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Characterization of HCV-like particles produced in a human hepatoma cell line by a recombinant baculovirus

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Abstract

Although processing of the hepatitis C virus (HCV) polyprotein and characterization of each of its viral proteins have been described in detail, analysis of the structure and assembly of HCV particles has been hampered by the lack of a robust cell culture system to support efficient replication of HCV. In this study, we generated HCV-like particles (HCV-LP) using a recombinant baculovirus encoding structural and a part of non-structural proteins in a human hepatoma cell line. The HCV-LP exhibited a buoyant density of 1.17 g/ml in CsCl equilibrium gradient and particles of 40 to 50 nm in diameter. Binding of the HCV-LP to human hepatoma cells was partially inhibited by the treatment with anti-hCD81 antibody, in contrast to the hCD81-independent binding of HCV-LP produced in insect cells. These results indicate that HCV-LP generated in different types of cells exhibit different cellular tropism for binding to target cells.

Keywords: Hepatitis C virus; Baculovirus; Virus-like particle; Human hepatoma cell line; hCD81

Hepatitis C virus (HCV) is the most important causative agent of post-transfusion and sporadic non-A non-B hepatitis, infecting at least 3% of the world population [1]. HCV infection becomes chronic in most cases and may eventually lead to chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma [2]. Current treatments for chronic HCV infection are still limited, and there is no vaccine available to prevent HCV infection [3]. Although processing of the HCV polyprotein and characterization of each of its viral proteins have been described in detail [2], the infection mechanisms of HCV are still unclear due to the lack of a robust and reliable cell culture system and small animal models for HCV replication. To circumvent this problem, soluble truncated E2 protein [4,5], HCV-like particles (HCV-LP) produced by recombinants of baculovirus [6–11] and vesicular stomatitis virus (VSV) [12], an RNA replicon system of Semliki forest virus (SFV) [13], an in vitro assembly system [14], and pseudotype viruses based on VSV [15,16] and retroviruses [17,18] have been developed and used to examine the binding and entry receptors for HCV. It has been shown that the expression of the major structural proteins by various viral vectors leads to the formation of virus-like particles (VLP) [6–10,12]. Production of VLP has mostly succeeded in insect cells using a recombinant baculovirus expression system [6–10,19,20]. VLP are demonstrated to be useful not only for subunit vaccine [19] but also for study of virus–cell interactions [20]. Several groups have succeeded in producing HCV-LP in insect cells

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using recombinant baculoviruses [6,9,10], and the resulting particles have been suggested to have properties similar to those of authentic HCV particles based on morphologic, biophysical, and antigenic analyses [7,10,11], in addition to dose-dependent and saturable binding to human lymphoma and hepatoma cell lines [8]. Although HCV-LP exhibit several properties similar to those of the recombinant E2 protein and pseudotype viruses, including involvement of cell surface glycosaminoglycans and neutralization by specific antibodies for binding to target cells, the binding of HCV-LP did not correlate with the expression of hCD81, which is a candidate receptor for HCV [8,11,21]. Therefore, the interaction of putative HCV receptors with HCV-LP is still controversial.

Recombinant baculoviruses were shown to be able to deliver foreign genes not only into insect cells, but also into mammalian cells without extensive cytopathic effects [22–24], and expression of the HCV genome in mammalian cells has been reported by use of the recombinant baculovirus system [25,26]. Recently, we developed modified recombinant baculoviruses possessing the VSV envelope G protein (VSVG) on the virion surface, and enhanced gene transfer efficacy has been demonstrated in a variety of cell lines [27,28]. In this study, we produced HCV-LP in a human hepatoma cell line, FLC4, by using the recombinant baculovirus system and characterized the properties of the HCV-LP.

Materials and methods

Plasmids. The HCV cDNA (genotype 1b) used in this study was originally isolated from a blood sample of an HCV carrier, which was infectious for both humans and chimpanzees (NIHJ1 clone) [29]. The puromycin resistance gene was excised from pPUR (BD Biosciences Clontech Laboratories, Mountain View, CA) and cloned into pCAGGS [30] under the SV40 early promoter. To create multiple cloning sites, a DNA fragment was synthesized by two oligonucleotides, 5′-AATTAATT AAGTTTAAACGGCGCGCCGATATCTTAAGATCTTCG-3′ and 5′-AATTCGAAGATCTTAAGATATCGGCGCGCCGTTTAAACTTAA TT-3′; the fragment contains 8 restriction sites, *Pac*I, *Pme*I, *Asc*I, *Eco*RV, *Afl*II, *Bgl*II, *Bst*BI, and *Eco*RI, inserted into the *Eco*RI site of pCAGGS, and the plasmid was designated pCAGPM. The PCR fragment of each region indicated in Fig. 1 was amplified from pBRT703′X containing a whole HCV cDNA into pBR322 (Promega, Madison, WI) under the T7 promoter and cloned into the *Eco*RV and *Bgl*II sites of pCAGPM. The whole HCV genome was excised from pBRT703'X and cloned into the *Eco*RI and *Pme*I sites of pCAGPM.

