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Ficolin-2 Inhibits Hepatitis C Virus Infection, whereas Apolipoprotein E3 Mediates Viral Immune Escape

Yinglan Zhao,*1# Yushan Ren,*1# Xuping Zhang,* Ping Zhao, † Wanyin Tao, ‡ Jin Zhong, ‡ Qiao Li, § and Xiao-Lian Zhang*1#

Human ficolin-2 (L-ficolin/p35) is a lectin-complement pathway activator that is present in normal human plasma and is associated with infectious diseases; however, little is known regarding the roles and mechanisms of ficolin-2 during chronic hepatitis C virus (HCV) infection. In this study, we found that ficolin-2 inhibits the entry of HCV at an early stage of viral infection, regardless of the viral genotype. Ficolin-2 neutralized and inhibited the initial attachment and infection of HCV by binding to the HCV envelope surface glycoproteins E1 and E2, blocking HCV attachment to low-density lipoprotein receptor (LDLR) and scavenger receptor B1, and weakly interfering with CD81 receptor attachment. However, no interference with claudin-1 and occludin receptor attachment was observed. The C-terminal fibrinogen domain (201–313 aa) of ficolin-2 was identified as the critical binding region for the HCV-E1-E2 N-glycans, playing a critical role in the anti-HCV activity. More importantly, we found that apolipoprotein E (ApoE3), which is enriched in the low-density fractions of HCV RNA–containing particles, promotes HCV infection and inhibits ficolin-2–mediated antiviral activity. ApoE3, but not ApoE2 and ApoE4, blocked the interaction between ficolin-2 and HCV-E2. Our data suggest that the HCV entry inhibitor ficolin-2 is a novel and promising antiviral innate immune molecule, whereas ApoE3 blocks the effect of ficolin-2 and mediates an immune escape mechanism during chronic HCV infection. HCV may be neutralized using compounds directed against the lipoprotein moiety of the viral particle, and ApoE3 may be a new target to combat HCV infection. The Journal of Immunology, 2014, 193: 783–796.

*State Key Laboratory of Virology, Department of Immunology, Hubei Province Key Laboratory of Allergy and Immunology, Wuhan University School of Medicine, Wuhan 430071, China; 1Department of Microbiology, Second Military Medical University, Shanghai 200433, China; †Key Laboratory of Molecular Virology and Immunology, Institute Pasteur of Shanghai, Shanghai 200025, China; and ‡University of Michigan Medical Center, Ann Arbor, MI 48109.

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Abbreviations used in this article: ApoE, apolipoprotein E; CLDN1, claudin-1; GlcNAc, N-acetyl-D-glucosamine; HA, hemagglutinin; HCV, hepatitis C virus; HCVcc, hepatitis C virus cell culture; HCVpp, hepatitis C virus pseudotyped particle; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; MBL, mannan-binding lectin; MOI, multiplicity of infection; OCLN, occludin; p.i., post-infection; qRT-PCR, quantitative RT-PCR; shRNA, short hairpin RNA; SR-B1, scavenger receptor B1; TMB, tetramethylbenzidine; VSVGpp, pseudotyped particles harboring glycoprotein of the vesicular stomatitis virus.

Hepatitis C virus (HCV) is a major cause of liver disease and infects nearly 200 million people worldwide. Approximately 50–80% of patients are estimated to be chronically infected, which leads to liver fibrosis, cirrhosis, and eventually hepatocellular carcinoma (1). Pegylated IFN-α in combination with ribavirin is the current standard treatment; however, eventually hepatocellular carcinoma (1). Pegylated IFN-α in combination with ribavirin is the current standard treatment; however, approximately 40% of treated patients are resistant. Therefore, new therapeutic strategies for HCV are needed. A number of antiviral drugs used in combination with pegylated IFN-α are effective in a substantial proportion of patients, with response rates ranging from 40% to 60% (2). IFN-α induces interferon-supported genes (3) and mediates the innate immune response to HCV infection. Interferon-stimulated gene (ISG) expression leads to exposure of CLDN1 and OCLN binding sites to HCV. CD81-bound HCV particles then migrate to tight junctions and in-
two types of complement lectins that can recognize the surface carbohydrate molecules of microorganisms and subsequently activate the lectin-complement system, which plays a pivotal role in innate immunity (15–17). Thee members of the human ficolin family have been characterized: ‐ficolin/p35 (ficolin‐2), H‐ficolin (ficolin‐3), and M‐ficolin (ficolin‐1). Similar to MBL, ficolin‐2 contains a collagen‐like stem structure; however, unlike MBL, ficolin‐2 has a fibrinogen‐like domain and a common binding specificity for N‐acetyl‐d‐glucosamine (GlcNAc), β‐(1,3)‐d‐glucan, lipoteichoic acid, and various acylated compounds (15–17). HCV contains two conserved, highly N‐glycosylated envelope glycoproteins: E1 and E2 (3). Recently, we found that the binding of ficolin‐2 to the N‐glycans of HCV‐E1–E2 glycoproteins leads to activation of the lectin‐complement pathway (18). However, because little is known regarding the roles of ficolin‐2 in antiviral activity, we sought to determine whether ficolin‐2 possesses anti‐HCV activity and to elucidate the mechanism of ficolin‐2 in HCV infection.

Apolipoprotein E (ApoE) is a class of apolipoprotein found in chylomicrons and intermediate‐density lipoproteins that is essential for the normal catabolism of triglyceride‐rich lipoprotein constituents (19). Significant quantities of ApoE are produced in the liver and brain and, to some extent, in almost every organ. ApoE exists in three major isoforms, E2, E3, and E4, which differ from one another by a single amino acid substitution. E3 is the most common isoform and is present in 40–90% of the human population (6, 20). ApoE receptors are members of the LDLR gene family, and ApoE‐containing very low‐density lipoproteins are a major LDLR ligand (6, 20). ApoE3 is enriched in the low‐density fractions of HCV RNA‐containing particles, and HCV virions can be precipitated or neutralized by anti‐ApoE and –HCV‐E2 Abs, suggesting that ApoE is important to the HCV life cycle (2, 6, 21–23). Although ApoE is required for HCV entry and infection, little is known regarding whether ApoE3 blocks HCV entry inhibitor ficolin‐2 in HCV infection.

