THE EXCHANGEABLE APOLIPOPROTEIN APOC-I PROMOTES MEMBRANE FUSION OF HEPATITIS C VIRUS

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Cell entry of hepatitis C virus (HCV) is strikingly linked to lipoproteins and their receptors. Particularly, HDL enhances infectivity of HCV particles by involving the lipid-transfer function of the scavenger receptor BI, a receptor for both HDL and HCV. Here, we demonstrate that ApoC-I, an exchangeable apolipoprotein that predominantly resides in HDL, increases the fusion rates between viral and target membranes via a direct interaction with HCV particles. We identify the hyper-variable region-I of HCV E2 as an essential viral component that modulates ApoC-I-mediated enhancement of HCV fusion properties. In contrast to the saturable infection-enhancement by HDL, excess ApoC-I provided as lipid-free protein induced the disruption of the HCV particles and loss of infectivity, through disruption of the viral membrane. This indicates that ApoC-I induces HCV infection-enhancement through a fine-tuned mechanism that is regulated via a triple interplay between HVRI, HDL and SR-BI, which results in optimal ApoC-I recruitment on the viral membrane. These results provide the first description of a host serum factor helping the fusion process of an enveloped virus.

With an estimated 170 million infected individuals, hepatitis C virus (HCV) has a major impact on public health (2). HCV is an enveloped, positive-stranded RNA virus of the Flaviviridae family. Its genome encodes a single polyprotein processed by viral and cellular proteases into three structural (core, E1 and E2 glycoproteins) and seven non-structural proteins (3,4).

For a long time, the study of HCV cell entry has remained limited because the ex vivo characterisation of HCV derived from plasma has proven extremely difficult. This is due in large part to the low infectivity of the virus in cultures of primary hepatocytes, to its high genetic heterogeneity and to its association, through different forms, with lipoproteins. Thus, to overcome these severe limitations toward the molecular characterisation of HCV infection, several surrogate assays have been developed. Two relevant and complementary in vitro cell entry assays consist of cell culture-grown genuine HCV (HCVcc) derived from a fulminant hepatitis C, JFH-1 (5-7), and of HCV pseudoparticles (HCVpp) harbouring authentic E1E2 glycoproteins, which are particularly amenable to mutagenesis analysis (8-10). While HCVcc further permit investigation of the late infection steps, HCVpp, which can be produced in non-hepatic cells that can be readily complemented with hepatic factors, offer a particularly flexible plate-form to study the structure/function relationship of HCV glycoproteins both in cell culture and in liposome fusion assays in vitro (11). Thus, both HCVcc and HCVpp infection assays reproduce some cell entry features of native HCV and allow a precise dissection of the cellular and viral factors involved in the early events of HCV infection (reviewed in (12-14)). The viral surface glycoproteins, E1E2, and their receptors mediate the cell entry processes of HCV. At least three receptors for HCV E2 have been identified that mediate concentration, binding and cell entry of viral particles. They include glycosaminoglycans, the CD81 tetraspanin and the scavenger receptor B-I (SR-BI), a major receptor of high-density lipoprotein (HDL). Using HCVpp and HCVcc infection assays as well as in vitro membrane fusion assays, HCV entry has been shown to occur in a pH-dependant manner (10,11,15-17), through endocytosis of the viral particles (18,19). Like for other Flaviviridae (20), the low endosomal pH may induce conformational rearrangement of HCV glycoproteins, leading to fusion of the viral membrane with that of the endosome.

The steps following the initial encounter of the HCV glycoproteins with the target cell surface remain ill-defined. Glycosaminoglycans such as highly sulfated heparan sulfate allow viral particles to adhere to target cells before specific receptors induce cell entry (21). As shown by genetic complementation, down-regulation and blocking experiments (5,6,10,16,22-25), CD81 appears an essential receptor in both HCVpp and HCVcc infection assays; yet its role in cell entry remains elusive. Through its down-
regulation and blocking, SR-BI has been shown as an important cell entry factor that can boost cell entry of HCVpp and HCVcc, cooperatively with CD81 (15,16,26,27). Additional host components contribute to cell entry, as recently highlighted by the finding that Claudin-1, a tight junction protein, is required for HCV infection of human hepatoma cell lines (28).

The involvement of SR-BI during cell entry of HCV seems closely related to its physiological function and to its natural ligands. SR-BI mediates binding and lipid transfer from different classes of lipoproteins (29), particularly HDL, accounting for its multiple functions in cholesterol metabolism such as removal of peripheral unesterified cholesterol, steroidogenesis and bile acid synthesis and secretion. SR-BI mediates direct binding of E2 (30,31) and, as a multiligand lipoprotein receptor, can also induce binding of HCV associated to β-lipoproteins (32). Intriguingly, we and others have demonstrated that HDL enhances infectivity of HCVpp and HCVcc (26,33-37). HDL-mediated enhancement of infection does not occur through a direct binding of HDL to HCV particles but rather, involves the lipid-transfer function of SR-BI (26,33,36). This original mechanism is controlled by the HCV glycoproteins, and, more particularly, by conserved residues of the hypervariable region-1 (HVR1) (33,36), a 27 amino-acid peptide located at the amino-terminus of E2. As SR-BI-mediated lipid transfer from HDL locally increases cholesterol content of the lipid membrane (38,39), it may enhance internalisation, membrane rearrangement of components of the HCV receptor complex and/or membrane fusion of HCV (11,26,27).

On the other hand, an essential component of HDL that seems responsible for infection enhancement is the apolipoprotein C-I (ApoC-I) (35). ApoC-I is a small plasma protein (57 amino-acids) composed of two amphipathic α-helices. It is the smallest of the exchangeable apolipoproteins (A-I, A-II, A-IV, C-I, C-II, C-III and E) and circulates in the bloodstream associated with HDL, mainly, and with very low-density lipoprotein (VLDL) and chylomicron particles (40,41). Its capacity to interact with lipid surfaces underlies a number of its functional properties and its important role in regulating plasma lipoprotein metabolism (42,43).

Here we have investigated the mechanisms underlying the enhancement of the early steps of HCV infection by HDL. We demonstrate that ApoC-I increases HCVcc and HCVpp membrane fusion via a direct and specific interaction with HCV particles. We show that the HVR1 region is an essential viral component that modulates this interaction and enhancement of HCV fusogenicity. Our data indicate that lipoprotein-associated ApoC-I induces HCV infection-enhancement through a mechanism that is regulated via a triple interplay between HVR1, HDL and SR-BI, which results in optimal ApoC-I recruitment on the viral membrane. These results provide the first description of a host soluble factor helping the fusion process of an enveloped virus.

