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Human Apolipoprotein E Peptides Inhibit Hepatitis C Virus Entry by Blocking Virus Binding

Shufeng Liu,[1] Kevin D. McCormick,[1] Wentao Zhao,[2] Ting Zhao,[3,4] Daping Fan,[2] and Tianyi Wang[1]

Hepatitis C virus (HCV) entry is a multiple-step process involving a number of host factors and hence represents a promising target for new antiviral drug development. In search of novel inhibitors of HCV infection, we found that a human apolipoprotein E (apoE) peptide, hEP, containing both a receptor binding fragment and a lipid binding fragment of apoE specifically blocked the entry of cell culture grown HCV (HCVcc) at submicromolar concentrations. hEP caused little cytotoxicity *in vitro* and remained active even if left 24 hours in cell culture. Interestingly, hEP inhibited neither human immunodeficiency virus (HIV)-HCV pseudotypes (HCVpp) nor HIV and Dengue virus (DENV) infection. Further characterization mapped the anti-HCV activity to a 32-residue region that harbors the receptor binding domain of apoE, but this fragment must contain a cysteine residue at the N-terminus to mediate dimer formation. The anti-HCV activity of the peptide appears to be dependent on both its length and sequence and correlates with its ability to bind lipids. Finally, we demonstrated that the apoE-derived peptides directly blocked the binding of both HCVcc and patient serum-derived virus to hepatoma cells as well as primary human hepatocytes. *Conclusion*: apoE peptides potently inhibit HCV infection and suggest a direct role of apoE in mediating HCV entry. Our findings also highlight the potential of developing apoE mimetic peptides as novel HCV entry inhibitors by targeting HCV-host interactions. (HEPATOLOGY 2012;56:484-491)

Hepatitis C virus (HCV) is an important human pathogen that primarily infects human hepatocytes and causes many chronic liver diseases. Without a prophylactic vaccine, combination therapy with pegylated interferon (IFN)-α and ribavirin is only effective in 40%-80% of patients and has severe side effects that result in poor patient compliance. The recent approval of boceprevir and telaprevir by the Food and Drug Administration (FDA) highlighted the success of developing small molecule inhibitors to treat chronic HCV infection. Resistance to these inhibitors, however, is expected to emerge rapidly in clinics, due to the high mutation rate of the virus.[1] As with HIV treatment, a successful treatment of HCV is expected to involve a combination of multiple inhibitors of different targets. Therefore, new antiviral drugs are urgently needed to treat HCV infection in combination with current therapies.

Research on HCV was revolutionized by the advent of the Japanese fulminant HCV strain (JFH-1) that can be cultivated in cell culture (HCVcc) and hence permits the study of the entire viral life cycle.[2-5] HCV entry requires at least four cellular membrane proteins, including CD81,[6] scavenger receptor BI (SR-BI),[7] claudin-1 (CLDN1),[8] and occludin (OCLN).[9,10] Remarkably, another host factor, human apolipoprotein E (apoE), appears to be assembled into infectious virions and plays a crucial role in conferring virus infectivity.[11-14] The 299-residue apoE is a main

Abbreviations: apoE, apolipoprotein E; HCV, hepatitis C virus; HCVcc, cell culture grown HCV; HCVpp, lentiviral particles pseudotyped with HCV envelope proteins; MOI, multiplicity of infection; VSVpp, human immunodeficiency virus (HIV) particles pseudotyped with vesicular stomatitis virus envelope protein G.

From the [1]Departments of Infectious Diseases and Microbiology, University of Pittsburgh, Pittsburgh, PA; [2]Department of Cell Biology and Anatomy, School of Medicine, University of South Carolina, Columbia, SC; [3]Mass Spectrometry Platform, Cancer Biomarkers Facility, University of Pittsburgh Cancer Institute, Pittsburgh, PA; and [4]Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA.

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Address reprint requests to: Dr. Tianyi Wang, Department of Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh, 130 DeSoto Street, Pittsburgh, PA 15261. E-mail: tywang@pitt.edu; fax: 412-383-8926.