**Materials and Methods**

Reagents, Abs, and cell lines

Mannan, a linear polymer of the sugar mannose from *Saccharomyces cerevisiae*, and GlcNAc were purchased from Sigma‐Aldrich. HCV NS5B mAbs (9G2) were purchased from Abcam and Santa Cruz Biotechnology (Dallas, TX). ApoE3 recombinant protein was obtained from R&D Systems (Minneapolis, MN). Anti‐ApoE mAb (E6D7) and goat anti‐ApoE polyclonal Ab were purchased from our laboratory (18). The anti‐ficolin‐2 mAb G5 was purchased from Hycult Biotech (Plymouth Meeting, PA). The anti‐GST mAb was purchased from EarthOx (San Francisco, CA). An HCV NS5A polyclonal Ab was provided by Dr. Jin Zhong at the Institute Pasteur of Shanghai. PE‐labeled streptavidin was obtained from Sigma‐Aldrich. Human hepatocellular liver carcinoma (Huh7.5.1) cells, human 293T embryonic kidney cells, and HeLa cells were cultured in complete DMEM medium with 10% (v/v) FBS (HyClone Laboratories, Logan, UT). HCVcc‐JFH1 from the Huh7.5 cell line was provided by Wen‐Zhe Ho from the Temple University School of Medicine (26).

**Titration of infectious HCV**

Infection of Huh7.5.1 cells and the titration of infectious HCV were performed as previously reported (27). The infectious supernatants were serially diluted 10‐fold in complete DMEM and used to infect 105 naive Huh7.5.1 cells per well in 96‐well plates (Corning). The virus was incubated with the cells for 2 h at 37˚C and then supplemented with fresh complete DMEM. The level of HCV infection was determined 3 d post‐infection (p.i.) by immunofluorescence staining for HCV NS5A. The viral titer is expressed as focus‐forming units per milliliter of supernatant, determined by the average number of NS5A+ foci detected at the highest dilutions. The multiplicity of infection (MOI) is the average number of virus particles infecting each cell and indicates focus‐forming units of virus used for infection per number of cell (28).

**Amplification of HCVcc viral stocks**

For large‐scale generation of viral stocks, infectious supernatants were diluted in complete DMEM and used to inoculate 15% confluent Huh7.5.1 cells at an MOI of 0.01 in a T75 flask (Corning). Infected cells were trypsinized and replated before confluence at days 4–5 p.i. Supernatants from infected cells were then harvested 7–8 d p.i., filtered through a 0.22‐μm filter, concentrated 100‐fold using an Amicon Ultra‐4 (Millipore, Billerica, MA), and aliquoted for storage at −80˚C. The titer of the viral stock was determined as described above.

**Purification of intracellular and extracellular HCV (HCVcc) virions using sucrose gradient ultracentrifugation**

HCV was concentrated and purified with sucrose density‐gradient ultracentrifugation according to a previously reported method (27, 28). For preparation of intracellular HCV, infected Huh7.5.1 cells were harvested and lysed in PBS three times and then repeatedly frozen and thawed from −80˚C to 37˚C three times. Supernatants from cell lysates were centrifuged at 12,000 rpm for 5 min to remove cellular debris. Pretreated supernatants were pelleted through a 20% sucrose cushion at 28,000 rpm for 4 h using an SW28 rotor in a Beckman Coulter Optima L‐100 XP ultracentrifuge. The pellets were resuspended in 1 ml TNE buffer (10 mM Tris‐HCl [pH 8.0], 150 mM NaCl, 2 mM EDTA), loaded onto a 10–60% sucrose gradient (12.5 ml total volume), and centrifuged at 14,000 rpm for 16 h at 4˚C in an SW41 Ti rotor (Beckman Coulter). Fractions of 1.3 ml were collected from the top of the gradient. The fractions were analyzed by quantitative RT‐PCR (qRT‐PCR) to detect HCV RNA. To determine the infectivity titer of each fraction, fractions were titrated on Huh7.5.1 cells as described above.

**Production of HCVpp**

Production and infection of Huh7.5.1 cells using infectious HCV JFH1 (a genotype 2a isolate) or murine leukemia virus–based HCVpp were performed as described (29, 30). Pseudotyped particles harboring glycoprotein of the vesicular stomatitis virus (VSVGpp) onto murine leukemia virus were used as a control.

**HCVcc and HCVpp infection and neutralization**

The HCVcc infection and neutralization experiments were essentially performed according to a previously published method (27). Huh7.5.1 cells in 24‐well plates were infected with HCVcc (MOI of ~2; 1.6 × 106 copies/ml) at 37˚C for 4 h. The supernatants were discarded, and the infected cells were washed twice with PBS and incubated in DMEM containing 10% FBS for 72 h. Neutralization of HCV infection was performed using quantitative RT‐PCR and Western blotting. The neutralization effect of ficolin‐2 on six genotypes of HCVpp was assessed. The cells were exposed to HCV‐containing supernatants and incubated in the presence of neutralizing Ab. The cells were washed twice with PBS and incubated in DMEM supplemented with 10% FBS for 72 h. HCV was identified using FITC‐conjugated streptavidin IgG and biotin‐labeled anti‐E2 aptamer ZE2 Ab and observed under a confocal fluorescence microscope. Alternatively, the cells were lysed and HCV RNA and protein were measured by qRT‐PCR and Western blotting.

**Construction of recombinant plasmids**

Full‐length HCV‐E1 and –E2 (GenBank accession no. M67463) were amplified and subcloned in‐frame into pcDNA3.1(–)‐MYC‐His(1)‐A‐hemagglutinin (HA) to generate the plasmids pcDNA3.1(–)‐MYC‐His(1)‐A‐E2‐HA and pcDNA3.1(–)‐MYC‐His(1)‐A‐E1‐HA, respectively. The HA tag

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sequence 5'-AGCATAGTCAGATCTAGCTATAAGGATA-3' was cloned in-frame into pCDNA3.1 (+) vector. The sequences of the constructs were verified by DNA sequencing. The pDisplay eukaryotic expression vector from Clontech Laboratories was used to display the expressed protein on the surface of cell membranes. Full-length HCV-E2 was amplified and subcloned in-frame into pDisplay to generate the plasmid pDisplay-E2 using the E2 specific primers P1 (5'-AAAGAATGATACCCCTTACACTAC-3') and P2 (5'-TGCTCTGTACATTCCCTTAGCTGCC-3'). The underlined nucleotides represent the mutation sites.