**EXPERIMENTAL PROCEDURES**

**Expression constructs and production of HCVpp.**

Expression vectors for the E1E2 glycoproteins of HCV strain H77 (AF009606), for the HVR1 deletion mutant (ΔG384-N411) and for the HVR1 point mutants were described previously (8,15,33). The murine leukemia virus (MLV) packaging and GFP-transfer vectors, the phCMV-RD114 expression plasmid encoding glycoproteins of cat endogenous virus RD114 were described elsewhere (8). The phCMV-VSV-G, phCMV-MLV-A and phCMV-HA expression plasmids encoding the glycoprotein of vesicular stomatitis virus, amphotropic MLV and an avian influenza virus hemagglutinin (HA H7N1) respectively, were described previously (44). The phCMV-712-HIV expression plasmids encoding the glycoprotein of human immunodeficient virus (HIV) was described previously (45).

Viral pseudo-particles named HCVpp, RD114pp, VSV-Gpp MLVpp, HApp and HIVpp harboured the glycoproteins of HCV, RD114, VSV, influenza virus and HIV. They were produced (8) by transfection in 293T cells of vectors encoding viral glycoproteins, packaging proteins, and GFP-transfer vector. Prior to harvest viral particles-containing supernatants, producer cells were incubated in DMEM containing 0.1% FCS for 24 hrs. Viral particles-containing supernatants were used to infect Huh-7 hepatoma cells directly or upon purification by ultracentrifugation through a 20%-sucrose cushion.

**Expression constructs and production of HCVcc.**

The pFK-venus-Jc1 is a chimeric J6C6/JFH1 HCV genome consisting of codons 1–846, derived from J6C6 (AF177036), and codons 847–3033, derived from JFH1 (AB047639) (46,47). HCVcc were produced by electroporation of Huh7-Lunet cells (6,47) in a L3 laboratory, according to European safety regulation. 24 hrs prior to harvest viral particles-containing supernatants, electroporated cells were incubated in DMEM containing 2% lipoprotein-deficient foetal bovine serum (Sigma). Viral particles-containing supernatants were used directly in infection assays using Huh7-Lunet target cells or after purification, as described above for HCVpp.

**Reagents and antibodies.** The HDL (Calbiochem) preparation (density 1.063-1.2 mg/ml) contained a mixture of HDL₂ and HDL₃. Purified apolipoproteins were purchased from Athens Research and Technology (Athens, GA). BLTs (48) were obtained...
from Chembridge. The rabbit anti-ApoC-I antibody was from Biodesign. The 9/27 (10), AP33 (49), H53 (8,50) and the E2mAb-1 (Granier et al., unpublished data) are E2-specific mAbs. A4 (51) is a E1-specific mAb. The 83A25 mAb (kind gift of L. Evans), 2F5 mAb (NIH AIDS Research and Reference Reagent Program) and RD114 SU goat antiserum (ViroMed Biosafety Labs) are antibodies against MLV, HIV and RD114 envelope glycoproteins, respectively. Lectin from Galanthus Nivalis (GNA) was obtained from Sigma. The recombinant CD81-LEL fragment (amino-acids 112-202) and a truncated soluble form of E2 glycoprotein (sE2) (amino-acids 384-664) were fused to a His-tag, produced in cells and purified on Ni-NTA resin (Qiagen). The protein A and G-coupled to sepharose beads were purchased from Amersham. Phosphatidylethanolamine from egg yolk (99% pure), cholesterol (chol, 99% pure), and Triton X-100 were from Sigma. Phospholipid oxidation was routinely checked by spectrophotometry. Octadecyl rhodamine B chloride (R<sub>18</sub>) was from Molecular Probes, and N-(lissamine rhodamine B sulfonyl)-phosphatidylethanolamine (N-Rh-PE) and N-(7-nitro-2,1,3-benzoxadiazol-4-yl)-phosphatidylethanolamine (N-NBD-PE) were purchased from Avanti Polar Lipids.

**Infection assays.** For infection assays with HCVpp and HCVcc, Huh-7 and Huh-7-Lunet cells were respectively seeded 24 hr prior to inoculation (6,8). 2 hrs before infection, target cells were pre-incubated in DMEM containing 0.1% FCS. Medium was then removed and dilutions of viral supernatants and various compounds were added to the cells as indicated. After 4 hrs, supernatants were removed and the infected cells were kept in regular medium (DMEM, 10% FCS) for 72 hrs before analysis. The infectious titres were deduced from the percentage of GFP-positive cells, as determined by FACS analysis (8). Infections with HCVpp were controlled by pseudo-particles devoid of E1E2 glycoproteins, which resulted in background titres below 5x10<sup>2</sup> i.u. (infectious units)/ml.

**Binding assays.** Binding of HCVpp was performed as previously described for HCVpp or for other types of pseudo-particles (26,52,53). Briefly, 50 µl of virus particles purified on a 20%-sucrose cushion were incubated with 10<sup>6</sup> CHO cells expressing SR-B1 and/or CD81 or Huh-7 cells in the presence of 0.1% sodium azide for 1 hr. Cells were then washed twice with PBFA (PBS, 2% fetal bovine serum, and 0.1% sodium azide) and incubated with the H53 anti-HCV-E2 (40 µg/ml) or the A4 anti-HCV-E1 mAbs (40 µg/ml) for 1 hr at 4°C. After two washes, cells were incubated with a goat anti-mouse-alkaline phosphocyanine antibody (Jackson Immunoresearch) diluted in PBFA (5 µg/ml) for 45 min at 4°C. Fluorescence of living 10,000 cells was determined by FACS analysis in the FL4-H channel.

**Surface plasmon resonance (SPR) binding assays.** Biomolecular interactions were studied using a BIAcore-3000 instrument (BIAcore AB, Uppsala, Sweden). Purified ApoC-I (200 µg/ml in 10 mM acetate buffer, pH4) was covalently immobilised via its primary amino groups to the dextran matrix of a CM4 sensor chip (amine coupling kit, BIAcore AB) at a flow rate of 5 µl/min. Activation and blocking steps were performed as described previously (26,54). A control flow cell was prepared according to the same procedure by omitting ApoC-I in the coupling buffer. It was used to assess non-specific binding to the sensor chip surface. Purified HCVpp and control viral particles (CONpp), HIVpp, MLVpp or RD114pp, were injected over immobilized ApoC-I in PBS containing 0.005% P20 surfactant (BIAcore AB) at a flow rate of 5 µl/min at 25°C. Sensorgrams obtained on the control flow cell were automatically subtracted from the sensorgrams obtained over immobilized ApoC-I. The surface was then regenerated with a pulse of 2 M guanidinium chloride.