Cells. HEK293T, HeLa, Huh7, HepG2, BRL3A, BHK, NMuLi, CHO, and RK-13 cell lines were obtained from the ATCC (Rockville, MD) and maintained in Dulbecco's modified Eagle's medium (DMEM; Sigma–Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS). FLC4 cell line was maintained as described above. The *Spodoptera frugiperda* (Sf9) cell line was purchased from the ATCC (Rockville, MD) and maintained in Sf900II-SFM (Invitrogen Life Technology, Carlsbad, CA).

Construction of recombinant baculoviruses. Viral constructs were prepared with the pFastBac1 plasmid and the Bac-To-Bac baculovirus expression system (Invitrogen Life Technology) as described previously [31]. A cDNA encoding the region from the HCV core to NS2 was cloned into pFB-VSVG/CAG [31] under the CAG promoter, and a recombinant baculovirus AcVSVG-CACN2 was generated following a protocol provided by the manufacturer. AcVSVG-CAGFP [28] was used as a negative control.

Reagents and antibodies. GST fusion and purification of a large extracellular loop of hCD81 (hCD81LEL) were performed as described previously [4]. Mouse monoclonal antibody against hCD81 was purchased from BD Biosciences Pharmingen (San Diego, CA). The anti-core (c-11-7, c-11-10, and c-11-14) monoclonal antibodies have been described previously [32]. Anti-E1 (299 and 384) and anti-E2 (187) monoclonal antibodies were kindly provided by Dr. M. Kohara. The anti-E2 (AP33) monoclonal antibody has been described previously [10]. Anti-E1 (0726cb1) monoclonal antibody was prepared by immunization with cell lysates of CHO/chiJ1 [15]. Control mouse IgG1 and IgG2a antibodies, anti-E1 (JMAb80) and anti-E2 (JMAb70) human monoclonal antibodies were provided by JT laboratory (Osaka, Japan). Endoglycosidase H (Endo H) and peptide-*N*-glycosidase F (PNGase F) were purchased from Roche-Diagnostics (Mannheim, Germany).

Expression of HCV proteins in mammalian cells. Culture supernatants of cells transfected with the expression plasmids were harvested at 48 h post-transfection, and cells were lysed with TNE lysis buffer (50 mM Tris, 50 mM NaCl, 0.1% NP-40, and 0.5 mM EDTA, pH 7.5) containing protease inhibitor (Complete EDTA-free; Roche-Diagnostics). Expression of HCV proteins in supernatants and cell lysates was analyzed by immunoblotting, quantitative HCV core ELISA (Ortho-Clinical Diagnostics, Tokyo, Japan), and ELISA for E2 protein as described previously [33]. FLC4 cells [34] infected with AcVSVG-CACN2 or AcVSVG-CAGFP at an moi of 250 were harvested at 12 to 96 h post-infection, and expression of HCV proteins in supernatants and cell lysates was analyzed as described above. Furthermore, FLC4 cells infected with the recombinant baculovirus were immunoprecipitated with anti-E1 (JMAb80), anti-E2 (JMAb70), or control human monoclonal antibody, and HCV structural proteins were detected by immunoblotting using specific monoclonal antibodies.

HCV-LP. Purification of HCV-LP from FLC4 cells infected with the recombinant baculovirus was carried out as described previously [6]. Briefly, FLC4 cells infected with AcVSVG-CACN2 were homogenized and subjected to low-speed centrifugation at 15,000*g* for 30 min at 4 °C, and the supernatant was spun down through 30% (wt/vol) sucrose in a phosphate-buffered saline (PBS) cushion by centrifugation at 200,000*g* for 1.5 h at 4 °C. The pellets were resuspended in PBS containing the protease inhibitor, homogenized, and mixed with 33% (wt/wt) cesium chloride and centrifuged at 300,000*g* for 48 h at 4 °C. After centrifugation, 10 fractions (0.5 ml each) were collected from the top and pelleted through a 30% (wt/vol) sucrose cushion by centrifugation at 200,000*g* for 1 h at 4 °C. The pellets were resuspended in PBS containing the protease inhibitor and analyzed by immunoblotting, ELISA, PCR, and electron microscopy. To examine the incorporation of viral RNA into HCV-LP, each of 10 fractions was treated with 50 mU/μl DNase and 0.6 μg/μl RNase for 30 min at 37 °C to remove free RNA and DNA. Total RNA was isolated from each sample by using TRIzol Reagent (Invitrogen Life Technology) and subjected to reverse transcription by using a First-Strand cDNA Synthesis kit (Amersham Biosciences, Piscataway, NJ) following the manufacturer's protocol. Synthesized cDNA was used as a template for HCV-specific PCR.

Electron microscopy. Five microliters of purified HCV-LP was absorbed onto carbon-coated copper 200 mesh grids (NisshinEM, Tokyo, Japan) for 5 min, washed with water, and negatively stained with 2% (wt/vol) uranyl acetate. For immunogold labeling, HCV-LP absorbed on the grids were incubated with HCV-specific mouse monoclonal antibodies, washed with PBS, incubated with a droplet of 10-nm gold particles conjugated to goat anti-mouse IgG antibody (British Biocell International, South Glamorgan, UK), washed quickly with water, and negatively stained. The grids were examined with a Hitachi electron microscope (Hitachi, Tokyo, Japan) at 75 kV.