Human ApoE3 cDNA (GenBank accession no. NC_000019.9) was amplified and subcloned in-frame into pCDNA3.1 (+) vector. The specific primers were as follows: primers F (5'-ATATGATCCTAGAAGGTTGGAACAGCGTTGGA-3') and R (5'-ATATAACATTGTAGTGGCTCCTGGCCACCAGG-3'). The underlined oligonucleotides represent BamHI and HindIII sites, respectively. Human ApoE2 and ApoE4 clones were generated by introducing single point mutations into ApoE3 by PCR using the following primers: ApoE2 forward (5'-GACCTGGCAAGTGCCCTGAGCAGTG-3'), ApoE2 reverse (5'-ACATCCGGACACATTCTTG-3'), ApoE4 forward (5'-ATGGAGGAAGCTGGCCCGGCGCTCTG-3'), and ApoE4 reverse (5'-CCAGCCGCGCCGACCTTCCTCCATA-3'). The underlined oligonucleotides represent the mutation sites.

The eukaryotic expression plasmids pLenti-6-CD81, -SR-B1, -CLDN1, -OCLN, and -LDLR were constructed as reported in our previous publications (29, 30). Full-length cDNAs of human CD81-SR-B1, -CLDN1, -OCLN, and -LDLR were amplified and subcloned in-frame into the eukaryotic expression plasmid pEGFP-C1 to generate pEGFP-C1-CD81, -SR-B1, -CLDN1, -OCLN, and -LDLR.

Excessive GlcNAc were added. After incubating at 37˚C for 1 h, the wells were then stained using the biotin–anti-E2 aptamer ZE2 (24), and the bound primary ZE2 Ab was detected using PE-streptavidin IgG; the nuclei were stained with Hoechst 33342 dye. The control groups included an GFP receptor cells only group, a GFP receptor cells plus ficolin-2 group, and a GFP receptor cells plus ApoE3 group. To demonstrate the specificity of the anti-E2 ZE2, PE-labeled streptavidin and biotin-labeled ZE2 aptamer were added to each group. The stained cells were observed and analyzed using confocal microscopy (PerkinElmer UltraVIEW Vox).

EC50 of ficolin-2

HCV 1 cells (1 x 10^6) were seeded into 96-well plates and incubated at 37˚C overnight. Different concentrations of ficolin-2 protein were mixed with HCVcc (MOI of 1) at 37˚C for 1 h and then added to Huh7.5.1 cells and incubated at 37˚C for 4 h. The cells were washed with PBS, fresh medium was added, and the cells were incubated at 37˚C for another 72 h. HCV was identified using PE-donkey anti-mouse IgG and mouse anti-NS3 mAb (Abcam) under immunofluorescence microscopy. The EC50 of ficolin-2, the minimal concentration of ficolin-2 that neutralizes 50% of HCVcc-infected Huh7.5.1 cells, was then determined.

Western blot analysis

The neutralization of ficolin-2 to HCVcc was detected by Western blot. Huh7.5.1 cells (2 x 10^5) were cultured in 24-well plates at 37˚C for 24 h. HCVcc (MOI of 1.5) was mixed with equimolar doses (1.0 μM) of GST–ficolin-2, GST-D1, GST-D2, GST-D3, and GST proteins at 37˚C for 1 h and then added to Huh7.5.1 cells for 4 h. Fresh DMEM was added and cells were cultured for 72 h. HCV-NS3 protein expression was detected by Western blot using an anti-NS3 mAb. To determine the effects of mann and GlcNAc on ficolin-2’s inhibition of HCV infection, ficolin-2 (0.1 μM) was incubated with different concentrations of mann and GlcNAc for 1 h. The mixture was then incubated with HCVcc (MOI of ~2, 1.6 x 10^5) for another 1 h and added into Huh7.5.1 cells. After 72 h, whole cells were harvested and HCV-NS3 was detected by Western blot analysis.

ELISA

For determining the binding ability of ficolin-2 to HCV particles, an indirect ELISA method was used (24, 30). ELISA (96-well) plates were pretreated with GNA lectin (34) (500 ng/ml, Sigma-Aldrich) (GNA binds to the HCV-E1 and -E2 glycoproteins) and blocked with 2.5% non-fat dry milk and 2.5% normal goat serum. HCVcc (1 x 10^6 copies) was added to each well and incubated at 37˚C for 1 h. The wells were washed three times with PBST and ficolin-2 protein (0.3 μM), and 10 and 40 μg/ml mann or GlcNAc were added. After incubating at 37˚C for 1 h, the wells were washed with PBST, and anti–ficolin-2 mAb (GN5, HyClone Laboratories, 1:1000) was added to each well, followed by HRP-goat anti-mouse IgG

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Ab. Color development was achieved by adding 100 μl/well tetramethylbenzidine (TMB) chromogen substrate (Sigma-Aldrich), and the reaction was stopped by adding 100 μl 0.5 M H₂SO₄. The absorbance at OD₄₅₀ was measured with a PerkinElmer 2030 multilabel reader.

The binding ability of ficolin-2 to E2-HeLa or HeLa was measured by ELISA, as previously reported (24, 30). ELISA (96-well) plates were pretreated with polylysine (0.1 mg/ml) and then coated with 2 × 10⁵ of each cell type at 4˚C overnight. The plates were then blocked with 2% BSA at 37˚C for 2 h. The plates were washed three times with PBS (containing 0.1% Tween 20), and ficolin-2 (0.3 μM) with HCV (MOI of 0.3, 3.0 × 10⁴ copies/100 μl) was added to each well and incubated at 37˚C for 1 h. An anti-ficolin-2 mAb (GNS, HyClone Laboratories, 1:1000) was added to each well, followed by HRP-goat anti-mouse. The absorbance at OD₄₅₀ was measured as described above.

For analysis of the blocking effect of ficolin-2 on HCV binding to the receptor-expressing HeLa cells or receptor knockdown cells, ELISA (96-well) plates were pretreated with polylysine (0.1 mg/ml) and then coated with 2 × 10⁵ of each cell type at 4˚C overnight, followed by blocking with 2% BSA. The plates were then washed with PBS, and ficolin-2 (0.3 μM) in the presence of HCV (MOI of 0.3, 3.0 × 10⁴ copies/100 μl) or in the presence of different concentrations of ApoE3 was added to each well and incubated at 37˚C for 1 h. A biotin-labeled anti-HCV-E2 aptamer ZE2 Ab (100 nM) (24) was added, followed by the addition of HRP-streptavidin. The absorbance at OD₄₅₀ and the binding ability were measured as described above.

For analysis of the interaction between ApoE3 and ficolin-2, ELISA plates were coated with ficolin-2 protein (0.15 μM, 100 μl/well) at 4˚C overnight. Then, the plate was blocked with 1% BSA at 37˚C for 1 h. After washing with PBS, different concentrations of ApoE3 protein (0.01–1.25 μM) were added, followed by incubation at 37˚C for 1 h. Mouse anti-ApoE3 mAb (1:1000) was added and incubated at 37˚C for 1 h, followed by HRP-goat anti-mouse IgG (1:5000). Color development was achieved by adding 100 μl/well TMB chromogen substrate (Sigma-Aldrich), and the reaction was stopped with 100 μl 0.5 M H₂SO₄. The OD values at 450 nm were measured with a PerkinElmer 2030 multilabel reader.