The AP33, 9/27, E2mAb-1, H53, 83A25, 2F5 antibodies (100 µg/ml in 10 mM acetate buffer, pH 4.5) and GNA lectin (200 µg/ml in 10 mM acetate buffer, pH4) were covalently immobilised to the dextran matrix of a CM3 sensor chip via their primary amino groups as described above. A control flow cell was prepared by immobilizing an irrelevant antibody (mouse anti-IL2) according to the same procedure. Purified HCVpp, MLVpp, HIVpp or sE2 were injected over the antibodies in PBS containing 0.005% P20 surfactant (BIAcore AB) at a flow rate of 5 µl/min at 25°C, as described previously (26) resulting in capture levels ranging from 500 up to 1000 resonance units (RU). Sensorgrams obtained on the control flow cell representing non-specific binding to the sensor chip surface were automatically subtracted from the sensorgrams obtained with captured HCVpp. Binding assays of ApoC-I, CD81-LEL were performed at 25°C in PBS with 0.005% P20 surfactant at a flow rate of 5 µl/min. The surface was then regenerated with pulse of 0.025% SDS or 2 M guanidinium chloride.

**RT release assays.** The reverse transcriptase (RT) release assays were performed as described previously (55,56) using an RT assay kit (RetroSys™, Innovagen, Sweden) following the manufacturer’s instructions but using a sample dilution buffer devoid of Triton x100.
HCV particles, either the HCV pseudo-particles (HCVpp) or the HCV pseudo-particles (HCVpp) were produced in vitro in media devoid of serum lipoproteins. As shown in Figure 1A, addition of purified HDL during infection of Huh-7 target cells increased the infectivity of HCVpp and HCVcc but not of control viral particles. One of the protein components of HDL that induced this enhancement of infection is apolipoprotein C-I (ApoC-I). Indeed, antibodies against ApoC-I (Fig. 1A), but not antibodies against the other HDL apolipoproteins (i.e., ApoA-I, A-II, C-II, and C-III; data not shown), abrogated the effect. Moreover, pre-incubation of HCV particles with purified, lipoprotein-free ApoC-I increased cell infection of HCVpp and HCVcc in a manner similar to HDL (Fig. 1B and data not shown). This enhancement of infection was specific of HCV E1E2 glycoproteins since it was not observed with viral particles bearing the surface glycoproteins from alternative enveloped viruses, such as RD114 cat endogenous virus, vesiculovirus, influenza virus or murine leukaemia virus (MLV) (Fig. 1B), in keeping with the HCV-specific infection enhancement detected with HDL (Fig. 1A and (33)). Of note, no stimulation of infection was detected with purified ApoC-II, another exchangeable apolipoprotein of HDL structurally related to ApoC-I (Fig. 1B).

Finally, addition of ApoC-I antibodies to HCVcc or HCVpp pre-incubated with purified ApoC-I resulted in specific inhibition of their infectivity (Fig. 1C), presumably by neutralisation of the HCV particles complexed with ApoC-I, as shown below. These results therefore extended previous data obtained with HCVpp (33,35,36) and validated them using the HCVcc infection assay.

We then sought to unravel the viral determinants and the cellular receptors through which HCV infection is enhanced by either lipoprotein-free ApoC-I or HDL. HDL mediates HCV infection enhancement via interaction with their common receptor, the scavenger receptor BI (SR-BI) (26,33,36). This triple interplay is controlled by the hypervariable region 1 (HVR1) of HCV E2 and, more particularly, via a framework of conserved residues within this region (33,36). To better put into evidence the differences in enhancement of infection using various HCV pp mutants and reagents modulating stimulation, we standardised the 3-5 fold increase of infection of wild type HCVpp detected with HDL or ApoC-I to 100% (first columns of the left and right panels of Fig. 2A, respectively). Note furthermore that in order to allow a direct comparison of the stimulation observed with HDL and Apo-CI, experiments in Fig. 2 were performed with 1.4 µg/ml of purified ApoC-I which corresponds to the physiological ApoC-I concentration in the HDL preparations used here (i.e., 6 µg/ml cholesterol-HDL), which is the optimal amount for maximal infection enhancement (33,36).

Compared to the standardised infection-enhancement observed with wild-type HCVpp, the infectivity of HCVpp harbouring a deletion of HVR1 (∆HVR1-HCVpp) was not or was barely enhanced by HDL as well as Apo-CI (Fig. 2A). We then performed infection assays with HCVpp bearing changes in conserved amino-acid positions of HVR1 (33) that are thought to be essential for its conformation (57). As compared to parental HCVpp, mutation of some of these conserved residues, i.e. residues G389 and L399, induced gain (G389R) or loss (L399R) of HDL-mediated infection enhancement (Fig. 2A), as reported before (33). Consistently, while HCVpp displaying the G389L point mutant were more intensively stimulated by ApoC-I than wild-type HCVpp, we found that HCVpp harbouring the L399R mutation were not enhanced by ApoC-I (Fig. 2A). Altogether, these findings assigned to HVR1 a
critical role in modulating infection enhancement by both soluble ApoC-I and HDL and pointed out to key amino-acid residues mediating this mechanism. Stimulation of infection induced by ApoC-I was detected for all HCVpp susceptible cell types, including Huh-7, Hep3B, HepG2-CD81 and PLC hepato-carcinoma cells (data not shown). Like for HDL, the mechanism by which soluble ApoC-I stimulates cell entry may involve SR-BI, a multi-ligand receptor that mediates lipid transfer from HDL and that is expressed in these different cell types (33). To address this possibility, we blocked SR-BI functions using either SR-BI antibodies (33), BLT-4, a compound that inhibits SR-BI-mediated lipid transfer (48), or via SR-BI down-regulation (16). While either SR-BI-blocking method abrogated the infection-enhancement of HCV particles by HDL, as reported previously (33), they did not inhibit infection-enhancement by ApoC-I (Fig. 2B). Thus, these results suggested that unlike HDL, infection-enhancement by soluble, lipoprotein-free ApoC-I does not require an interaction with SR-BI.

Like for HDL (33), infection enhancement by lipoprotein-free ApoC-I was dose-dependent and reached a maximal level at 0.7-1.4 µg/ml (Fig. 2C). However, the stimulating effect progressively disappeared at higher ApoC-I concentrations (Fig. 2C), in contrast to the saturable infection enhancement at high HDL concentrations (33). Furthermore, ApoC-I concentrations higher than 7 µg/ml induced a dose-dependent inhibition of infectivity for both HCVpp (Fig. 2C) and HCVcc (data not shown). Such an inhibition was specific of ApoC-I and HCV particles since it was neither detected with ApoC-II, whatever the concentration was, nor with control viral particles bearing alternative surface glycoproteins (data not shown).

**ApoC-I specifically binds HCV particles.** Since lipoprotein-free ApoC-I does not require SR-BI to modulate HCV infection, we reasoned that it could exert its activity through a direct interaction with the surface of HCV particles. Thus, to study HCV/ApoC-I interactions, we performed surface plasmon resonance (SPR) assays. This was achieved using HCVpp since for trivial safety reasons, live HCVcc could not be investigated in our BIAcore plate-form. ApoC-I was covalently immobilised to the dextran matrix of a CM4 BIAcore sensor chip through activation of its amine groups. We found that upon injection in the BIAcore system, HCVpp bound to immobilised ApoC-I, as shown by the increase of SPR signal during HCVpp injection (Fig. 3A). Furthermore, this binding was stable since no decrease of SPR signal was detected after the injection of HCVpp was stopped and during buffer flows on the sensor chip surface (Fig. 3A). Finally, this interaction was specific since no significant binding to CM4-immobilised ApoC-I could be detected upon injection of control pseudo-particles bearing the glycoproteins from HIV (Fig. 3A), murine leukaemia virus (MLV), influenza virus or RD114 (data not shown). Note that identical inputs of HCVpp and control pseudo-particles were compared (Fig. 3A, inset).

