by human DCs. We demonstrate that (i) SR-BI is required for the binding and uptake of HCV by human DCs and (ii) SR-BI-mediated uptake results in trafficking into the MHC class I pathway, followed by efficient cross-presentation to HCV-specific CD8+ T-cells. Taken together, our results reveal a novel function for SR-BI for antigen uptake and presentation and identify a novel mechanism whereby DCs can capture and process viral antigens.

SR-BI and its splicing variant SR-BII are physiologically relevant HDL receptors with an identical extracellular loop. SR-BII differs from SR-BI at the C terminus, which is reported to confer an intracellular localization on SR-BII (64). Using defined antibodies targeting the cytoplasmic tail or extracellular loop of SR-BI, we could show that human immature DCs express SR-BI. These findings are in line with the results of two previous studies demonstrating that SR-BI is expressed on monocyte-derived DCs, as well as on plasmacytoid and myeloid DCs (10, 67). In contrast to our findings, Yamada et al. (67) observed a higher level of SR-BI expression on the surface of monocytes using a different anti-SR-BI antibody. These differences could be due to different epitopes recognized by the antibodies or different protocols of monocyte isolation used in their study and ours. In our study, as well as in the study of Buechler et al. (10), SR-BI expression was induced during the differentiation of monocytes into DCs, indicating that SR-BI may play a specific role for DC function. Since SR-BI has been shown to represent a host cell entry factor for HCV infection of human hepatoma cells (25, 31, 69), we explored its role in viral antigen capture and presentation by DCs. Using an HCV-LP-based model system (5), we demonstrate that SR-BI is required for the binding and uptake of HCV-LP into DCs. Since previous results have shown that C-type lectins, such as mannose receptor or DC-SIGN, were not sufficient to mediate HCV-LP binding to DCs (5), SR-BI may represent one of the key DC surface proteins binding HCV particles on DCs. This novel SR-BI function is further supported by the observation that HDL enhanced the binding of HCV-LP to DCs, whereas oxidized LDL and polyanionic ligands reduced HCV-LP binding. Since the presence of HDL did not inhibit but rather enhanced HCV-LP binding, it is unlikely that HCV and HDL compete for the SR-BI HDL binding domain. The highly reproducible enhancement of HCV-LP binding by HDL may rather point to a more-efficient interaction of SR-BI with HCV, e.g., as a result of a conformational change induced by HDL. These findings are in line with findings observed for the infection of human hepatoma cells with recombinant HCVpp.
and HCVcc (6, 19, 34, 43, 61). The significant modulation of HCV-LP binding by HDL and LDL provides a link between lipid metabolism and antigen recognition and may suggest that lipoproteins may interfere with the DC-antigen interaction.

Antigen cross-presentation offers a solution by permitting DCs to crossover exogenous antigens for access to the class I MHC peptide-loading machinery. This mechanism enables DCs to raise immune responses against pathogens, like viruses, that do not infect them (1). Since robust HCV infection of DCs has not been documented either in vivo (49) or in vitro (17), it is likely that the cross-presentation of HCV antigens represents an important mechanism for the induction of antiviral CD8+ T-cell responses. This hypothesis is further supported by our data clearly demonstrating that productive infection of

FIG. 9. SR-BI is involved in HCV-LP cross-presentation to HCV-specific CD8+ T cells. (A) HCV-LP cross-presentation in the presence of anti-SR-BI antibody. DCs were incubated with anti-SR-BI, control serum, or lactacystin prior to the addition of HCV-LPs, as described in Material and Methods. DCs incubated with HCV core peptide core36-53 or an insect cell lysate control preparation (GUS) served as positive and negative controls, respectively. After 24 h, DCs were cocultured with autologous HCV core-specific CD8+ T cells (recognizing an epitope in the HCV core protein comprising amino acids 36 to 53) and analyzed by flow cytometry after staining with antibodies to CD8 and IFN-γ. The percentages of CD8+ T cells that produced IFN-γ in the respective quadrants are indicated on the dot plots. FITC, fluorescein isothiocyanate. (B) HCV-LP cross-presentation in the presence of SR-B ligands, anti-SR-BI, and anti-CD81. Data are shown as percent HCV-LP cross-presentation relative to HCV-LP cross-presentation in the absence of the respective antibodies or SR-B ligands (100%). Mean percentages ± standard deviations of the results of three independent experiments are shown for anti-SR-BI and preimmune serum. Statistical significance of differences between DCs preincubated with anti-SR-BI and control serum was determined by the two-tailed t test.
studies suggest that CD40 is provided by NK lymphocytes in an early DC-NK lymphocyte interaction (21). Since this interaction likely takes place at the site of infection and in secondary lymphoid organs, the maturation of HCV-LP-pulsed DCs by CD40L could reflect the scenario for antigen presentation in an acute HCV infection.

HCV-LP cross-presentation was markedly inhibited in the presence of anti-SR-BI, suggesting that SR-BI is involved in the trafficking of viral antigens toward the MHC class I pathway. This finding suggests that SR-BI may act as an immuno-receptor facilitating the intracellular accumulation of viral antigens and triggering processing and cross-presentation. This hypothesis is further supported by recent data demonstrating that SR-BI mediates bacterial adhesion and cytosolic accumulation (60). Moreover, other members of the growing SR family, SR-A and LOX-1, have been shown to be involved in the uptake and trafficking of exogenous antigens toward the MHC class I pathway (18, 27). Since the anti-SR-BI antibody used in this study may also target the large extracellular loop of SR-BII, we cannot exclude a role for SR-BII in viral antigen uptake and cross-presentation.