Binding assay. Binding of HCV-LP to the target cells was determined by the amount of core proteins detected by quantitative core ELISA. HepG2 or Huh7 cells were incubated with various amounts of HCV-LP for 2 h at 4 °C, harvested after extensive washing with DMEM containing 2% FBS, and examined by core ELISA. To determine the effects of chemical modification of cells on the binding of HCV-LP, Huh7 cells were pre-incubated with various concentrations of phospholipase C, Pronase, or sodium periodate as described previously [27]. Binding of HCV-LP to

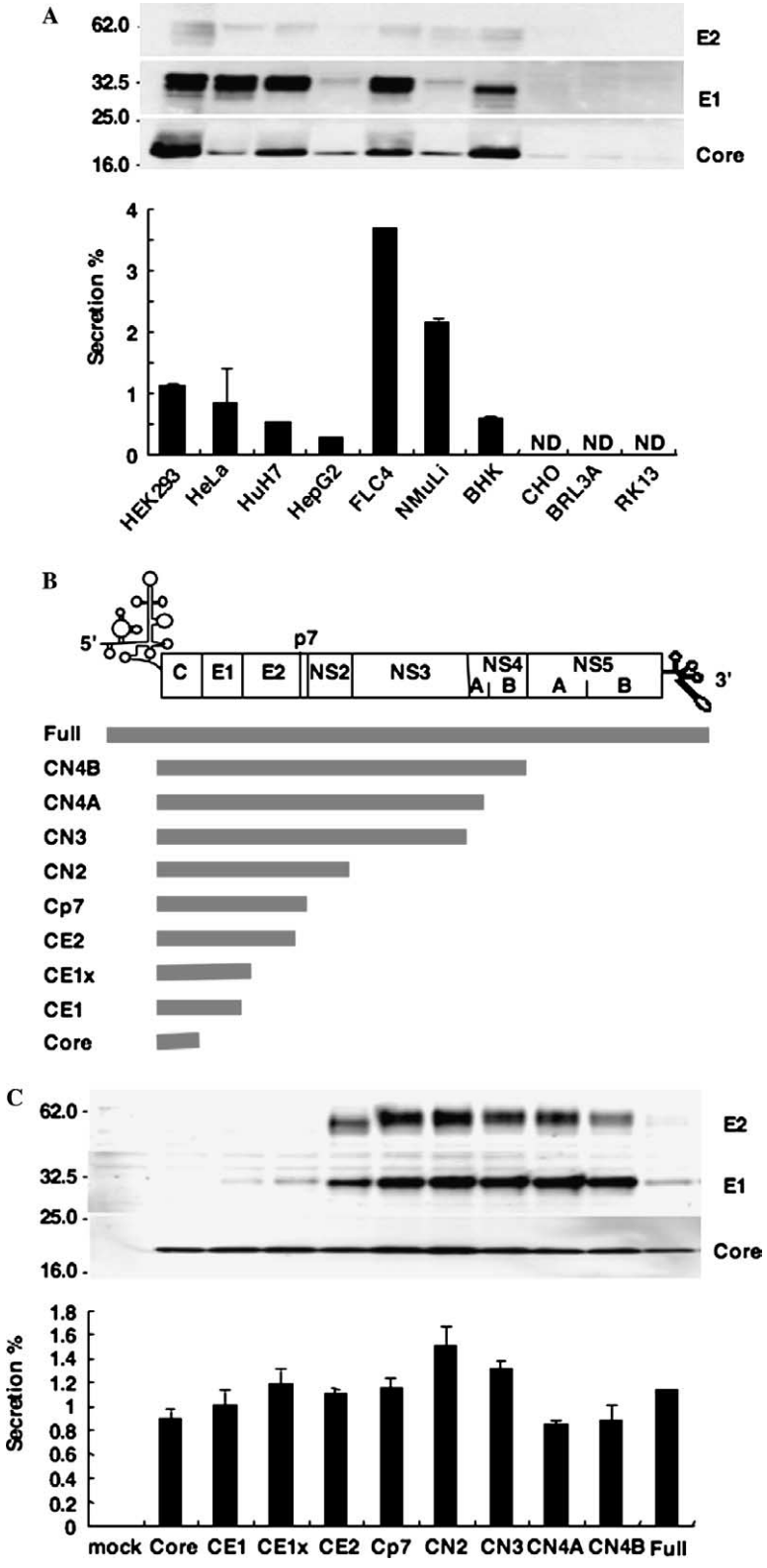

Fig. 1. (A) Expression of HCV structural proteins in various cell lines transfected with the expression plasmid, pCAG-Cp7. (top) Immunoblot analysis of structural proteins. (bottom) Secretion % of core proteins from each cell line. (B) Various cDNA clones used for transient expression in FLC4 cells. (C) Expression of HCV structural proteins in FLC4 cells transfected with the expression plasmids encoding various regions of HCV. (top) Immunoblot analysis of structural proteins. (bottom) Secretion % of core proteins in FLC4 cells transfected with each construct.