To confirm ApoC-I binding to HCV particles, we pre-incubated HCVpp or control pseudo-particles with, or without, 1.4 µg/ml soluble ApoC-I or HDL at equivalent ApoC-I concentrations; i.e., 6 µg/ml cholesterol-HDL (see apoC-I bands in “input” western-blot panel of Sup. Fig. 1A). These samples were then immuno-precipitated using ApoC-I antibodies and the pellets were analysed by immunoblotting. We could detect the E1 and E2 proteins in samples from HCVpp pre-incubated with lipoprotein-free ApoC-I, but not with HDL (Sup. Fig. 1A). In contrast, control particles harbouring RD114 glycoproteins pre-incubated with ApoC-I were not immuno-precipitated with ApoC-I antibodies, as shown by the lack of detection of RD114 surface glycoproteins, Env, in the pellets (Sup. Fig. 1B). These results indicated that soluble ApoC-I, but not HDL-embedded ApoC-I, could specifically associate to HCV particles.

Next, to investigate the properties of HCVpp/ApoC-I complexes, we purified HCVpp on a sucrose cushion following incubation with soluble ApoC-I or HDL. Again, we detected ApoC-I on the viral particles pre-incubated with ApoC-I but not with HDL (data not shown), indicating that soluble ApoC-I, but not HDL-embedded ApoC-I, could stably associate to the HCV particles. These HCV particles retained the ApoC-I-mediated infection enhancement after purification (78.8% ± 4.15% of the infection enhancement detected before purification), suggesting a stable and functional HCVpp/ApoC-I association. Furthermore, the infectivity of these purified HCV/ApoC-I complexes could be neutralised by ApoC-I antibodies (Table 1), which suggested a sterical hindrance induced via binding of the antibodies onto the viral particles. Similar results were obtained when using HCVcc rather than HCVpp in such neutralisation assays of purified HCV/ApoC-I complexes (Table 1).

Altogether, the results of infection inhibition, BIAcore and immunoprecipitation analysis indicated that HDL-free ApoC-I can associate to the surface of HCV particles and explained the results of HCVcc and HCVpp neutralisation by ApoC-I antibodies in Fig. 1C.

**ApoC-I induces the disruption of HCV particles at high concentrations.** ApoC-I immobilised on a sensor chip may not have the flexibility necessary to
mediate an effect on HCV particles beyond inducing their mere association (Fig. 3A). Thus, to further investigate the characteristics of HCVpp/ApoC-I interaction, we performed alternative BIAcore studies by injecting lipoprotein-free ApoC-I on captured pseudo-particles. HCVpp were captured via an E2-specific mAb covalently immobilised to a CM3 sensor chip (Fig. 3B). In those conditions, as reported before, HCVpp interacted with E1E2 ligands such as E1E2 antibodies or the large extra-cellular loop of CD81, CD81-LEL, but not with HDL (26). This indicated that while a part of HCVpp surface is occupied by the CM3-immobilised E2 mAb, a significant portion of the surface of the viral particles remains accessible for interacting with E1E2 partners. No or very poor binding of ApoC-I to captured HCVpp could be detected upon its injection at low concentrations, in the range of 0.1-3 µg/ml (data not shown). Strikingly, the injection over the captured HCVpp of ApoC-I at higher concentrations, above 5 µg/ml, induced a rapid decrease of the SPR signal, suggesting a loss of mass from the chip surface via a partial removal of CM3-bound HCVpp (Fig. 3B). Such an effect was ApoC-I dose-dependent (Fig. 3C), leading to up to 50-60% decrease of HCVpp-specific SPR signal (Sup. Fig. 2A) as deduced from the ratio of SPR decrease upon ApoC-I injection relative to SPR signal after HCVpp capture (Fig. 3B). HCVpp loss was detected neither when ApoC-II (Fig. 3C) nor when HDL (26) was injected, even at high concentrations. Furthermore, whatever the concentration, ApoC-I did not induce the loss of control particles displaying the surface glycoproteins of HIV (Fig. 3D) or MLV (data not shown) that were captured at levels similar to HCVpp via their respective CM3-immobilised Env antibodies, hence establishing the specificity of this particular HCVpp/ApoC-I interaction. Then, to investigate whether the HVR1 determinant of HCV E2 glycoprotein plays a role in the disruption of CM3-bound HCVpp induced by ApoC-I, we captured HVR1-deleted HCVpp (ΔHVR1-HCVpp) on the CM3 sensor chip and we analysed SPR signals upon ApoC-I injection. As compared to wild type HCVpp, ApoC-I only marginally reduced the SPR signal of the ΔHVR1-HCVpp (Sup. Fig. 2B). This confirmed that HVR1 is a critical determinant of HCVpp/ApoC-I interplay, which can be visualised in both cell entry (Fig. 2A) and SPR assays.

The loss of SPR signal could be induced by partial release of the HCVpp from the immobilised E2 antibody via a competition of injected ApoC-I for the same E2 binding site. However, other experimental set-ups to capture HCVpp on the CM3 sensor chip via different E2 antibodies that bind alternative epitopes or via Galanthus Nivalis lectins that bind E1E2 glycans also resulted in similar losses of HCVpp mass upon ApoC-I injection (Sup. Fig. 2A). This indicated that ApoC-I induced a disruption of HCVpp via interactions with the accessible portion (non antibody-bound) of the E1E2 glycoproteins of the captured viral particles. Finally, ApoC-I did not induce a modification of the SPR signal when soluble forms of the HCV glycoproteins, rather than whole HCVpp, were captured on the CM3 sensor chip via the same antibodies (Sup. Fig. 2C and data not shown). This indicated that the loss of CM3-bound HCVpp was specifically induced by ApoC-I interaction with HCV glycoproteins as displayed in a native conformation on the membrane of viral particles.

That ApoC-I induced the disruption of sensor chip-captured HCVpp (Fig. 3A-D and Sup. Fig. 2A) but not of captured sE2 (Sup. Fig. 2C) suggested that ApoC-I exerts an effect on the HCV particle itself. To directly address this possibility, we incubated purified HCVpp with ApoC-I and subsequently assessed the release of one of the inner components of these viral particles, the reverse-transcriptase (RT), using an enzymatic assay that reflects disruption of the viral membrane (55). Little RT release was detected when HCVpp were incubated with ApoC-II or when control viral particles were incubated with either ApoC-I or ApoC-II (Fig. 3E). In contrast, ApoC-I could induce the disruption of HCV particle at concentrations above 10 µg/ml (Fig. 3E). Altogether, the loss of SPR signal and the release of RT upon ApoC-I treatment of the HCVpp may explain the inhibition of infection at high ApoC-I concentrations (Fig. 2C) by membrane disruption.