The supernatants from plasmids pcDNA 3.1–ApoE3, –ApoE2, and –ApoE4-transfected Huh7.5.1 cells for 72 h were collected and the secreted ApoE proteins were measured using a sandwich ELISA according to previous publications (35, 36). Briefly, supernatants of each sample were loaded into a 96-well plate coated with a polyclonal anti-ApoE (goat anti-human ApoE; 1:400 dilution, Calbiochem). Following incubation at 37˚C for 1 h,amples were removed and the plates were washed three times with PBST prior to incubation with a mouse anti-ApoE mAb (6E7, recognizes E3, E2, and E4 isoforms of ApoE; Calbiochem) (1:2000) at 37˚C for 1 h. The plates were then washed again three times with PBST and incubated with HRP-goat anti-mouse IgG (1:4000) for an additional 1 h at 37˚C. The immunocomplex was reacted with TMB substrate and detected, washed, and resuspended in PBS containing 1% BSA and anti-GST mAb for 30 min. The cells were pelleted, washed, and resuspended in PBS containing 1% BSA and PE-labeled goat anti-mouse IgG Ab (1:100). After incubation, the cells were washed, and cell-bound GST–ficolin-2/GST protein was analyzed using a BD Accuri C6 flow cytometer (BD Biosciences).

Flow cytometry analysis
To analyze the ability of ficolin-2 protein to bind to E2-HeLa cells or HeLa cells, 0.5 μM GST–ficolin-2 or GST protein in a final volume of 100 μl PBS was incubated with 1 × 10⁵ E2-HeLa cells or HeLa cells at 37˚C for 30 min. The cells were then washed in PBS and stained in 100 μl PBS containing 1% BSA and anti-GST mAb for 30 min. The cells were pelleted, washed, and resuspended in PBS containing 1% BSA and PE-labeled goat anti-mouse IgG Ab (1:100). After incubation, the cells were washed, and cell-bound GST–ficolin-2/GST protein was analyzed using a BD Accuri C6 flow cytometer (BD Biosciences).

The binding affinity of GST–ficolin–2, GST, or ApoE3 protein to target cells (E1-HeLa, E2-HeLa, HeLa cells) was measured by flow cytometry by monitoring the mean fluorescence intensity of the target cells bound to PE-labeled anti-GST or PE-labeled anti-ApoE3, as previously described (24, 29). E1-HeLa, E2-HeLa, and HeLa cells (1 × 10⁶ cells) were incubated with different concentrations of GST–ficolin, GST, or ApoE3 protein (100 μg/ml) at 4˚C overnight. The binding affinity was analyzed using a BD Accuri C6 flow cytometer (BD Biosciences).

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**Results**

**Ficolin-2 blocks HCVcc entry and infection at an early stage**

To determine the role of ficolin-2 in HCV infection, we tested the antiviral effects of recombinant ficolin-2 protein in an HCV cell culture model. HCV (JFH-1) was preincubated with recombinant ficolin-2 protein prepared as previously reported (18) and then used to infect HuH7.5.1 human hepatocytes. We found that ficolin-2 significantly inhibited HCV infection of HuH7.5.1 cells, as determined by real-time fluorescence qRT-PCR analysis (Fig. 1A). Ficolin-2 prevented viral entry into HuH7.5.1 cells at an early stage (preincubated with HCVcc for 1 h) in a dose-dependent manner (Fig. 1A), but it had only a mild inhibitory effect at later stages (addition of ficolin-2 at 48 h p.i.). The control protein GST did not have any effect on viral infection. Western blotting also showed that HCV-NS3 expression was decreased in the presence of ficolin-2, which indicated that ficolin-2 blocked and neutralized HCVcc infection (Fig. 1B). To determine whether ficolin-2 inhibits HCVcc entry by interacting with cell-surface sugars or with viral-surface sugars, HuH7.5.1 cells were preincubated with ficolin-2, followed by a wash with PBS prior to infection. As shown in Fig. 1B, ficolin-2 preincubated with HuH7.5.1 cells had no effect on HCVcc infectivity, suggesting that the anti-HCV activity of ficolin-2 is primarily due to its effect on viral particles rather than on an interaction with cell-surface sugars (Fig. 1B). The neutralization efficiency of ficolin-2 on HCV infection occurred in a ficolin-2 dose-dependent manner, whereas the control protein GST did not have any effect (Fig. 1C).

We further analyzed the neutralization effect of ficolin-2 on HCVpp. As shown in Fig. 1D, the infectivity of all six genotypes of HCVpp (genotypes 1a–6a) was dramatically decreased in the presence of ficolin-2 compared with the control groups. The control serum, which was the preimmunized mouse serum and did not contain anti-HCV Ab, and VSVGpp were used as negative controls. Ficolin-2 exhibited a much stronger antiviral activity against HCVcc (Fig. 1D) than against HCVcc (Fig. 1C).

**Both mannann and GlcNAc competitively block the anti-HCV effect of ficolin-2**

Mannan is a linear polymer of the sugar mannose with molecular masses ranging from 34 to 62.5 kDa. GlcNAc is a monosaccharide derivative of glucose. Previous reports have shown that ficolin-2 binds to acetylated compounds and carbohydrates (e.g., GlcNAc, β-(1,3)-d-glucan) (15–17) and the heavy N-glycans of HCV E1E2 heterodimers (18). Therefore, we sought to determine whether such interactions were responsible for the anti-HCV activity of ficolin-2 by assessing whether the infectivity of HCVcc was modified in the presence of ficolin-2 (0.1 μM) preincubated with increasing amounts of mannan or GlcNAc. At 3 d p.i., the protein expression levels of HCV NS3 and the relative RNA levels of HCV were measured by Western blotting (Fig. 2A) and qRT-PCR (Fig. 2B), respectively. Both mannan and GlcNAc restored HCVcc infectivity in a concentration-dependent manner (Fig. 2A, 2B). Both 4 μg/ml mannan and 15 μg/ml GlcNAc significantly blocked the binding of ficolin-2 to cell surface–expressed E2 glycoprotein
by flow cytometry analysis (Fig. 2C). Interestingly, we found that the anti-HCV activity of ficolin-2 was more sensitive to mannan than to GlcNAc, as mannan could restore HCVcc infectivity at a much lower concentration compared with GlcNAc (Fig. 2A–C). In an indirect ELISA, virus particles from HCVcc were added and captured by GNA lectin–coated ELISA plates and incubated with ficolin-2 (0.3 μM) or control cell culture medium and different concentrations of mannan or GlcNAc, followed by extensive washing to remove unbound samples. The ficolin-bound viral particles on the ELISA plates were revealed using an anti–ficolin-2 mAb. From our data shown in Fig. 2D, 10 μg/ml (0.16–0.29 μM) mannan significantly blocked ficolin-2 binding to HCVcc, whereas 10 μg/ml (45 μM) GlcNAc (which molar concentration is almost 155- to 250-fold higher than that of 10 μg/ml mannan) did not significantly block ficolin-2 binding to HCVcc. These results suggest that the inhibitory effect of mannan on the binding of ficolin-2 to HCVcc is almost 155 to 250-fold stronger than that of GlcNAc. Therefore, we proposed that the binding of ficolin-2 to the HCVcc particles was remarkably blocked by mannan and only slightly by GlcNAc (Fig. 2D, *p < 0.05, versus GST–ficolin-2).
Note that mannan and GlcNAc alone had no effect on HCVcc infectivity at the highest concentrations tested (15 μg/ml) (data not shown), which indicates that mannan and GlcNAc competitively block antiviral activity of ficolin-2.