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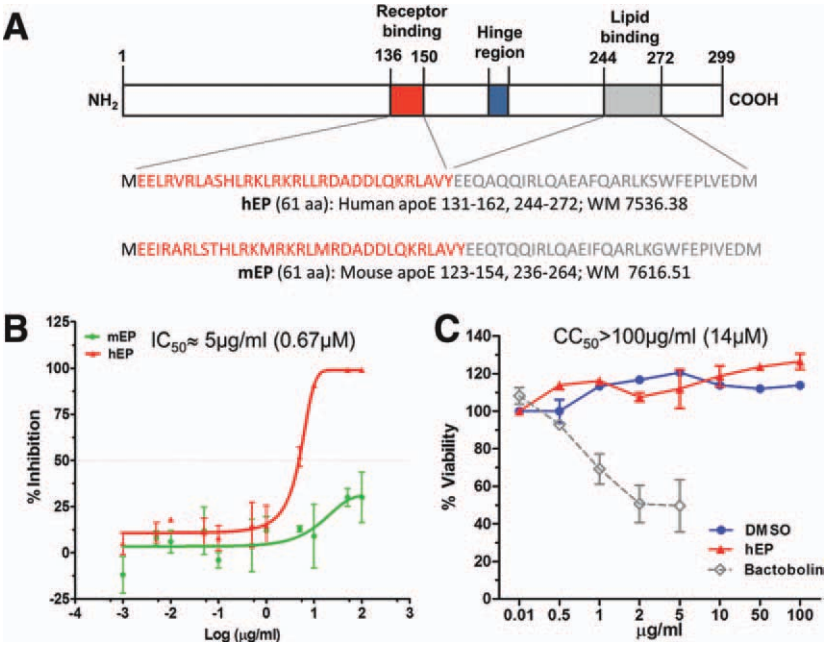

Fig. 1. A human apoE-derived peptide (hEP) suppresses HCV infection. (A) Schematic illustration of apoE protein domains and the amino acid sequences of hEP and mEP peptides. (B) Dose-dependent inhibition of HCVcc infection by hEP. Huh7.5.1 cells were infected with JFH-1 HCVcc-Luc (MOI ≈0.5) in the presence of hEP or mEP for 2 hours. After removal of virus and peptides, cells were further incubated for 48 hours prior to luciferase assay. Data are presented as percent inhibition relative to control infections in which cells were treated with dimethyl sulfoxide (DMSO) (0%). Results are expressed as mean ± standard deviation (SD) (n = 3). (C) Huh7.5.1 cells were treated with hEP or DMSO at indicated doses for 48 hours. The effect of hEP on cell viability was measured by a CellTiter Glo assay kit. Bactobolin, a protein synthesis inhibitor, induced significant cell death at the indicated concentrations.

component of lipoproteins in plasma and participates in lipid transport by way of its ability to bind to multiple cell surface receptors, including low-density lipoprotein receptor (LDLR), apolipoprotein E receptor 2 (apoER2), very-low-density lipoprotein receptor (VLDLR), SR-BI, low-density lipoprotein receptor-related protein 1 (LRP1), and heparan sulfate proteoglycan (HSPG),[15] some of which have been implicated in HCV entry (reviewed[16]). Here we report that novel peptides derived from human apoE specifically block HCV binding to the cell surface.

Materials and Methods

Full details are available in the Supporting Materials.

Cells and Reagents. The human kidney epithelial cell line Lenti-X 293T was purchased from Clontech. The Huh7.5.1 line generated from a cured HCV replicon cell line was provided by Dr. Francis Chisari (Scripps Research Institute).[4] Maintenance of cell lines has been described.[17] Normal human hepatocytes were either obtained through the Liver Tissue Cell Distribution System (Dr. Stephen Strom, Pittsburgh, PA), which was funded by NIH contract N01-DK-7-0004/HHSN267200700004C, or purchased from Celsis and maintained as described.[17] Antibodies were purchased from BD Biosciences (anti-CD81, JS-81 clone; anti-LDL-R, #550495). Secondary antibodies were purchased from Jackson ImmunoResearch Laboratories and Molecular Probes (Invitrogen). Heparin and bafilomycin A1 were purchased from Sigma.

Statistical Analysis. Bar graphs were plotted to show the mean ± standard deviation (SD) of at least two independent experiments. Statistical analyses were performed using Graphpad Prism 5. $P < 0.05$ in Student test was considered statistically significant.

Results

Rationale Design of a Human apoE Peptide (hEP) as HCV Entry Inhibitor. Previous studies have revealed that the majority of E2-containing viral particles also contain apoE on their surface.[11,12,14] In the lipid-free state, apoE contains two independently folded structural domains: the N-terminal four-helix-bundle structure that harbors the LDLR binding region (residues 136-150), and the C-terminal domain containing the major lipid-binding region (residues 245-266).[18] Based on this knowledge, we designed a human apoE dual-domain peptide, named hEP, that contains nearly the entire amphipathic helix 4 of N-terminal domain and the major lipid-binding region of the C-terminal domain (Fig. 1A). We reasoned that such a peptide will compete with virions-associated apoE for cellular receptors or lipids. For comparison, we also designed a similar peptide using sequences derived from the mouse apoE (mEP) (Fig. 1A). Both peptides were expressed in bacteria, affinity-purified to >95% purity as determined by HPLC, and were free of endotoxin.