**ApoC-I stimulates HCV membrane fusion.**

Infection-enhancement by low concentrations of ApoC-I (Fig. 1B and 2C) may also be explained by less dramatic alterations of the HCV particle. Particularly, upon association with HCV particles, ApoC-I may modify their binding properties to cell surface receptors. To address this possibility, we performed binding assays on Huh-7 cells or on CHO cells that individually expressed the CD81 or SR-BI HCV receptors. The binding of purified HCVpp was assessed by flow cytometry using either anti-E2 or anti-E1 antibodies, as described elsewhere (26,53). No difference in cell binding of HCVpp (Fig. 4) or of control particles (data not shown) was observed whether the viral particles were incubated, or not, with HDL or ApoC-I. Similar results were obtained in binding assays performed with sE2 (data not shown).

Since ApoC-I does not modify HCVpp or sE2 binding to HCV receptors, an alternative possibility is that it stimulates the viral particles for membrane fusion processes. Owing to its amphipathic α-helix structure, ApoC-I may indeed interact with the viral
surface, in a manner dependent on E1E2 presence, and may predispose it for fusion with a target membrane, via alterations of the viral membrane. To address this possibility, we investigated the membrane fusion process induced by HCVcc or HCVpp by using a recently developed HCVpp/liposome fusion assay (11,53). This fusion assay is based upon direct measurement of lipid mixing between virion and liposome membranes. Briefly, the assay relies on the relief of fluorescence self-quenching probes used to measure virus/cell fusion kinetics, i.e., either the pair of N-Rh-PE and N-NBD-PE probes (58), based on phospholipids stably inserted within the target membranes via their two long fatty acid chains, or the R18 probe (59). Lipid dilution upon fusion between liposome and viral membranes at low pH leads to fluorescence dequenching of these probes. Thus, the fusion rates can be measured by the initial rates of fluorescence, as shown here for pH-dependent viruses such as HCV and influenza, but not for pH-independent viruses such as MLV (Table 2; see Fig. 5A and Sup. Fig. 3, as representative curves for N-Rh-PE/N-NBD-PE and R18 probes, respectively). As reported before, fluorescence dequenching was observed only when the pH was decreased to 5.0 and no significant dequenching could be detected at neutral pH even after long incubation times (11).

When ApoC-I was pre-incubated with HCVpp (Table 2 and see Fig. 5B, as representative curves) at concentrations around 1 µg/ml, we found that lipid mixing with liposomes was stimulated, as shown by the increased initial rates of the fusion kinetics. The enhancement of fusogenicity remained acid pH-dependent since no fusion could be detected at neutral pH (data not shown), indicating that ApoC-I promotes HCV fusogenicity in a specific manner. ApoC-I concentrations higher than 6 µg/ml resulted in inhibition of lipid mixing (Table 2 andFig. 5B), which reflected the inhibition of infection at the same concentrations (Fig. 2C) and the disruption of HCVpp detected in SPR and RT release assays (Fig. 3). The stimulating effect of ApoC-I on membrane fusion was highly specific of ApoC-I interaction with HCV envelope since pre-incubation of ApoC-I with control pseudo-particles harbouring an influenza virus hemagglutinin (HApp) (Table 2 and Fig. 5C) and pre-incubation of ApoC-II with either HCVpp or HA (Table 2) did not change the fusion rates. Of note, the results obtained with the N-Rh-PE and N-NBD-PE probes were fully confirmed using the R18 probe (Sup. Fig. 3). Moreover, we also confirmed that low concentrations of ApoC-I could also enhance membrane fusion by replicative HCVcc particles (Sup. Fig. 3B).

No effect of ApoC-I could be detected on membrane fusion when ApoC-I was pre-incubated with the liposomes, rather than with the HCVpp (Sup. Fig. 3E). This suggested that the fusion-enhancing effect of ApoC-I requires a prior interaction with the viral particles to promote fusion enhancement, most likely via its interplay with the HCV glycoprotein. Finally, we found that ApoC-I could not stimulate liposome fusion of HCVpp harbouring a deletion of HVR1 (Table 2 and Sup. Fig. 3F), congruent with the notion that HVR1 controls infection enhancement by ApoC-I and HDL (Fig. 2A).

DISCUSSION

HCV biology is strikingly linked to lipoproteins, which act at several steps of viral replication, including assembly, cell entry and protection from the host immune response (26,32,33,37,60-71). Several groups have purified HCV from plasma to investigate virus/cell interactions in vitro. However, in addition to the inherent difficulties to measure viral infectivity of HCV purified from plasma, the current approaches to study viral particles isolated from patients do not allow the unequivocal molecular analysis of their interactions with serum components. Using novel infection assays that consist of infectious HCV particles produced in vitro, in defined medium compositions, our results reveal novel features of the interplay between HCV and lipoproteins. Our findings uncover an original mechanism by which HDL stimulates cell entry of HCV, at a post-receptor-binding stage. We demonstrate here that ApoC-I, a protein sub-component of HDL, is specifically recruited by HCV glycoproteins on the viral surface and intrinsically increases the fusogenicity of HCV particles. ApoC-I can be recruited either as HDL-bound component, through a triple interplay with SR-BI, a receptor for both HDL (29) and HCV (15,31) or directly as lipoprotein-free protein, in our experimental conditions. To our knowledge, this is the first description of a host component that is specifically recruited to the surface of an enveloped virus to promote membrane fusion events. Interestingly, high ApoC-I concentrations induced the disruption of the HCV particles and loss of infectivity, most likely through alteration of the viral membrane, indicating that a fine-tuning of ApoC-I recruitment is required for optimising infection enhancement.

The flexibility of the HCVpp assay, which facilitates the substitution or the modification of the viral surface glycoproteins, established the high specificity of the interaction between ApoC-I and HCV viral surface and the subsequent effect of this interaction on infectivity. Significantly, the most salient features of our findings could be confirmed with the J6/JFH-1 replicating HCV (HCVcc). Furthermore we demonstrate that the HVR1 domain of HCV-E2 is a
crucial viral component mediating the effect of ApoC-I on virions. The importance of this HCV determinant is suggested in vivo by the attenuated phenotype of HVR1-deleted virus in chimpanzees and by abrogation of infectivity by HVR1 antibodies (72-74). The analysis of HVR1 sequences from different HCV strains indicated the involvement of this region during cell entry (57). Furthermore, the presence of basic residues in HVR1 was found to facilitate virus entry (75). Finally, HVR1 also modulates the interaction of HCV-E2 with SR-BI since deletion of HVR1 abrogates both SR-BI binding of soluble E2 (31) and HCV infection enhancement by HDL (33,36). Our results indicate that the same HVR1 conserved residues are involved in infection-enhancement by either HDL or lipoprotein-free ApoC-I. This substantiates the notion that HVR1 sequences harbour a conserved function that modulates the effect of ApoC-I on infectivity/fusogenicity (Fig. 2A and Sup. Fig. 2B & 3F) and/or its recruitment, either directly, when ApoC-I is provided at optimal concentrations as a lipoprotein-free protein to HCV particles, or, alternatively, from HDL, most likely following HDL/SR-BI interaction, as discussed below.