The C-terminal fibrinogen domain of ficolin-2 is the region critical for binding to HCV-E2

To determine which region of ficolin-2 is critical for the binding of HCV envelope glycoproteins, different deletions of both the CLR and FBG regions of ficolin-2 were constructed in a pGEX-KG background, as shown in Fig. 3A. Recombinant ficolin-2 and ficolin-2 D1, D2, and D3 proteins were prepared and quantified by SDS-PAGE and Western blot analysis (data not shown). We established stable expression of E2 in HeLa cells and confirmed that the E2 glycoprotein was expressed on the surface of E2-HeLa cells by flow cytometry (Fig. 3B). We determined that ficolin-2 did not bind to HeLa cells (Fig. 3C). We incubated the recombinant ficolin-2 protein or its mutants with E2-expressing HeLa cells or normal HeLa cells and examined the proteins that were associated with the E2 glycoprotein. The binding of ficolin-2 or its mutant proteins to the E2 glycoprotein were detected by flow cytometry. Ficolin-2 and the mutants D1 (from 113 to 313 aa) and D2 (from 201 to 313 aa) could bind to the E2 glycoprotein expressed on the surface of HeLa cells in a dose-dependent manner, whereas D3 (from 25 to 111 aa) and GST did not exhibit binding (Fig. 3D, 3E). Flow cytometry analysis confirmed that ficolin-2 and mutants D1 and D2 bound to E2-HeLa cells much more strongly than did D3 and GST (Fig. 3D, 3E). Because ficolin-2 and the mutants D1 (from 113 to 313 aa) and D2 (from 201 to 313 aa) could bind to the E2 glycoprotein, we speculated that the C-terminal FBG domain (from 201 to 313 aa) of ficolin-2 is the critical region of the HCV-E2 glycoprotein. Moreover, Western blot analysis showed that equal moles of ficolin-2 and mutants D1 and D2 could significantly inhibit HCV-NS3 protein expression and neutralize HCVcc infection, whereas D3 and GST did not exhibit neutralizing effects (Fig. 3F, 3G, *p < 0.05 compared with only HCVcc group). Real-time fluorescence qRT-PCR also showed that equal moles of ficolin-2 and mutants D1 and D2 could significantly decrease HCV RNA expression in Huh7.5.1 cells, whereas D3 and GST did not significantly block HCV infection (Fig. 3H, *p < 0.05 compared with only HCVcc group).

Ficolin-2 interferes with HCVcc binding to LDLR and SR-B1 receptors

We next sought to determine whether ficolin-2 is able to prevent the interaction of E2 with cellular receptors. HCVcc infection requires several receptors to coexist in Huh7.5.1 cells. The LDLR and SR-B1 receptors are required for the early step of HCV cell entry (7, 14), whereas the CLDN1 and OCLN receptors function in the late step of HCV entry (10–12). Huh7.5.1 cells contain all of HCV receptors and are susceptible to HCV infection; however, HeLa...
cells are not an HCV target cell and are not susceptible to HCV infection. Endogenous CLDN1 was only found in Huh7.5.1 and Huh 7 cells, but not in HeLa cells (37). The endogenous ectopic HCV receptor expression levels and densities in HeLa cells are much lower than those of Huh7.5.1 cells (37–39). We established HeLa cell lines that overexpressed the receptors LDLR, SR-B1, CD81, CLDN1, or OCLN and then measured whether ficolin-2 interfered with HCVcc binding to any of these cells. shRNA expression vectors against the HCV receptors CD81, SR-B1, CLDN1, OCLN, and LDLR were constructed according to previously reported methods (32). The expression of receptors was reduced after transfection with shRNA against each receptor, as determined by SDS-PAGE and Western blot analysis using anti-SR-B1, anti-CLDN1, anti-CD81, anti-CLDN1, and anti-LDLR in both Huh7.5.1 cells (Fig. 4A) and receptor overexpressing HeLa cells (Fig. 4B). Each receptor-expressing HeLa cell line was mixed with ficolin-2 plus HCVcc, and the binding ability of HCVcc to each receptor-expressing HeLa cell line was determined by ELISA. We observed that the binding ability of HCVcc to LDLR and SR-B1 receptor-expressing HeLa cells was significantly higher than to CD81, CLDN1, and OCLN receptor-expressing HeLa cells, which suggested that HCVcc binds to LDLR and SR-B1 receptors that are required for the early steps of HCV cell entry (Fig. 4C). We further found that ficolin-2 significantly reduced the interaction of HCVcc-LDLR and HCVcc–SR-B1 by 2-fold compared with cells in the absence of ficolin-2; the interaction between HCVcc and CD81 was also slightly reduced (Fig. 4C). However, the binding of HCV to CLDN1 and OCLN, which occurs in the late stages of HCV infection, was not affected (Fig. 4C). Our results are consistent with others’ reports that the binding of CLDN1 and OCLN to HCV occurs only after CD81 bound HCV particles movement, and this cell surface trafficking to the sites of CLDN1 and OCLN led to this cellular surface conformation change and exposure of CLDN1 and OCLN binding sites to HCV (4). Thus, HeLa cells that only overexpressed CLDN1 or OCLN do not lead to increased HCVcc binding to HeLa cells. Moreover, ficolin-2 did not affect the interaction between HCVcc and each receptor-expressing HeLa cell line after the cells were transfected with the respective shRNA against CD81, SR-B1, CLDN1, OCLN, and LDLR (Fig. 4C). These results suggest that ficolin-2 interferes with HCVcc binding to LDLR and SR-B1 receptors, which is required for the early steps of HCV cell entry. Ficolin-2 also interferes weakly with HCVcc binding to the CD81 receptor (Fig. 4C). Therefore, we chose LDLR, SR-B1, and CD81 as target receptors to study the blocking effect of ficolin on HCV binding to receptor-expressing cells in the following experiments.