Huh7 cells was examined by the amount of core proteins in the cell lysates after 2 h incubation. Infection of AcVSVG-CAGFP was examined by the expression of GFP after 24 h incubation. Cell viabilities were determined by trypan blue staining. To determine the inhibitory effects of binding of HCV-LP to the target cells by soluble proteins or antibodies, HCV-LP were pre-incubated with soluble hCD81LEL (50 μg/ml) or anti-E2

antibody (AP33, 50 μg/ml) for 16 h at 4 °C, and the mixture was incubated with HepG2 or Huh7 cells for 2 h at 4 °C. To assess the effect of anti-hCD81 antibody on the binding of HCV-LP, HepG2 or Huh7 cells were incubated with anti-hCD81 antibody (20 μg/ml) for 1 h at 26 °C prior to the addition of HCV-LP. The binding of HCV-LP to HepG2 or Huh7 cells was examined by core ELISA as described above.

Results and discussion

Determination of a cell line and cDNA construct for the production of HCV-LP

Although previous studies have produced HCV-LP in mammalian cells using various expression systems, including a recombinant VSV [12] and an SFV RNA replicon [13] system, the levels of production of HCV-LP in mammalian cells were lower than those in insect cells [6–8,10,11]. In this study, we tried to produce HCV-LP in human hepatoma cells using a novel baculovirus vector for an efficient transduction of foreign genes into mammalian cells [22–24]. To determine a cell line suitable for production of HCV-LP, various mammalian cell lines were transfected with an expression plasmid, pCAG-Cp7, encoding HCV structural proteins (Figs. 1A and B). Among the cell lines examined, the human hepatoma cell line FLC4 was selected due to its high level expression of HCV structural proteins and its high level secretion of core proteins into culture supernatants (Fig. 1A). FLC4 cells have previously been shown to efficiently translate HCV RNA [34] and to produce infectious HCV particles in a three-dimensional radial-flow-bioreactor system [35]. We then compared expression plasmids encoding various regions of HCV proteins to determine a cDNA construct for HCV-LP production (Fig. 1B). Among the clones examined, an expression plasmid encoding the region from the core to NS2 (CN2) under the CAG promoter exhibited the highest expression of structural proteins and secretion of core proteins in FLC4 cells (Fig. 1C). Therefore, we selected the CN2 construct encoding the region from the core to NS2 for construction of a recombinant baculovirus.

Expression of HCV structural proteins in FLC4 cells infected with a recombinant baculovirus

Recombinant baculovirus possessing VSVG protein on the virion surface exhibited higher efficiency of gene transfer into a variety of cell lines, including FLC4 cells [27,28]. To achieve an efficient gene transduction of the CN2 construct into FLC4 cells, we constructed a recombinant baculovirus AcVSVG-CACN2 encoding a cDNA encoding the region from the core to NS2 under the control of the CAG promoter (Fig. 2A). HCV structural proteins properly processed as reported previously in both mammalian and insect cells [6,15,36] were detected in cells by immunoblotting analysis using specific monoclonal antibodies (Fig. 2B). To examine the kinetics of HCV-LP production, the level of secretion of core proteins in culture supernatants and the expression of core proteins in FLC4 cells infected with AcVSVG-CACN2 were determined (Fig. 2C). The expression of core proteins in cells reached the highest level (approximately 500 pg/μg of total protein) at 24 h post-infection and then decreased, whereas the level of core proteins secreted into the culture supernatants gradually increased up to 96 h post-infection (approximately 115 pg/ml) and then decreased. Similar results were obtained in the expression of envelope proteins by immunoblotting analyses (data not shown). No significant cytopathic effect was observed in the infected cells (data not shown). Co-immunoprecipitation of not only E1 and E2 proteins but also core proteins was observed by anti-E1 and -E2 monoclonal antibodies (Fig. 2D). No precipitation was detected in cells infected with AcVSVG-CAGFP or in the precipitates with a control antibody. Immunofluorescence analyses of the infected cells revealed co-localization of structural proteins in the cytoplasm, primarily in the ER (data not shown). These results indicate that properly processed HCV structural proteins were generated in FLC4 cells by infection with AcVSVG-CACN2.