As ApoC-I in vivo is present in HDL, mainly, and in VLDL, but not as a lipoprotein-free form (40), it could be released from either lipoprotein type, in theory, to allow a subsequent recruitment on HCV particles. As expected, owing to its association to LDL/VLDL as "lipo-viro-particles (LVP)" (61,69,71), we found that plasma-derived HCV contains ApoC-I (data not shown). Although this suggests that in vivo, HCV may also recruit ApoC-I from VLDL, besides HDL, this possibility remains difficult to test experimentally. Indeed, due to the technical difficulties to unambiguously characterise LVPs in biochemical and functional assays, it is not possible to demonstrate if VLDL-bound ApoC-I can be transferred to the viral membrane of LVP and if it regulates membrane fusion. Nevertheless, our data support the notion that ApoC-I is most likely recruited from HDL since only the latter lipoprotein and not LDL/VLDL enhance the infection of HCVpp and HCVcc (26,33,35,36,82) and since HDL-mediated infection-enhancement was fully blocked by ApoC-I antibodies.

HDL itself does not directly interact with virions (26,33,36) and does not spontaneously exchange ApoC-I (Sup Fig. 1). We propose that in vivo ApoC-I could be recruited on HCV particles following the interaction between HCV, HDL and SR-BI. Indeed, recent studies indicate that HDL/SR-BI interaction results in consequent changes in the size, the density and the lipid composition of HDL (76). Such changes modulate the affinity and stability of the exchangeable apolipoproteins on the lipoprotein and hence, induce their dissociation, shedding and/or transfer to neighbouring lipid surfaces (76-81). Owing to the proximity of SR-BI-bound HDL with HCV particles, ApoC-I desorbed from HDL could then be captured by the HCV glycoproteins and subsequently transferred to the viral lipid membrane. Such a model is compatible with our observation that the lipid transfer functions of SR-BI are required for HDL-mediated infection enhancement (26,33,36), but not for stimulation by lipoprotein-free ApoC-I, which was fully maintained when SR-BI functions were inhibited by chemical compounds or blocking antibodies or by its down-regulation (Fig. 2B).

Our results indicate that ApoC-I influences the infectivity of HCV particles at a post-binding stage, by promoting their membrane fusion properties. On the one hand, ApoC-I may modify the HCV glycoproteins conformation, thereby assisting a limiting transition stage in the refolding of the glycoproteins, and consequently, increase the fusion rate. However, we did not find evidence for modification of the conformation of HCV glycoproteins following interaction of HCV particles with ApoC-I (data not shown). Alternatively, our results indicate that ApoC-I alters the viral membrane in a dose-dependent manner: it increases membrane fusion at low concentrations (Table 2, Fig. 5 and Sup. Fig. 3) but disrupts the viral particles at high concentrations (Fig. 3 and Sup. Fig. 2). These two effects may reflect the same property of ApoC-I to interact with lipid membranes and are both reminiscent of a particular feature of membrane fusion processes.

The fusion between viral and cellular membranes involves a complex, multi-step conformational change of the viral glycoproteins (20). HCV entry is pH-dependent (10,11,15,17), suggesting that the low-pH induces the refolding of the E1E2 glycoprotein complex. The critical domains and the molecular events that mediate HCV membrane fusion remain poorly defined (53). For pH-dependent viruses, such as alphaviruses and flaviviruses, protonation of the ectodomain of the viral glycoproteins in the acidic endosomal environment triggers the first refolding event, which induces the dissociation of the alphavirus E2-E1 heterodimers or of the flavivirus E-E homodimers, insertion of the fusion peptide in the target cell membrane, apposition of the viral and cell membranes and ultimate merging of the outer lipid leaflets (20).

While the essential components of the fusion apparatus reside on the extra-virion side of the virion membrane (20), additional discrete segments or determinants of the viral glycoproteins modulate the extent of membrane fusion. For example, the transmembrane domains of several class I (83-85)
and II (86) fusion proteins are essential for their activity at different stages of the fusion process, most likely during lipid mixing of the viral and cellular membranes. Additional segments located immediately before the transmembrane domains can also contribute in perturbation of the viral outer lipid leaflet and hence influence fusion with the cell membrane. Notably, a membrane-proximal tryptophan-rich region of HIV gp41 has been suggested to aid in the disruption of membrane during the gp41-mediated fusion process (87-89). This peptide forms a partially amphipathic helix (89), which, by positioning in the outer membrane bilayer of the viral membrane (90), is believed to disrupt the water-phospholipid interface in a manner similar to the proposed mechanism of amphipathic structures present in antimicrobial peptides or exchangeable apolipoproteins (91-97). We propose that the recruitment of ApoC-I on HCV particles similarly influences membrane fusion, by facilitating the disruption of the outer phospholipid bilayer of the viral envelope. The α-helical amphipathic structure of ApoC-I, its affinity to phospholipid surfaces and its direct interaction with HCV particles are features shared with the aforementioned peptides. Indeed, like for the other exchangeable apolipoproteins, ApoC-I contains a cluster of charged amino acids that interact with the polar head of negatively charged lipids and a cluster of hydrophobic amino acids that are embedded within the lipid aliphatic chains (96,98-102). Furthermore, the strong interaction of exchangeable apolipoproteins with phospholipid bilayers is accompanied by microsolvilisation of artificial as well as cell membranes through the modification of the lipid bilayer properties (93,96) and, at high peptide/lipid ratios, disruption of the vesicles to form smaller discoidal complexes (103). Thus, binding of ApoC-I to the surface of the HCV particle at a limited extent may induce small perturbations or deformations of the outer phospholipid layer, which may expose the hydrophobic interiors of bilayers and contribute to generate attractive forces between membranes that facilitate membrane fusion (Table 2, Fig. 5 and Sup. Fig. 3). At high ApoC-I concentrations however, extended disruption of the viral membrane would release the non lipid-linked viral components, e.g., the nucleocapsid (Fig. 3E), only leaving patches of lipid-embedded E1E2 glycoproteins bound to the sensor chip (Fig. 3 and Sup. Fig. 2). These results therefore indicated the possibility that ApoC-I-derived molecules could be used to develop HCV-specific inhibitors.