Next we assessed the effects of both peptides on HCVcc infection. Notably, hEP blocked HCVcc infection with a 50% inhibitory concentration (IC_{50}) of 0.67 μM, but mEP did not exert significant inhibitory

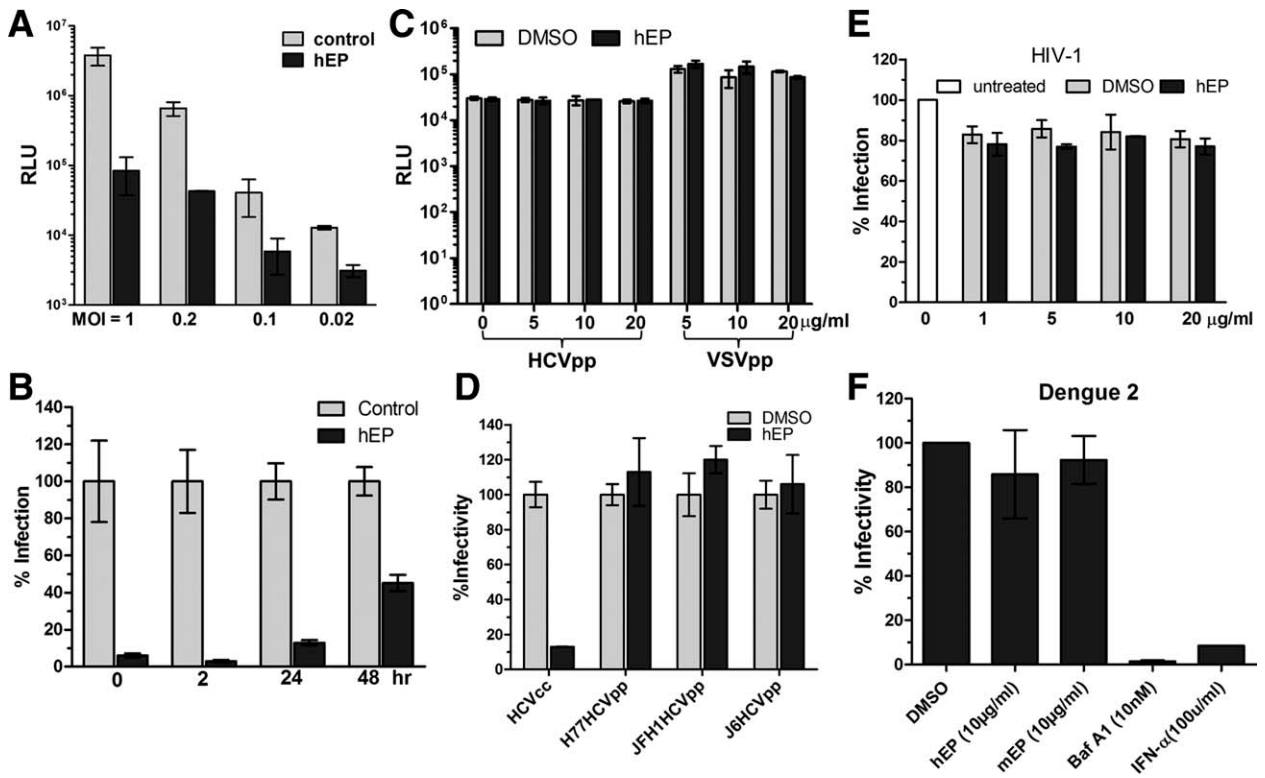

Fig. 2. Characterization of the hEP-mediated inhibition of HCVcc infection. (A) hEP inhibited HCVcc infection at multiple MOIs. DMSO was added as the negative control. (B) 20 μg/mL hEP or DMSO was incubated with Huh7.5.1 cells for specified periods of time prior to exposure to HCVcc-luc. Results were calculated as percent infection relative to counts obtained from the DMSO-treated samples (set to 100; n = 3). (C,D) Huh7.5.1 cells were infected by HCVpp bearing HCV envelope proteins derived from H77, JFH1, and J6, or VSV-Gpp (MOI $\approx$0.5) in the presence of DMSO or hEP at the indicated concentrations. (D) The hEP was 20 μg/mL and the infectivity of DMSO-treated samples was arbitrarily set to 100%. (E) HIV-1 was produced by transfecting the proviral construct pNL4.3 into 293T cells. An equal amount of virus (MOI $\approx$0.5) was added to an HIV reporter cell line TZM-bl that harbors an HIV TAT-dependent luciferase reporter construct in the presence of DMSO or hEP at the indicated concentrations. At 48 hours postinfection luciferase activity was measured. (F) DENV (Thailand 16681) was added to a Huh7.5.1 cells in the presence of DMSO, hEP, mEP, IFN-α, and bafilomycin A1 for 2 hours and then replaced with fresh media. At 48 hours postinfection the infectivity of each virus was determined by calculating the 50% tissue culture infection dose ($TCID_{50}$/mL) following a standard protocol based on immunostaining of DENV Prm/E protein (2H2 antibody, American Type Culture Collection [ATCC]).