Interestingly, HCV-LP cross-presentation could not be completely inhibited by anti-SR-BI, suggesting that additional receptors are involved in targeting HCV-LPs into the MHC class I pathway. Recent studies have shown that the initiation of HCV infection is dependent on a cooperativity between SR-BI and CD81 (31). In contrast to the findings for HCV infection, CD81 did not appear to play a major role in HCV-LP binding and cross-presentation in DCs. These data suggest that SR-BI is the main HCV capture receptor on DCs, while a cooperative action of SR-BI and CD81 is required for efficient HCV infection of hepatocytes. Furthermore, these data illustrate the difference in HCV entry pathways in hepatocytes and DCs. In hepatocytes, HCV enters by clathrin-mediated endocytosis, followed by an HCV envelope membrane fusion process for the delivery of the HCV genome into the cytosol (3, 8). In contrast, classical MHC class I presentation requires the transfer of the exogenous antigens from the endosome or phagosome into the cytosol, where the antigens are degraded by proteasomes into oligopeptides. The peptides are then transported by the transporter associated with antigen processing into the endoplasmic reticulum and are bound to MHC class I molecules. In an alternative pathway, peptides may be generated within the endocytotic compartment and the resulting peptides are then bound to recycling MHC class I molecules (1). Further studies analyzing the molecular mechanisms of HCV-LP processing and presentation are in progress. Preliminary studies demonstrated that lactacystin, a highly specific inhibitor of proteasomal antigen processing, did not inhibit HCV-LP cross-presentation (Fig. 9A). These results may indicate that alternative MHC class I processing and presentation pathways could be involved in HCV-LP cross-presentation or that additional, as-yet-unidentified cytosolic proteases downstream of the proteasome could participate in HCV-LP processing and presentation. Interestingly, several viral epitopes have been identified that are produced or presented more efficiently when proteasome activity is impaired or altered, including viral epitopes from influenza virus (39, 66) and HIV (14). Studies are under way to analyze these mechanisms in detail.
In this study, we used an HCV-LP-based model system to assess the molecular mechanisms of HCV particle uptake and presentation by human DCs (5). HCV-LPs are generated by self-assembly of HCV structural proteins in insect cells (7) and are characterized by morphological, biophysical, and antigenic properties similar to those of infectious virions (22, 63). Furthermore, the binding and uptake of HCV-LPs to target cells appear to require a set of viral epitopes and cellular host factors similar to that required by infectious HCV (2, 4, 55, 59). Although we cannot exclude the possibility that the virus-like particle concentration in our in vitro experiments may exceed the concentration of circulating infectious viral particles interacting with DCs in vivo, studies in animal models, including mice and chimpanzees, have shown that HCV-LPs used in amounts as in this study are appropriate for HCV-LP uptake and presentation by DCs in vivo. Indeed, in vivo studies have demonstrated that HCV-LPs induce a strong antiviral humoral and cellular immune response, including HCV-specific T-helper cells and cytotoxic T lymphocytes, in primates, including chimpanzees (30, 35, 46, 51). The quantity and quality of HCV-LP-induced cellular immune responses against the HCV structural proteins appear to be similar to the immune responses induced by the infectious virus (30, 35, 46, 51). Moreover, HCV-LP-induced T-cell responses result in control of HCV infection in the chimpanzee in vivo (20). These findings and the successful use of virus-like particles of other viruses, including HIV (11), hepatitis B virus (56), papillomavirus (36, 53), and parvovirus (44), for the study of virus uptake and antigen presentation in DCs indicate that the interaction of HCV-LPs with DCs represents an appropriate model system to study the molecular mechanisms of HCV particle uptake and presentation of HCV structural proteins.

To confirm the validity of the HCV-LP model system, as well as the role of SR-BI for HCV uptake into DCs, we produced high-titer, gradient-purified HCVcc and studied HCVcc uptake by using anti-E2-specific immunofluorescence and confocal LSM. Using this method and purified anti-SR-BI IgG, recently shown to inhibit HCVcc infection of hepatoma cells (69), we demonstrate that anti-SR-BI IgG specifically inhibits the uptake of HCVcc into DCs (Fig. 10). These findings demonstrate the relevance of the HCV-LP model system for the study of HCV particle uptake and confirm the specificity of the anti SR-BI serum used for the study of HCV-DC interaction.

In conclusion, we have demonstrated that SR-BI mediates HCV-LP and HCVcc uptake into human DCs, indicating that SR-BI may represent a cell-surface receptor for the recognition of viral antigens. The inhibition of HCV-LP cross-presentation by anti-SR-BI antibody suggests that SR-BI is implicated in trafficking exogenous viral antigens toward the MHC class I presentation pathway. Taken together, these findings support a novel function of SR-Bs for viral antigen uptake and recognition. In addition, the SR-BI–viral antigen interaction may represent a novel target for therapeutic or preventive strategies aiming at the induction of efficient antiviral immune responses.

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