To further confirm whether ficolin-2 is able to prevent the interaction of E2 with the cellular receptors LDLR, SR-B1, or CD81, we performed an immunofluorescent confocal microscopy analysis. LDLR-, SR-B1−, and CD81-expressing HeLa cells were incubated with HCVcc. A biotin-labeled ZE2 aptamer (ZE2, an anti–HCV-E2 glycoprotein aptamer Ab) was added to detect HCV. HCV was stained with a red PE-conjugated streptavidin, which binds to the biotin-labeled anti-E2 ZE2 aptamer Ab, and the receptors were labeled in green for HeLa cells transfected with pEGFP-LDLR, −SR-B1, and −CD81. To demonstrate the specificity of ZE2 (an anti-HCV-E2 aptamer Ab), PE-conjugated streptavidin and biotin-labeled ZE2 was added to each group. We observed that the addition of ficolin-2 decreased HCVcc binding to the cells, as displayed by less HCV-E2 (red) on the surface of the LDLR-HeLa cells (Fig. 4D, row 4 versus row 3), SR-B1–HeLa cells (Fig. 4E, row 4 versus row 3), and CD81–HeLa cells (Fig. 4F, row 4 versus row 3), indicating that ficolin-2 reduced HCVcc binding to these receptors, which is consistent the results of the ELISA (Fig. 4C). We observed that PE-labeled ZE2 did not stain the cells in all control groups and only stained HCVcc as shown in Fig. 4D–F, which indicated that the anti-E2 ZE2 is specific to HCV. Because E2 is both a target for ficolin-2 and anti-E2 ZE2, we further analyzed whether the anti-E2 ZE2 aptamer Ab interfered with binding or with the assay readout by steric hindrance of ficolin-2. Using flow cytometry analysis, we found that different amounts of ZE2, as indicated in Fig. 4G, did not block ficolin-2 binding to E2-HeLa cells, which indicated that ficolin-2 and the anti-E2 ZE2 aptamer Ab do not occupy overlapping binding sites of E2 glycoprotein. Ficolin-2 and ZE2 recognize different epitopes of HCV-E2 (Fig. 4G).

ApoE3 binds to both E1 and E2 glycoproteins

Our previous data convincingly showed that HCV infection causes increased expression of ficolin-2 (18, 40) and that increased ficolin-2 concentrations are associated with the efficacy of antiviral therapy in chronic hepatitis C patients (40). Our present data confirm that ficolin-2 blocks HCV infection of hepatocytes in vitro. Because HCV-associated ApoE3 may facilitate viral entry into cells via LDLR and plays an important role in viral formation/egression and infectivity of HCV (6), we speculated that ApoE3 is involved in the effects of ficolin-2 on HCV.

First, we analyzed whether ApoE3 binds to ficolin-2 using an ELISA method. We found that ApoE3 did not directly bind to ficolin-2 (Fig. 5A). We further determined the interaction between ApoE3 and E1/E2 glycoprotein–expressing HeLa cells using flow cytometry analysis. We found that ApoE3 could bind to E2-HeLa cells and E1-HeLa cells but not to HeLa cells (Figs. 5B). Ficolin-2 protein could bind to E2-HeLa cells and E1-HeLa cells, but not to HeLa cells (Fig. 5C). The control protein GST did not bind to E1-HeLa, E2-HeLa, or HeLa cells (Fig. 5D). The following binding affinities were determined as the equilibrium $K_d$ by flow cytometry as described in Materials and Methods (41, 42): ApoE3–E2 ($K_d$ 8 ± 0.8 nm) > ficolin-2–E2 ($K_d$ 40.8 ± 4.15 nm) > ApoE3–E1 ($K_d$ 58 ± 2.5 nm) > ficolin-2–E1 ($K_d$ 172.3 ± 10.9 nm) > ApoE3–ficolin-2 (Fig. 5B, C). These results indicate that the binding affinity of ApoE3 for E2 (Fig. 5B) was stronger than that of ficolin-2 for E2 (Fig. 5C) and much stronger than that of ficolin-2 for E1 (Fig. 5C). Additionally, using flow cytometry, we found that ApoE3 bound to E2 and E1 glycoprotein–expressing HeLa cells in a dose-dependent manner (Fig. 5E) and exhibited a much higher binding affinity than ApoE3 to HeLa cells (Fig. 5E). These data further demonstrate that ApoE3 can bind to HCV-E2 and HCV-E1. Interestingly, we found that both mamman and GlcNAc blocked the binding of ApoE3 to the E1 glycoprotein (Fig. 5F) but did not block binding to the E2 glycoprotein (Fig. 5F), which suggests that ApoE3 binds to the N-glycans of E1 and the peptide portion of E2. Conversely, ApoE3 did not bind to the N-glycans of the E2 glycoprotein.

ApoE3 competitively blocks the interaction between ficolin-2 and HCV-E2

We further found that exogenous ApoE3 significantly blocked ficolin-2 binding to HCVcc (extra-HCV) in an ApoE3 dose-dependent manner using flow cytometry analysis (Fig. 6A). We also observed a certain inhibitory effect of exogenous ApoE3 on binding of ficolin-2 to intracellular HCV (intra-HCV), but with no statistical significance (Fig. 6A). The inhibitory effect of exogenous ApoE3 on ficolin-2 binding to extra-HCV was much stronger than to intra-HCV (Fig. 6A). We speculate that this phenomenon was probably due to more ApoE in extra-HCV than that in extra-HCV. Our data further demonstrate that ApoE3 can bind to extra-HCV (Fig. 5B) and can bind to extra-HCV, but not to intra-HCV, as shown in Fig. 6A. We also observed that the anti-E2 ZE2 is specific to HCV. Because E2 is both a target for ficolin-2 and anti-E2 ZE2, we further analyzed whether the anti-E2 ZE2 aptamer Ab interfered with binding or with the assay readout by steric hindrance of ficolin-2. Using flow cytometry analysis, we found that different amounts of ZE2, as indicated in Fig. 4G, did not block ficolin-2 binding to E2-HeLa cells, which indicated that ficolin-2 and the anti-E2 ZE2 aptamer Ab do not occupy overlapping binding sites of E2 glycoprotein. Ficolin-2 and ZE2 recognize different epitopes of HCV-E2 (Fig. 4G).
was then added (Fig. 6B, 6C). Furthermore, ApoE3 competitively blocked the interaction between ficolin-2 and E2 but did not block the interaction between ficolin-2 and E1 (Fig. 6D). These results also confirm that the binding affinity of ApoE3 for E2 was stronger than the binding affinity of ficolin-2 for E2.