effect until a much higher concentration of peptide was used (Fig. 1B). hEP also showed no cytotoxicity even at the highest dose we could possibly test (100 μg/mL, $\approx$14 μM; Fig. 1C). Further characterization demonstrated that hEP robustly suppressed HCVcc infection at various multiplicities of infection (MOIs) (Fig. 2A). The peptide is also rather stable, as its anti-HCV activity largely retained even after being left in the culture for 24 hours (Fig. 2B). By contrast, hEP did not affect the infectivity of lentiviral particles pseudotyped with HCV envelope proteins (HCVpp), human immunodeficiency virus (HIV)-1, or Dengue virus (DENV) (Fig. 2C-F). Therefore, hEP appears to act on a target that is specifically required for HCVcc infection.

hEP Binds Lipid and Lowers Plasma Cholesterol Level. The design of hEP and mEP enables both lipid binding and receptor binding. To verify this we assembled dimyristoylphosphatidylcholine liposome (DMPC vesicles) *in vitro* and found both hEP and mEP efficiently bound to DMPC, transforming the large vesicles (turbid solution) into smaller particles (clear solution) (Fig. 3A). In addition, peptide-DMPC complexes competed with DiI-labeled LDL in binding to cells (Fig.3B), suggesting they compete for LDLR. Finally, to test whether the two peptides promote endocytic clearance of plasma lipoproteins, we measured the plasma cholesterol level in mice administered peptides or phosphate-buffered saline (PBS). Shown in Fig. 3C, hEP and mEP comparably mediated plasma cholesterol clearance in mice. Taken together, these data indicate that both peptides possess lipid binding and receptor binding activity.

Sequence-Activity Analysis of the apoE Peptide. To investigate the requirement of lipid binding and receptor binding for hEP to inhibit HCV entry, we sought to fine map the region required for antiviral activity of hEP. To that end, six additional peptides

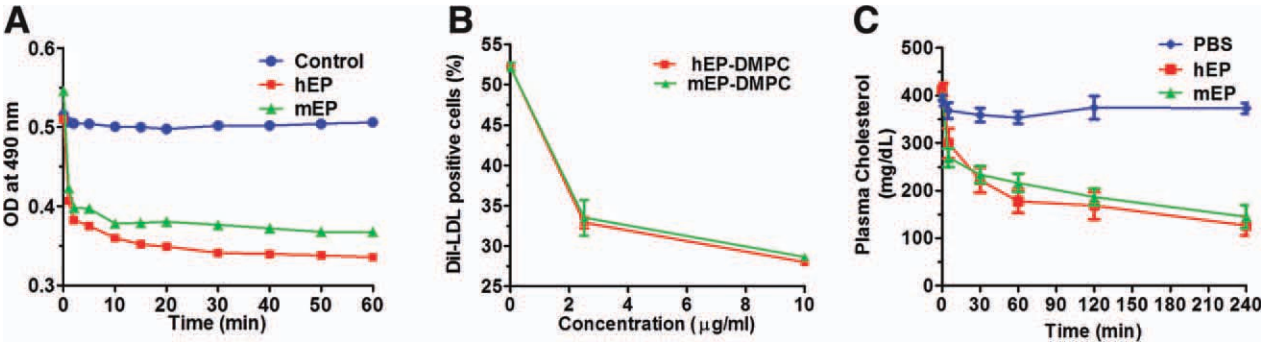

Fig. 3. Both hEP and mEP mediate plasma cholesterol clearance through hepatic lipoprotein receptors. (A) DMPC binding assay was conducted as described in the Supporting Information. The turbidity of DMPC vesicle solution was monitored by OD490nm. The curves were representative of two independent experiments. (B) HEK293T cells were loaded with Dil-LDL (5 μg/mL) along with apoE peptide-DMPC particles, which competed with Dil-LDL for LDLR binding and LDLR-mediated endocytosis (for 30 minutes); n = 3 for each group. (C) Each peptide (100 μg in 100 μL PBS) was retro-orbitally injected into 6-month-old apoE−/− mice (two mice each group). PBS (100 μL) was injected into two other mice as control. Before (set as 0 minute timepoint) and 5 minutes, 30 minutes, 1 hour, 2 hours, and 4 hours after the peptide or PBS injection, 30 μL blood samples were drawn for plasma cholesterol measurement using the total Cholesterol Reagent Kit (Raichem, San Diego, CA).

(hEP-1 through hEP-6, Table 1) were first designed and chemically synthesized such that the importance of the LDLR binding, and lipid binding, as well as the peptide length for inhibition of HCV entry could be determined. hEP-1 was essentially identical to hEP except it is synthesized. hEP-2 and hEP-3 contained the N-terminal and the C-terminal half of hEP, respectively. An extra cysteine residue was added to the N-terminus of hEP-2 and hEP-3 in order to increase peptide stability and potentially facilitate the dimerization so that the peptide length would be approximately the same as hEP-1. hEP-4 and hEP-5 are shorter versions of hEP-2 and hEP-3 that still contain the consensus LDLR binding region or major lipid binding region, respectively. hEP-6 is the combination of hEP-4 and hEP-5 sequences without the N-terminal cysteine residue. Shown in Table 1 and Supporting Fig. 1A, whereas hEP-1 and hEP-2 had similar activity to the original hEP peptide, hEP3 showed a dramatically reduced ability to inhibit HCVcc entry, likely due to the loss of the LDLR binding domain. The primary amino acid sequence is critical for antiviral activity because a scrambled peptide based on hEP-2 (scrambled hEP2), and mEP-2, a peptide derived from mouse apoE gene, both failed to inhibit HCV infection (Table 1). The length of the peptide appeared to