Characteristics of HCV-LP generated in FLC4 cells

Although a small amount of HCV-LP was secreted into the culture supernatant of FLC4 cells, most of them were detected in intracellular organelles, primarily the ER, as previously demonstrated for HCV-LP produced in insect cells and by an SFV replicon system [6,13]. According to the purification procedure of HCV-LP in insect cells, lysates of FLC4 cells infected with AcVSVG-CACN2 were subjected to CsCl equilibrium gradient centrifugation, and each fraction was analyzed by ELISA and immunoblotting (Fig. 3A). ELISA revealed two peaks of core and E2 proteins in fractions 4 and 7/8, corresponding to buoyant densities of 1.17 and 1.29–1.40 g/ml, respectively (Fig. 3A, bottom). Immunoblotting analyses revealed core proteins clearly and small amounts of E1 and E2 proteins in these fractions (Fig. 3A, top). The buoyant density of HCV-LP in fraction 4 is consistent with that of the high-density HCV particles in patient sera [37,38], HCV-LP produced in insect cells [6,8,9], and infectious HCV particles of the JFH-1 strain recently reported [39,40]. HCV-LP produced in insect cells have been shown to incorporate HCV RNA [6]. To examine the incorporation of viral RNA in each fraction, total RNA was extracted, reverse transcribed, and amplified by PCR using four sets of primers specific for the core, E1, E2 and NS2 proteins. Viral RNA was detectable only in fraction 4, in which HCV proteins were detected by immunoblotting (Fig. 3A, middle), suggesting that HCV-LP produced in FLC4 cells contain HCV genomes, as do HCV-LP produced in insect cells [6]. HCV RNA has also been detected in the lower-density particles (1.07 g/ml) in the supernatants of FLC4 cells cultured in a three-dimensional radial-flow-bioreactor system [35] and in purified particles from the sera of hepatitis C patients [37,41]. Although we do not know the biological meaning of the presence of these particles exhibiting different densities, it may be that the higher-density fractions (over

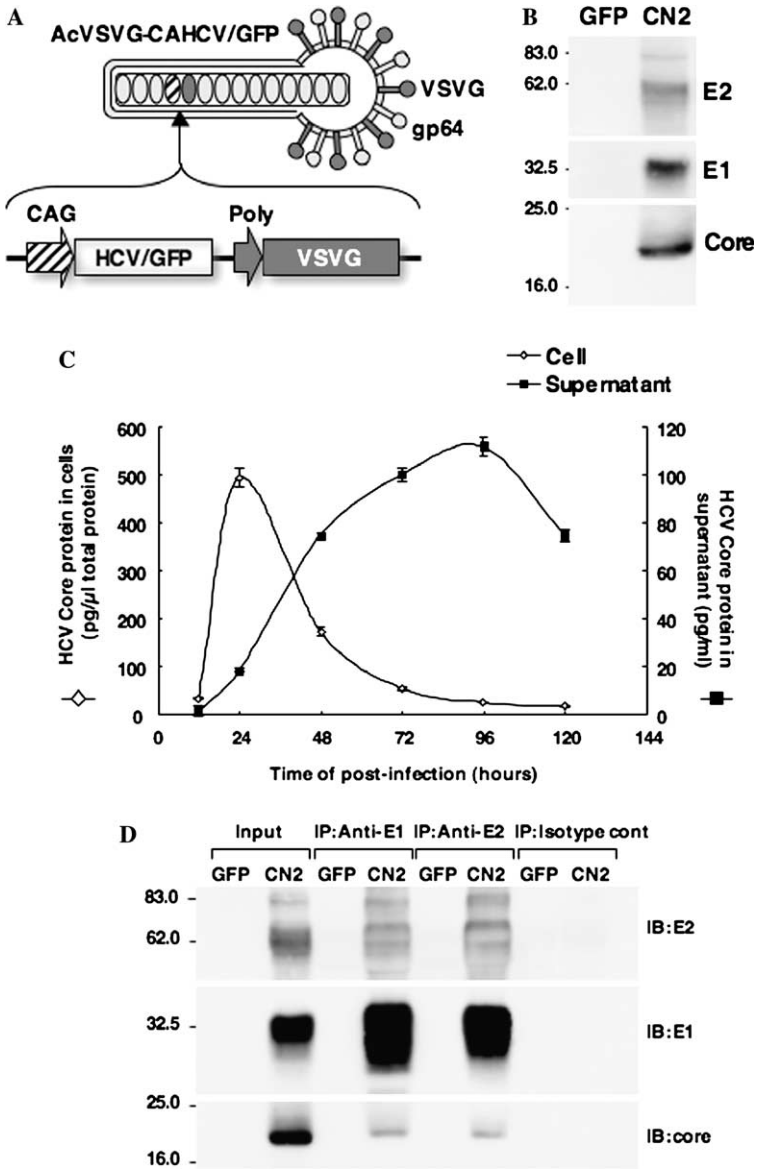

Fig. 2. (A) Schematic representation of recombinant baculoviruses used in this study. HCV or GFP and VSVG genes were inserted under the CAG and the polyhedrin (Poly) promoter, respectively. (B) Expression of HCV proteins in FLC4 cells by infection with a recombinant baculovirus. FLC4 cells were infected with AcVSVG-CAGFP (GFP) or AcVSVG-CACN2 (CN2) at an moi of 250. Expression of HCV proteins was determined by immunoblot analysis at 24 h post-infection. (C) Expression of core proteins in FLC4 cells (open diamond) and secretion into culture supernatants (closed square) upon infection with AcVSVG-CACN2. (D) Co-immunoprecipitation of HCV structural proteins in FLC4 cells. FLC4 cells were infected with AcVSVG-CAGFP (GFP) or AcVSVG-CACN2 (CN2) at an moi of 250 and cell lysates were immunoprecipitated with anti-E1 (JMAb70) or anti-E2 (JMAb80) human monoclonal antibody, or isotype control antibody (JMAb23). The immunoprecipitates were detected by immunoblotting with anti-core (c-11-7, 10, 14), anti-E1 (0726cb1), or anti-E2 (187) mouse monoclonal antibodies.