**ApoE3 promotes HCV infection and blocks the anti-HCV effect of ficolin-2**

We further demonstrated that the addition of exogenous ApoE3 increased HCV-NS3 protein expression (Fig. 7A) and HCV RNA expression (Fig. 7B, versus HCVcc group, p < 0.05; Fig. 7C, versus HCVcc group, p < 0.05).
ficolin-2 plus HCVcc group, \( p < 0.05 \) in an ApoE3 dose-dependent manner and blocked the antiviral effect of ficolin-2. Preincubation of GST with HCVcc did not influence HCVcc infectivity (Fig. 7A, lane 1). A qRT-PCR analysis also revealed that ApoE3 alone (150, 300 nM) significantly enhanced HCVcc infection of Huh7.5.1 cells (Fig. 7B, ApoE3 plus HCVcc versus HCV group, \( p < 0.05 \)).

Because ApoE2 and ApoE4 are similar isomers of ApoE3, with only one or two amino acid differences (6), we further determined the roles of ApoE2 and ApoE4 on the effects of ficolin-2 on HCV infection. The plasmids pcDNA3.1-ApoE2, -ApoE3, and -ApoE4 were transfected into Huh7.5.1 cells. After 24 h, GST–ficolin-2 or GST proteins were preincubated with HCVcc at 37˚C for 1 h and then added into ApoE-transfected Huh7.5.1 cells. The expression of HCV-NS3 protein was detected after 72 h p.i. by Western blotting analysis. The ApoE2, ApoE3, and ApoE4 proteins were expressed in Huh7.5.1 cells at similar levels (Fig. 7D). The secreted ApoE2, ApoE3, and ApoE4 proteins in the supernatants were also detected at similar levels by Western blotting (Figs. 7D, 7E) and ELISA analysis (Fig. 7F). However, we found that ApoE2 and ApoE4 did not block the inhibitory effects of ficolin-2 on HCV RNA (Fig. 7G, versus GST–ficolin-2 plus ApoE3 group, \( p < 0.05 \)) and HCV-NS3 protein (Fig. 7H, 7I, versus GST–ficolin-2 plus ApoE3 group, \( p < 0.05 \)) levels.

The EC50 values of ficolin-2 against HCVcc-Huh7.5.1 cells in the presence and absence of 300 nM ApoE3 at 72 h were determined by immunofluorescence, as described in Materials and Methods, to be 22.57 and 6.7 ± 0.7 μg/ml, respectively. Our data suggest that ficolin-2 displays a differential EC50 in the presence and absence of 300 nM ApoE3, and ApoE3 interferes with ficolin-2 independently of its ability to enhance HCV infection.

**ApoE3 blocks the effect of ficolin-2 and restores HCVcc binding to LDLR and SR-B1**

We further measured the effects of ficolin-2 and ApoE3 on HCV binding to its receptors. LDLR- and SR-B1–expressing HeLa cells were incubated with HCVcc plus ficolin-2 or ApoE3 and analyzed by confocal fluorescence microscopy. HCVcc was stained with a red PE-conjugated anti-E2 ZE2, and the receptors LDLR and SR-B1 were stained green in the HeLa cells transfected with pEGFP-C1–LDLR and pEGFP-C1–SR-B1. The addition of ficolin-2 decreased HCV binding to the cells, as shown by reduced HCV-E2 on the LDLR-HeLa cells (Fig. 8A, row 4 versus row 3), whereas the addition of exogenous ApoE3 enhanced HCV binding to cells, with more HCVcc found on the SR-B1–HeLa cells (Fig. 8A, row 5 versus row 4). An ELISA analysis also showed that ficolin-2 significantly blocked HCVcc binding to LDLR and SR-B1 (Fig. 8C, HCV plus...
ficolin-2 group versus HCV group, \( p < 0.05 \) and that exogenous ApoE3 blocked the binding of ficolin-2 to LDLR and SR-B1 in an ApoE3 dose-dependent manner (Fig. 8C). Although ApoE3 (600 nM) alone also significantly increased HCVcc binding to LDLR and SR-B1 (Fig. 8C, HCV plus ApoE3 group versus HCV group, \( p < 0.01 \), ficolin-2 only weakly interfered with HCVcc attachment to CD81 (Fig. 8C) and did not interfere with attachment to CLDN1 and OCLN (Fig. 8D). Ficolin-2 combined with different doses of ApoE3 had no effect on HCVcc attachment to CLDN1 and OCLN receptors (Fig. 8D). Taken together, these data suggest that exogenous ApoE3 blocked the antiviral effect of ficolin-2 and restored HCV binding to LDLR and SR-B1.

To our knowledge, this is the first report that reveals the anti-HCV effects of ficolin-2, the important role of ApoE3 in blocking the antiviral activities of ficolin-2, and the immune escape mechanism mediated by ApoE3 during chronic HCV infection. The overall model we propose based our results is shown in Fig. 9.

**Discussion**

In a previous study, we found that an early increase of ficolin-2 was highly correlated with hepatic inflammation and a rapid viral response in chronic HCV patients (40). In the present study, we identify ficolin-2 as a new inhibitor of HCV entry. We have demonstrated that this human lectin inhibited the entry of HCVcc (genotype 2a) and all six genotypes of HCVpp at the early stages of virus entry, regardless of the genotype.

Our present data showed that ficolin-2 blocked HCVcc entry and infection at an early stage (Fig. 1). Both LDLR and SR-B1 are required for the early steps of HCV cell entry (7, 14), whereas CLDN1 and OCLN function in the later steps of HCV entry (10–12). In the present work, we found that ficolin-2 blocked HCVcc entry at an early stage by blocking HCV binding to the early receptors LDLR and SR-B1, and weakly blocking HCV binding to CD81 (Fig. 4). Ficolin-2 did not block HCV binding to the late receptors CLDN1 and OCLN (Fig. 4).