Table 1. Synthetic Peptides Described in the Study

ID	Sequence	Corresponding to human apoE sequence	IC_{50} (μM)	DMPC binding	Solubility in water
hEP-1	EELRVRLASHLRKLRKRLLRDADDLQKRLAVYEEQAQQIRLQAEAFQARLKSWFEPLVEDM	131-162 + 244-272	0.67	+	low
hEP-2	CEELRVRLASHLRKLRKRLLRDADDLQKRLAVY	131-162	0.50	++	good
hEP-2/ΔCys	EELRVRLASHLRKLRKRLLRDADDLQKRLAVY	131-162	>10	++	good
Scrambled hEP-2	CVDRYEARLRARALKDRQKRLLKELSLLVRHLD	131-162	>10	±	good
mEP-2	CEEIRARLSTHLRKMRKRLMRDADDLQKRLAVY	131-162	>10	++	good
hEP-3	CEEQAQQIRLQAEAFQARLKSWFEPLVEDM	244-272	>10	+	low
hEP-4	CVRLASHLRKLRKRLLRDADDL	135-155	>10	-	low
hEP-5	CIRLQAEAFQARLKSWFEPLV	250-269	>10	n.d	n.d
hEP-6	VRLASHLRKLRKRLLRDADDLIRLQAEAFQARLKSWFEPLV	135-155 + 250-269	>10	n.d	n.d
hEP-7	LRVRLASHLRKLRKRLLRDADDLQKRLAVY	133-162	>10	n.d	n.d
hEP-8	EELRVRLASHLRKLRKRLLRDADDL	131-155	>10	n.d	n.d
hEP-9	VRLASHLRKLRKRLLRDADDLQKRLAVY	135-162	>10	n.d	n.d
hEP-10	CVRLASHLRKLRKRLLRDADDLQKRLAVY	135-162	0.80	++	good
hEP-11	CLRVRLASHLRKLRKRLLRDADDL	133-155	4	-	low
hEP-12	CLRKLRKRLLRC	141-150	>10	-	low

The DMPC binding assay requires all peptides to be dissolved in aqueous solution. The solubility of each peptide under such condition is determined by formation of visible precipitates at 0.22 mg/ml. "Good" represents no visible precipitate, whereas "low" indicates partially dissolved. For DMPC binding assays, the binding was quantified as the reduction (percentage) of the turbidity (OD490nm) of DMPC solution, which normally plateaus 10 minutes after the addition of a peptide. -, reduction <10%; +/-, reduction 10-25%; +, reduction 25-50%; ++, reduction >50%; n.d, not determined.

be important, because both hEP6 and hEP4 lost all the inhibitory activities compared with their longer counterparts, hEP-1 and hEP-2, respectively. To further test this hypothesis, we synthesized an hEP-2 peptide lacking the N-terminal cysteine (designated hEP-2/ΔCys). It was observed that hEP-2/ΔCys only existed as a monomer and failed to inhibit HCV infection (Supporting Fig. 1). Additionally, truncated peptides without N-terminal cysteine (hEP 7-9, Table 1), even though containing the essential LDLR binding region,[19] all failed to inhibit HCV. Moreover, three peptides that are shorter than hEP-2, even though they contain the N-terminal cysteine (hEP 10-12, Table 1), displayed reduced or no inhibitory effect, suggesting the hEP-2 contains the length essential to maintaining the maximal anti-HCV activity.

The above results indicate that the C-terminal lipid binding region of apoE is not required for peptides to inhibit HCV infection. However, in the subsequent DMPC binding assay most peptides, except hEP-4, 11, and 12, were able to bind DMPC efficiently (Table 1). Interestingly, hEP-4, 11, and 12 had marginal or no inhibition on HCV entry in comparison to hEP-2. Altogether, these results suggest that shorter peptides, such as hEP-2, still bind lipids. Moreover, the lipid-binding ability of a peptide appears to be necessary but not sufficient for inhibiting HCV.

hEP Does Not Decrease the Level of LDLR on Cell Surface. Because the administration of hEP resulted in clearance of plasma cholesterol in mice, it is possible that hEP reduces the surface level of LDLR and hence inhibits HCV infection. To test the hypothesis, we treated Huh7.5.1 cells with hEP for various time periods and quantified the level of LDLR on cell surface by flow cytometry. hEP did not cause detectable change of surface LDLR (Fig. 4), although we cannot completely rule out that hEP might have an effect on another receptor that is known to bind apoE. Similarly, hEP did not affect the levels of CD81, SR-BI, CLDN1, or OCLN.