1.24 g/ml) represent empty particles or those with broken envelope proteins [37].

To examine the morphology of HCV-LP, the gradient fractions were examined by electron microscopy. HCV-LP were detected in fraction 4 by negative staining and immunogold labeling. The majority of the particles had a diameter of 40 to 50 nm (Fig. 3B). The specificities were also confirmed by immunogold affinity labeling with anti-E1 (299 and 0726cb1; Figs. 3C and D) and anti-E2 (97 and 187; Figs. 3E and F) monoclonal antibodies under the electron microscopic analyses. The morphology of HCV-LP produced in the FLC4 cells was similar to that produced in insect cells [6,8,10] and authentic HCV particles [41], suggesting that HCV-LP retain immunoreactive envelope proteins on the particles. To determine the glycosylation of HCV envelope proteins, cell lysates of FLC4 cells infected with the recombinant baculovirus and purified HCV-LP were treated with Endo H or PNGase F. E1 and E2 proteins in both cells and HCV-LP were completely digested by both of the enzymes, suggesting that E1 and E2 proteins have high-mannose-type carbohydrates (Fig. 3G). E1 protein digested with PNGase F was hardly detected by anti-E1 antibodies, probably due to reduction of affinity to the anti-E1 antibody.

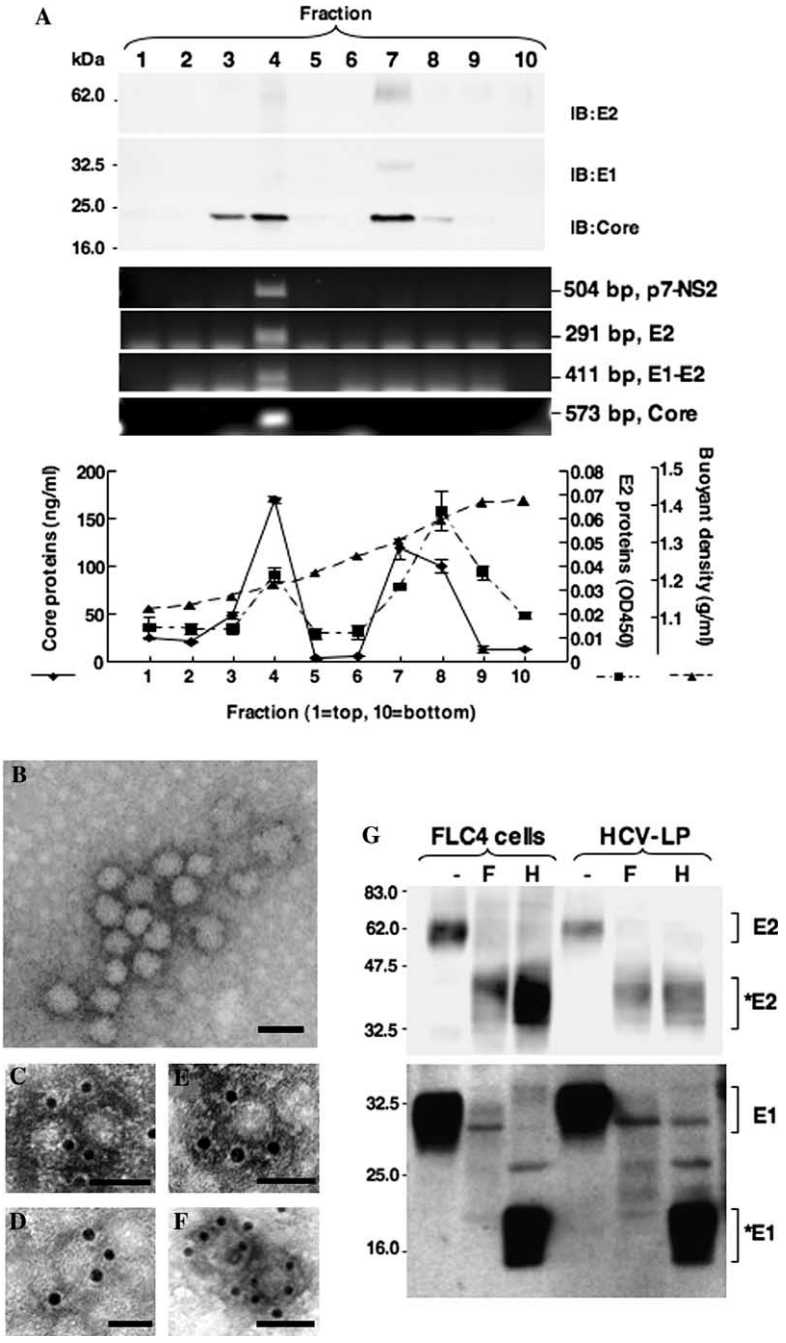

Fig. 3. (A) Purification of HCV-LP by CsCl equilibrium centrifugation. Supernatants of lysates of FLC4 cells infected with AcVSVG-CACN2 were spun down over a 30% (wt/vol) sucrose cushion and subjected to CsCl equilibrium centrifugation. Ten fractions were collected from the top and then analyzed by immunoblotting with anti-core (c-11-7, 10, 14), anti-E1 (0726cb1), and anti-E2 (187) monoclonal antibodies (top). Incorporation of HCV RNA into HCV-LP was examined by RT-PCR (middle). Core and E2 proteins in each fraction were determined by core ELISA (closed diamond) and E2 ELISA (closed square), respectively. Closed triangles indicate the buoyant density. (B–F) Electron microscopy of purified HCV-LP stained with 2% uranyl acetate (B). Immunogold labeling of HCV-LP with anti-E1 (C: 299; D: 0726cb1) and anti-E2 (E: 97; F: 187) mouse monoclonal antibodies. The bar represents 50 nm. (G) Deglycosylation of HCV envelope proteins. Cellular lysates and purified HCV-LP were treated with PNGase F (F), Endo H (H) or PBS (−) and subjected to immunoblotting with anti-E1 (299 and 384) and anti-E2 (187) monoclonal antibodies. Asterisks indicate deglycosylated envelope proteins.

Binding of HCV-LP to human hepatoma cell lines

The human hepatoma cell lines Huh7 and HepG2 are known to be target cells for the binding or infection of pseudotype viruses [15,16,42,43] or JFH-1 virus [39,40] as well as the HCV-LP derived from insect cells [8,11]. Therefore, the binding properties of HCV-LP generated in FLC4 cells were examined in Huh7 and HepG2 cells. The binding of HCV-LP occurred in a dose-dependent manner and was saturable to both Huh7 and HepG2 cells (Fig. 4A). Binding