It is important to highlight the high specificity and concentration-dependent ApoC-I effect on HCV particles. Indeed, neither membrane disruption nor fusion increase was detected with alternative viruses, or with ApoC-II, which has a amphipathic structure closely related to ApoC-I. Moreover, infection-enhancement vs. disruption of HCV particles were induced at well-defined concentrations, suggesting that HCV needs a regulatory mechanism to recruit the optimal dose of ApoC-I on virions and favour its propagation. Such regulation could be achieved via HDL interaction with SR-BI, as above discussed, and/or via change of HVR1 residues involved in this function. Indeed, it is interesting that some mutations of HVR1 conserved residues resulted in either loss of function or gain of function (Fig. 2A) and that no infection-inhibition was detected with HDL, even at saturating concentrations (33). This indicates that HCV interplay with HDL and SR-BI, which involves HVR1 conserved framework, results in release and subsequent recruitment of the appropriate quantity of ApoC-I on viral particles. Thus, the unexpected role of a serum protein in promoting fusion enhancement is another remarkable feature of the ability of HCV to highjack blood components to facilitate its replication and highlights the intricate relationship between HCV and lipoproteins.

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FIGURE LEGENDS

Figure 1. ApoC-I enhances the infectivity of HCV particles. Results of infection assays on HuH-7 cells using HCVcc (J6/JFH-1), HCVpp harbouring HCV E1E2 proteins or control viral particles (CONTpp), as specified below. The viral particles were produced in cell culture media devoid of serum lipoproteins and were diluted in serum-free medium to compare viral preparations containing equivalent input of infectious particles, i.e., ca. 10^4 infectious units (i.u.). A. Infection assays using HCVcc, HCVpp and CONTpp harbouring the glycoproteins of RD114 in the presence of 6 µg/ml cholesterol-HDL and anti-ApoC-I antibody, as indicated. The results show the increases of infection (mean±SD; n=3) determined by calculating the ratios between the average infectious titres in the presence of the indicated components relative to the average infectious titres in the absence of antibody and/or HDL, as specified (—). B. HCVpp and CONTpp harbouring the glycoproteins of RD114 (RD114pp), VSV (VSV-Gpp), influenza (HApp) and MLV (MLVpp) viruses were pre-incubated 45 min at room temperature with 1.4 µg/ml of ApoC-I or ApoC-II, as indicated. The results show the increases of infection (mean±SD; n=3) as determined by calculating the ratios between the average infectious titres in the presence of the indicated apolipoprotein relative to the average infectious titres in the absence of apolipoproteins (—). C. HCVcc, HCVpp and CONTpp harbouring the RD114 glycoproteins were pre-incubated 45 min at room temperature with 1.4 µg/ml of ApoC-I, before adding anti-ApoC-I antibody for 45 min at the...
indicated concentrations. The results are expressed as the percentage of neutralisation of the infectious titres relative to incubation with medium devoid of antibody.

**Figure 2. Parameters of ApoC-I-induced modulation of HCV infectivity.** Results of infection assays on Huh-7 cells using HCVpp harbouring wild type or mutant E1E2 proteins or control viral particles (CONTpp) harbouring the glycoproteins of RD114, as indicated. The viral particles were produced in cell culture media devoid of serum lipoproteins. The input of HCVpp or CONTpp, i.e., ca. 10^5 infectious units (i.u.), was adjusted by dilutions of either viral particle in serum-free medium. A. Effect of HDL or Apo-C-I on infectivity of HCVpp harbouring HVR1-deletion (ΔHVR1-HCVpp) or G389L, L399R point mutations in HVR1. The results are expressed as the average percentages of the infection enhancements of these mutants relative to that detected for wild-type HCVpp set to 100%, which corresponded to 4.5- and 3-fold increases of infection (see Fig. 1A and 1B) when using HDL and Apo-C-I concentrations of 6 µg/ml (as cholesterol-HDL) and 1.4 µg/ml, respectively (mean±SD, n=3). The infectious titres of parental and mutant HCVpp were within the range of 2x10^5-2x10^6 i.u./ml. B. Effect of HDL or Apo-C-I (CI) in HCVpp infected of Huh-7 cells pre-treated with a 1/100-diluted polyclonal anti-SR-BI mouse serum (anti-SR-BI) or with 50 µM BLT-4 (BLT), an SR-BI lipid-transfer inhibitor, or with a SR-BI siRNA vector (down-regulation). The results are expressed relatively to the average percentages of the infection enhancement detected for wild-type HCVpp in absence of SR-BI blocking antibody, BLT-4 or SR-BI siRNA set to 100%, which corresponded to 5- and 2.8-fold increases of infection (see Fig. 1A and 1B) when using HDL and Apo-C-I concentrations of 6 µg/ml (as cholesterol-HDL) and 1.4 µg/ml, respectively (mean±SD, n=3). Down-regulation of SR-BI was verified by western blotting of siRNA vector-treated Huh-7 cells (inset). C. HCVpp were pre-incubated for 45 min at room temperature with varying concentrations of Apo-C-I (as µg/ml), as indicated. The results are expressed relatively to the average percentages of the infection enhancement detected for wild-type HCVpp at the concentration of 1.4 µg/ml set to 100% (see Fig. 1B), which corresponded to a 3.5-fold increase of infection (mean±SD, n=5).

**Figure 3. ApoC-I interacts with HCVpp.** Detection of HCVpp/Apo-C-I interactions by surface plasmon resonance analysis (BLAcore). The viral particles, HCVpp or control particles (CONTpp), as specified below, were produced in cell culture media devoid of serum lipoproteins and purified through a sucrose-cushion. A. Apo-C-I was immobilised on the dextran matrix of a CM4 sensor chip, Purified HCVpp or control pseudo-particles displaying the glycoproteins of HIV (CONTpp), as indicated, were injected over immobilised Apo-C-I (pp injection) and the binding of either viral particle type was determined by the number of resonance units (RU). Equivalent input of either viral particle, i.e., ca. 10^5 infectious units (i.u.), was checked by the detection of their capsid protein (CA) by western blotting (inset). The spikes detected at the start and at the end of injection of the viral particles are due to change in buffer and/or to the online subtraction of the sensorgram recorded on the control flow cell. B. HCVpp were captured via the AP33 E2-mAb covalently immobilised to the dextran matrix of the CM3 sensor chip and the binding was determined by the number of resonance units (RU). Purified Apo-C-I was injected over captured HCVpp at 100 µg/ml. C. Injection of Apo-C-I at varying concentrations and of Apo-C-II at 100 µg/ml on HCVpp captured with the AP33 mAb, as in panel B. D. HCVpp and control pseudo-particles displaying the glycoproteins of HIV (CONTpp) were captured via the AP33 and 2F5 mAbs, respectively, covalently immobilised to the dextran matrix of the CM3 sensor chip. Equivalent capture levels of HCVpp and CONTpp (data not shown) were obtained by adjusting the input of viral particles. Purified Apo-C-I was injected over captured HCVpp and CONTpp at 100 µg/ml. E. Disruption of viral membrane was determined by the release of an inner component of the viral particles (the reverse transcriptase (RT)), using an enzymatic assay. HCVpp and CONTpp harbouring the glycoproteins of HIV (HIVpp), RD114 (RD114pp) and MLV (MLVpp) were preincubated with various concentrations of Apo-C-I (left panel) or Apo-C-II (right panel) in the absence of Triton x100 in the sample dilution buffer. Equivalent input of either virus particle was checked by the detection of their capsid protein (CA) by western blotting. The results show the ratio of the release of RT activity at each concentration of Apo-C-I or Apo-C-II relative to the release of RT in the absence of Apo-C-I and Apo-C-II. These data are representative of three independent experiments.