hEP Blocks HCV Binding. To investigate the mechanistic action of hEP, we conducted three sets of experiments. First, hEP or scrambled peptide-treated HCVcc were purified through ultracentrifugation to remove the peptide and then used to infect naïve Huh7.5.1 cells. Both samples displayed equal infectivity, indicating hEP does not directly inactivate virus (Fig. 5A). Second, to determine the kinetics of inhibition a time-of-addition experiment was conducted. HCV remained sensitive to bafilomycin A1, a fusion inhibitor that prevents endosome acidification, until 3 hours after the 37°C temperature shift (Fig. 5B),

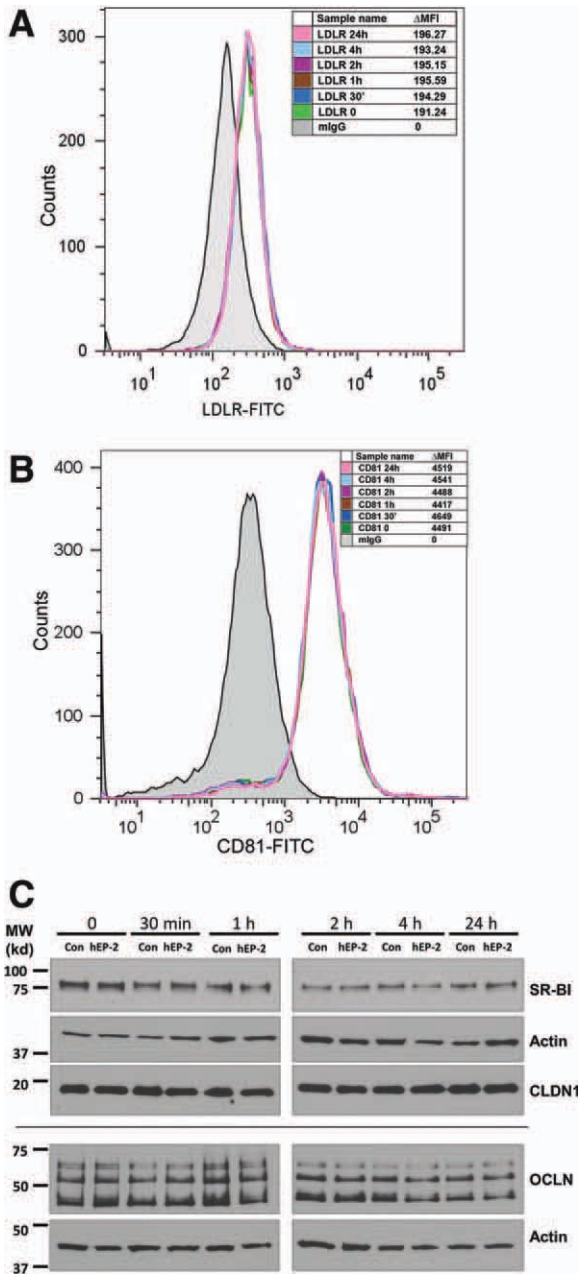

Fig. 4. (A) Huh7.5.1 cells seeded in a 12-well plate were treated with hEP-2 (20 μg/mL) for the indicated time periods. Cells were stained with a mouse IgG (isotype control) or the anti-LDLR antibody followed by flow cytometric analysis. An overlay of seven histograms is presented and the Δ median fluorescence intensity (ΔMFI, LDLR stain minus isotype control stain) of each sample is indicated to the upper right of the figure. (B) Cells were stained with anti-CD81 antibody and analyzed similarly as in (A). (C) Immunonoblots of SR-BI, CLDN1, and OCLN in the presence of hEP-2 or scrambled peptide.

which is consistent with previous reports.[20] By contrast, hEP activity disappeared almost completely when added after the temperature shift, indicating it acts on a very early step in virus entry. In order to determine whether hEP blocks infection at the initial attachment step or a downstream event in the HCV entry process,