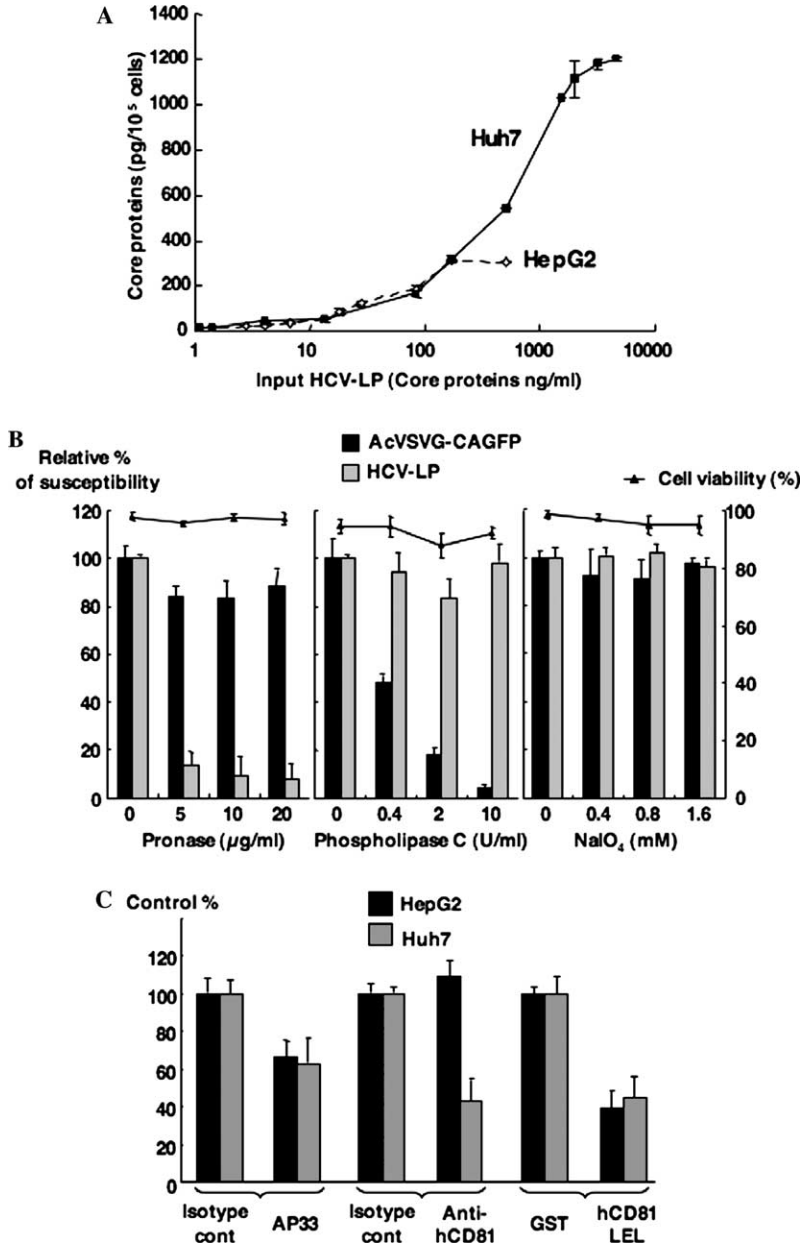

Fig. 4. (A) Binding of HCV-LP to HepG2 and Huh7 cells. Cells were incubated with increasing concentrations of HCV-LP, and bound HCV-LP were determined by core ELISA after extensive washing. (B) Effect of chemical modification of Huh7 on the binding of HCV-LP and the infection of AcVSVG-CAGFP. Huh7 cells were pre-incubated with various concentrations of Pronase, phospholipase C or sodium periodate. Binding of HCV-LP was determined by quantitative core ELISA. Infection of AcVSVG-CAGFP was examined by the expression of GFP at 24 h post-incubation. Cell viabilities were determined by trypan blue staining. (C) Effects of hCD81LEL or antibody to E2 or hCD81 on the binding of HCV-LP to HepG2 and Huh7 cells. HCV-LP were incubated with hCD81LEL (50 μg/ml) or anti-E2 (AP33, 50 μg/ml) overnight at 4 °C. HepG2 or Huh7 cells were incubated with anti-hCD81 antibody (20 μg/ml) for 1 h at 26 °C prior to inoculation of HCV-LP. The binding of HCV-LP to the target cells was determined by core ELISA. The results shown are the average of three independent assays with the error bars representing the SD.

of HCV-LP reached plateaus at concentrations of approximately 0.5 and 4.0 μg/ml of core protein for HepG2 and Huh7 cells, respectively. These results indicate that Huh7 cells have a greater capacity to bind HCV-LP produced in mammalian cells than HepG2 cells. HCV-LP produced in insect cells [8,10,11] and pseudotype VSV [15] exhibited higher affinity for HepG2 cells than Huh7 cells, in contrast to the pseudotype retrovirus, which exhibited infectivity to Huh7 cells but not to HepG2 cells [43]. To examine cell surface molecules involved in the binding of HCV-LP, Huh7 cells were pre-incubated with various concentrations of Pronase, phospholipase C or sodium periodate, and binding of HCV-LP was evaluated (Fig. 4B). Previously, it was demonstrated that infectivity of baculovirus [27] and binding of VLP of Norwalk virus [20] were decreased by the treatment with phospholipase C or Proteinase K. Treatment of Huh7 cells with Pronase, but not with phospholipase C or sodium periodate, decreased the binding of

HCV-LP, whereas infection of AcVSVG-CAGFP was inhibited by the treatment with phospholipase C, but not with Pronase or sodium periodate as reported previously [27]. These treatments exhibited no effect on cell viability. These results indicated that protein molecules on the cell surface play an important role for the binding of HCV-LP to Huh7 cells.