**Figure 4. Apo-C-I does not modify cell surface binding of HCVpp.** 50 µl of sucrose cushion-purified HCV particles produced in cell culture media devoid of serum lipoproteins were pre-incubated, or not (—), with 1.4 µg/ml of Apo-C-I or with 6 µg/ml cholesterol-HDL, as indicated, for 45 min at room temperature. The viral particles were then incubated for 1 hr at 37°C with Huh-7 (A) or with CHO-derived cells (B) expressing the human CD81 (CHO-CD81) and SR-BI (CHO-SR-BI)
molecules. The cell-bound HCVpp were detected with either anti-E1 (A4) or anti-E2 (H53) mouse antibodies, as indicated; then with allophycocyanin (APC)-conjugated anti-mouse antibodies. The fluorescence was analysed with a fluorescence-activated cell sorter (FACSCalibur; Beckton Dickinson). The background fluorescence was provided using pseudo-particles without glycoproteins (A) or using parental CHO cells incubated with HCVpp only (B). The data are representative of three independent experiments. No difference in cell binding of VSV-Gpp control particles could be detected whether HDL or ApoC-I was pre-incubated with such viral particles (data not shown).

Figure 5. **ApoC-I increases membrane fusion.** The fusion capacity of HCVpp harbouring wild type E1E2 proteins and control pseudo-particles harbouring influenza (HApp) glycoproteins, MLV glycoproteins (MLVpp) or no glycoprotein (noEnvpp), as indicated, was tested using lipid mixing assays. 40 µl of purified viral particles produced in cell culture media devoid of serum lipoproteins were added to liposomes labelled with N-Rh-PE and N-NBD-PE lipids in PBS, pH 7.4. After a 2-min equilibration at 37°C, fusion was initiated by decreasing the pH to 5 in the cuvette (time 0 of the fusion kinetics). No fluorescence dequenching was detected before acidification of the medium. The results are expressed as percentages of maximal fluorescence, obtained by addition of Triton X-100 (final 0.1% v:v) to the particle/liposome suspensions. 

**A.** Basic profiles of the fusion kinetics obtained with pH-dependent (HCVpp and HApp) and pH-independent (MLVpp) viral particles. Equivalent input of either virus particle was checked by the detection of their capsid protein (CA) by western blotting (inset). **B.** ApoC-I was pre-incubated at low (0.7 µg/ml) vs. high concentration (7 µg/ml) with HCVpp. **C.** ApoC-I was pre-incubated at low (0.7 µg/ml) vs. high concentration (7 µg/ml) with HApp.
Table 1. Neutralisation of HCV/ApoC-I complexes

| Step 1a | Step 2 | Step 3 | % neutralisationc |
|---------|--------|--------|------------------|
| HCVpp + ApoC-I | anti-ApoC-I Abb | - | 66.3±8.3 |
| HCVpp + ApoC-I | purificationc | anti-ApoC-I Abb | 59.4±13.8 |
| HCVcc + ApoC-I | anti-ApoC-I Abb | depletiond | 60.1±15.7 |
| HCVcc + ApoC-I | anti-ApoC-I Abb | - | 95.8±1.5 |
| HCVcc + ApoC-I | purificationc | anti-ApoC-I Abb | 92.3±3.9 |
| HCVcc + ApoC-I | anti-ApoC-I Abb | depletiond | 94.9±3.0 |

a: The supernatant of HCVpp- or HCVcc-producer cells was pre-incubated with ApoC-I at the concentration of 1.37 µg/ml for 45 min at RT.
b: The anti-ApoC-I antibody was added to the virus/ApoC-I mix at the concentration of 1 µg/ml for 45 min at RT.
c: Purification of viral particles after pre-incubation with ApoC-I upon ultracentrifugation through a 20%-sucrose cushion.
d: The ApoC-I-bound HCVcc or HCVpp were depleted by immuno-precipitation using ApoC-I antibody and a mix of A and G protein coupled to sepharose beads.
e: After infection of Huh-7 target cells, the percentage of neutralisation was determined by calculating the average infectious titres determined in the presence of anti-ApoC-I antibody relative to the infectious titres in the absence of anti-ApoC-I.

Table 2. Initial rates of lipid mixing in liposome fusion assaysa

| Conditionsb | HCVpp | HApp | ΔHVRI-HCVpp |
|-------------|-------|------|-------------|
| no apoC     | 0.44±0.05 | 0.64±0.04 | 0.51±0.08 |
| apoC-I 0.7 µg/ml | 0.97±0.08 | 0.64±0.03 | 0.49±0.07 |
| apoC-I 7 µg/ml  | 0.25±0.07 | 0.66±0.05 | 0.52±0.05 |
| apoC-II 0.7 µg/ml | 0.48±0.03 | 0.65±0.06 | 0.50±0.07 |
| apoC-II 7 µg/ml  | 0.49±0.03 | nd | nd |

a: the initial rates of lipid mixing (% max Fluorescence.min⁻¹) at pH5 were determined as the tangents of the lipid mixing kinetics at t=0 (see Fig. 5, as representative curves), which were averaged from 3 separate experiments.
b: the viral particles were pre-incubated 15 min at room temperature with the indicated concentrations of ApoC-I or ApoC-II before performing the liposome fusion assays. nd, not determined.
Fig. 1. Dreux et al. 2007
Fig. 2. Dreux et al. 2007
Fig. 3. Dreux et al. 2007
**A**

Huh-7, anti-E1

Huh-7, anti-E2

- Background
- HCVpp/
- HCVpp/HDL
- HCVpp/ApoC-I

**B**

CHO-SR-BI, anti-E2

CHO-CD81, anti-E2

- Background
- HCVpp/
- HCVpp/HDL
- HCVpp/ApoC-I

Fig. 4. Dreux et al. 2007
Fig. 5. Dreux et al. 2007
The exchangeable apolipoprotein APOC-I promotes membrane fusion of hepatitis C virus
Marlène Dreux, Bertrand Boson, Sylvie Ricard-Blum, Jennifer Molle, Dimitri Lavillette, Birke Bartosch, Eve-Isabelle Pécheur and François-Loïc Cosset

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