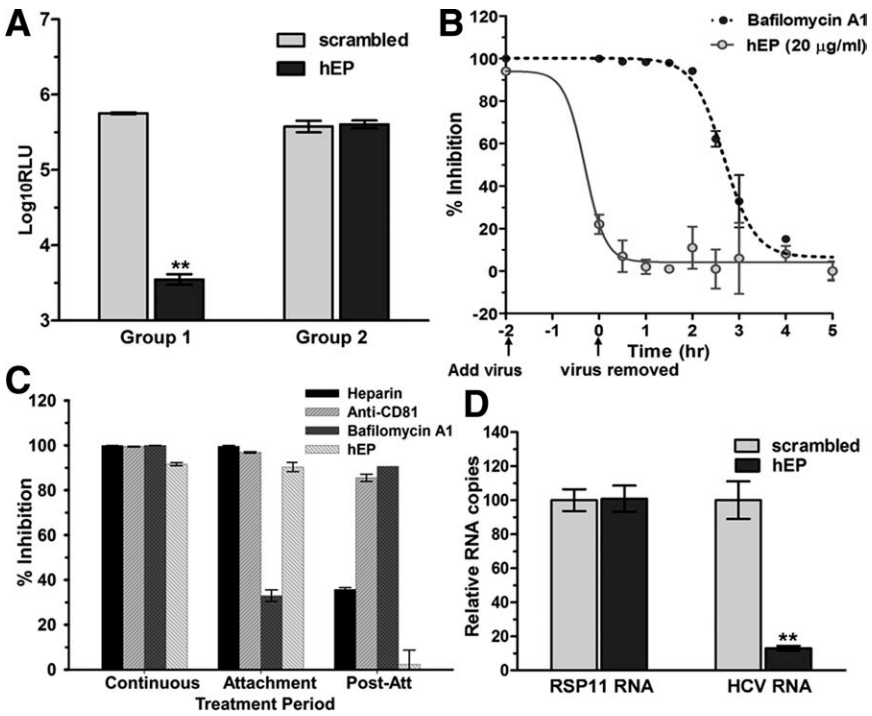

Fig. 5. hEP inhibits HCV entry at the attachment step. (A) HCVcc-luc was first mixed with either scrambled peptide or hEP-2 (20 μg/mL) for 1 hour at room temperature. In Group 1, mixed virus was directly added to Huh7.5.1 cells, whereas in Group 2 virus was purified through ultracentrifuge at 28,000*g* for 4 hours in order to remove peptide, and then used to infect Huh7.5.1 cells; $^{**}P < 0.005$. (B) The kinetics of hEP- and bafilomycin A1-mediated inhibition were determined by time-of-addition assays, as described in the Supporting Materials. Fitted lines represent sigmoidal time-dependent curves (mean of n = 3; error bars, SD). (C) HCVcc-Luc was added to Huh7.5.1 cells at 4°C and incubated for 1.5 hours. Unbound virus was removed by two washes with cold media, fresh media was added, and the cells were shifted to 37°C to allow synchronous infection to proceed. Heparin (200 μg/mL), anti-CD81 monoclonal antibody (5 μg/mL), hEP (20 μg/mL), or bafilomycin A1 (10 nM) were present in the media either continuously, during the 4°C incubation only (attachment), or during the 37°C incubation phase only (post-attachment). Percent inhibition was calculated as 100% infection relative to control infections containing DMSO or an isotype antibody control containing 0.01% sodium azide (for anti-CD81 only) (mean of n = 3; error bars, SD). (D) The effects of hEP (2.7 μM) or scrambled peptide on HCV binding were determined as described in the Supporting Materials. The results were calculated as relative RNA copies with numbers obtained from the scrambled peptide-treated wells set to 100 (mean of n = 3; error bars, SD). $^{**}P < 0.005$.

hEP was either added together with HCVcc to cells during the 4°C attachment step only, and then removed prior to shifting the temperature to 37°C, or added only after the temperature shift. As a positive control, hEP was present during the entire course of the experiment. Independent control inhibitors included heparin, the CD81 blocking antibody, and bafilomycin A1. Shown in Fig. 5C, all of the inhibitors and peptides suppressed HCV infection if present throughout the course of the experiment. Inhibition by heparin was predominant when added during the 4°C attachment step, whereas bafilomycin A1 was most effective during the postattachment stage. The anti-CD81 antibody was effective when added prior to the temperature shift and remained active even after the temperature shift, which is consistent with what has been reported.[8] hEP exhibited very little inhibition when added after the temperature shift, but strongly inhibited viral infection when added during the 4°C attachment step. Taken together, these data demonstrate that hEP blocks HCV entry at the attachment stage. Finally, we measured the direct binding of virions to cell surface using a real-time polymerase chain reaction (PCR)-based assay. Shown in Fig. 5D, the amount of viral RNA (vRNA) decreased significantly in samples isolated from hEP-treated cells, indicating a reduction of binding of HCV to cells. The same observation was made when hEP-2 was added (Supporting Fig. 2).

hEP Blocks Patient Serum-Derived HCV Binding to Primary Human Hepatocytes (PHHs). Evidence has suggested structural differences between virions produced *in vitro* and *in vivo* in their association with host lipoproteins.[14,21] PHHs then differ from hepatoma cells in many ways that could influence virus entry. To verify the above observations, we incubated serum-derived HCV (HCVser) from five patients with PHHs in the presence of hEP-2 or the scrambled peptide. Shown in Fig. 6, hEP-2 markedly reduced the binding of all five HCVser to PHHs albeit to slightly different degrees. The binding of JFH1 to PHHs was also significantly inhibited by hEP-2.