It is likely that ficolin-2 binds to the HCV envelope glycoproteins E2 and E1 glycans and inhibits HCV entry by blocking the interaction between HCV and LDLR, SR-B1, and CD81 though steric hindrance (43, 44). The interaction between ficolin-2 and the E1 glycoprotein was weaker than the E2 glycoprotein (Fig. 5C). However, owing to the lack of a three-dimensional structure of the HCV envelope glycoproteins, the spatial relationship between HCV glycans and their receptors has not been resolved. Several recent reports have shown that lectins such as cyanovirin-N and griffithsin bind to HCV glycans and inhibit HCV entry by blocking the interaction between E2 and CD81 (44, 45). These reports also suggest that a slight increase in steric hindrance could affect the E2-receptor interaction and that the lectin ficolin-2 may be a promising drug that targets the N-glycans of HCV-E2.

Both ficolin and IFN-\( \alpha \) are human innate immune molecules. In normal human serum, the IFN-\( \alpha \) concentration is 2.25 ± 0.88 pg/ml (43, 46), whereas the therapeutic dose of IFN-\( \alpha \) reaches the microgram level (180 \( \mu \)g/wk) in the clinical treatment of HCV (43, 46), much higher than the normal level. The mean concentration of ficolin-2 in healthy controls is ∼4.67–6.0 \( \mu \)g/ml (18, 40), and the EC\(_{50}\) value of ficolin-2 against HCVcc-Huh7.5.1 cells at 72 h was 6.7 ± 0.7 \( \mu \)g/ml. Thus, our data suggest that the ficolin-2 EC\(_{50}\) is slightly higher than or close to the mean concentration of serum ficolin-2 in normal controls, which indicates that ficolin-2 is a more effective innate immune molecule against HCV and demonstrates potential as an effective drug against HCV.

Our data revealed that both mannan and GlcNAc, which are ligands for the ficolin-2 FBG domain, could competitively inhibit the anti-HCV activity of ficolin-2 in a dose-dependent manner (Fig. 2). Mass spectrometry has clearly shown that all 11 glycosylation sites on the HCV-E2 protein are occupied by both high-mannose glycans and complex-type oligosaccharides (47), suggesting that the sugars attached to the E2 glycopeptides are primarily mannose and secondarily GlcNAc or other sugars. These reports are in agreement with our present results, in which mannan
which is primarily composed of high mannose) and, to a lesser extent, GlcNAc could competitively block the interaction between ficolin-2 and HCV-E2 N-glycans and inhibit the anti-HCV activity of ficolin-2.

We found that HCVpp was more sensitive to ficolin-2–mediated antiviral effects (Fig. 1). This result indicates that mature packaged HCVcc contains more ApoE than do unpackaged immature HCV and HCVpp, which lack apoproteins. Because HCVpp is not associated with lipoproteins, we propose that ApoE3 may competitively bind to HCVcc envelope glycoproteins and interfere with the interaction between ficolin-2 and HCVcc. The results shown in Fig. 5 demonstrated that the binding affinity of ApoE3 for E2 was stronger than ficolin-2 for E2 and much stronger than ficolin-2 for E1. Therefore, HCVpp is more sensitive to the antiviral effects of ficolin-2 than HCVcc. Recently, Uprichard and colleagues (48) reported that another cholesterol-uptake receptor, NPC1 L1, is an novel HCV entry factor. Whether ficolin-2 can block HCVcc binding to the NPC1 L1 receptor and whether ApoE3 can block the antiviral effect of ficolin-2 and restore HCVcc binding to NPC1 L1 remain to be further investigated.

ApoE is a component of both HCV lipoparticles and Huh7.5.1 cells. HCV particles are packaged as lipoviroparticles. ApoE3 is enriched in the low-density fractions of HCV particles, essential for binding to LDLR, and is important for HCV infectivity (6, 20). In the present work, it was found that exogenous ApoE3 could bind to E2 and E1 glycoproteins in a dose-dependent manner (Fig. 5B, 5E). We demonstrated that ApoE3 could block ficolin-2 binding to E2, even when ficolin-2 was preincubated with E2-HeLa cells for 2 h and ApoE3 was then added (Fig. 6B, 6C). Furthermore, ApoE3 did not directly bind to ficolin-2 (Fig. 5A). Our data strongly demonstrated that the binding affinity of ApoE3 for E2 was stronger than ficolin-2 for E2 and confirmed that ApoE3 competitively blocked the interaction between ficolin-2 and HCV-E2.

ApoE is polymorphic, with three major isoforms: ApoE2 (Cys 112, Cys158), ApoE3 (Cys 112, Arg158), and ApoE4 (Arg112, Arg158) (49). Although these allelic forms differ from each other by only one or two amino acids at positions 112 and 158, these differences alter the ApoE structure and function. Functional ApoE gene polymorphisms may be a determinant of the outcome of HCV infection (32, 50, 51). ApoE2 and ApoE4 protect against the severe liver disease caused by
HCV, whereas the ApoE3 allele is associated with persistent HCV infection. Our data show that ApoE3 blocks the neutralization of HCVcc by ficolin-2, but ApoE2 and ApoE4 did not (Fig. 7D, 7E). Abs targeting several different apolipoproteins exhibited neutralizing activity against lipoprotein-associated viruses. ApoE is detected in the low-density fractions of HCV, and Abs specific to ApoE can precipitate HCV virions (32, 50, 51). ApoE3 but not ApoB is required for formation/egression and is required for HCV infectivity though interactions with LDLR (25). As this association of host elements cannot evoke an immune attack against HCV particles, lipoprotein association may favor chronic HCV infection by increasing virus-specific infectivity and by shielding the virus from humoral immune responses. Previous data, as well as our own, suggest that lipoproteins play an important role in HCV infection and that ApoE3 mediates immune escape from antiviral innate immunity, promoting HCV infection. This could explain why increased concentrations of ficolin-2 in HCV patients (40) still result in chronic infection.

![Figure 8](https://example.com/figure8.png)

**Figure 8.** ApoE3 blocks the effects of ficolin-2 and restores HCVcc binding to LDLR and SR-B1. (A and B) Immunofluorescent detection of E2 and SR-B1 or LDLR receptor expression in SR-B1 or LDLR plasmid-transfected HeLa cells in the presence of ficolin-2 or ficolin-2 plus ApoE3 using confocal fluorescence microscopy. Original magnification ×600. (C and D) Exogenous ApoE3 restored HCV binding to its receptor and reversed the blocking effects of ficolin-2, as shown by ELISA. **p < 0.01.

![Figure 9](https://example.com/figure9.png)

**Figure 9.** A potential model of the ficolin-2 antiviral effects and ApoE3-mediated immune escape.
Disclosures
The authors have no financial conflicts of interest.

References

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