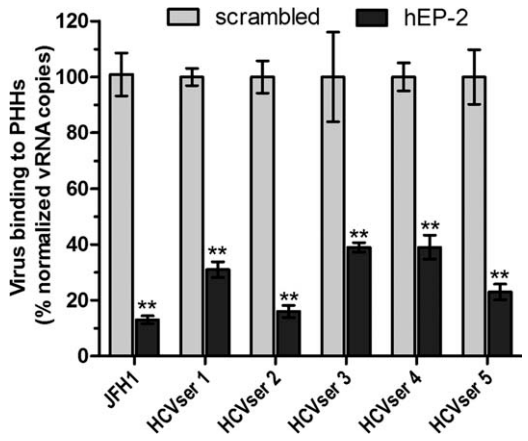

Fig. 6. hEP-2 inhibits binding of HCVser to PHHs. hEP-2 (2.7 μM) or scrambled peptide (2.7 μM) were added together with JFH1 or HCVser 1-5 to PHHs (seeded in a 24-well plate). The binding of virus to cell surface was measured by determining the vRNA copy numbers normalized to the RPS11 RNA level; $^{**}P < 0.001$.

Discussion

HCV entry is a multistep event involving a number of host factors, including HSPG, LDLR, SR-BI, CD81, CLDN1, and OCLN, all of which are located on the plasma membrane of permissive cells. Differing from those cellular factors listed above, the host cell-derived protein apoE is now recognized as a component of the infectious viral particles and contributes to virus infectivity. It has long been known that HCV circulating in blood is in complex with lipoproteins, including apoE.[16,22-24] Recent studies revealed dual roles of apoE in viral infectivity and assembly.[11,13] The formation of infectious HCV particles requires interaction of NS5A with apoE through a C-terminal α-helix domain of apoE.[25,26] As to the role of apoE in viral infectivity, it has been proposed that lipoviral particles (LVPs) can attach to cells by way of low-affinity interactions with HSPG or LDLR, which are likely facilitated by apoE packaged into virions.[27,28] Our results demonstrate that the hEP peptide blocks the binding of virus to cells, suggesting a role of apoE at the very early stage of HCV entry. Because the included sequences in hEP are known to bind both LDLR and HSPG,[15] we cannot distinguish whether hEP block an interaction with HSPG or LDLR. Regardless, our data support a model that hEP competes with viral particles for surface receptors during the attachment stage. Of note, the anti-HCV activity of apoE-derived peptides was retained in a 33-mer synthetic peptide that forms dimer but quickly lost in those shorter ones harboring the minimal receptor binding region of apoE. Those shorter peptides, while previously shown to display antimicrobial activity,[19,29] all failed to block HCVcc infection (Table 1). This is likely due to the influence of peptide length on its structure in solution. A number of apoE peptides have previously been reported to lower blood cholesterol[30] and alleviate inflammation.[31,32] The hEP used in this study could also mediate plasma cholesterol clearance. Interestingly, both altered lipid profile and chronic inflammation are major problems associated with chronic HCV infection.[33,34] In this regard, a pleotropic apoE peptide would be an ideal antiviral candidate.

Another interesting aspect of hEP is that its antiviral activity was limited to HCVcc. Although HCVpp is thought to enter cells in a manner analogous to authentic HCV, subtle differences between the two experimental systems have been reported.[35] Our finding that hEP inhibited HCVcc but not HCVpp underscores the difference between the two systems. HCVpp is typically produced in 293T cells, which do not produce endogenous apoE. In support, the anti-apoE antibody blocked HCVcc but not HCVpp infection of hepatoma cells.[36] We envision that the presence of apoE may potentially contribute to HCVcc entry in two ways. First, apoE binding to the LDLR is known to trigger endocytosis[15]; therefore, an intriguing question is whether apoE-containing viral particles become internalized by way of an apoE-mediated pathway. A recent report suggests, however, this pathway leads to degradation of internalized virions.[36] Alternatively, apoE may merely facilitate the initial attachment of the virus to the cell surface prior to the association between viral envelope proteins and SR-BI/CD81. In this case, apoE would function more like an adhesion molecule, similar to those found in many other virus entry processes that stabilize virus-cell contact to initiate entry.[37] Another observation worth mentioning is that two peptides derived from the mouse apoE sequence (mEP and mEP-2) failed to inhibit HCV entry. Because a very recent report suggests apoE does not represent a species-specific entry factor,[38] the subtle difference between hEP and mEP remains to be investigated.

In conclusion, the identification of apoE peptides now adds new tools in developing novel antiviral drugs that target HCV entry. These reagents will also aid in dissecting the molecular mechanisms of HCV entry. Although most of small molecule inhibitors that have advanced to the clinic target viral components, the apoE peptides described here may offer advantages, as they target a cellular protein that is important for HCV infection and hence reduce the likelihood of developing resistance. By virtue of its distinct mechanism of inhibition, hEP may be used in combination with other anti-HCV drugs for potential synergistic effects in treating HCV infections.

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