Involvement of hCD81 in the binding of HCV-LP

hCD81 has been suggested to be one of the major host factor candidates for the binding and infection of HCV [44]. Accumulating data from studies on binding or infection assays suggest that hCD81 is involved in the infection of HCV [8,10,16,18,33,40,42,43]. To determine the involvement of hCD81 in the binding of HCV-LP, target cells or HCV-LP were pre-treated with anti-hCD81 antibody or soluble hCD81LEL, and the effect on the HCV-LP binding was evaluated (Fig. 4C). The binding of the HCV-LP to both HepG2 and Huh7 cells was partially inhibited by the treatment with anti-E2 antibody (AP33) and hCD81LEL. The binding to Huh7 cells was also inhibited by the treatment with anti-hCD81 antibody, but no inhibition was observed in HepG2 cells due to the lack of hCD81 expression. The specific interaction of hCD81 with purified E2 protein has been reported [4,44,45]. Pseudotype retroviruses produced in 293T cells [18,42,43] and infectious HCV clones of the JFH-1 strain [39,40] were also shown to infect target cells through an hCD81-dependent pathway. However, HCV-LP derived from insect cells and pseudotype VSV produced in CHO cells exhibited an hCD81-independent binding [21] or infection [15]. In the case of HCV-LP derived from FLC4 cells, hCD81 did indeed play an important role in the binding to the target cells, but inhibition of binding by the anti-hCD81 and hCD81LEL was approximately 50%, suggesting that molecules other than hCD81 also participate in the binding. By contrast, in HCV-LP produced in insect cells, expression of hCD81 is not essential for binding [8,11,21]. Although we do not know the reason why the binding properties of HCV-LP derived from insect and human hepatoma cells are different, it may be that post-translational modifications, including glycosylation and maturation of envelope proteins, are different between insect and mammalian cells.

In conclusion, we could produce HCV-LP in a human hepatoma cell line using a recombinant baculovirus encoding HCV proteins and showed that the HCV-LP exhibited profiles different from those of HCV-LP derived from insect cells. The binding properties to the target cells were partially dependent on hCD81, but the involvement of other pathways was also suggested. The HCV-LP produced in FLC4 cells may help to clarify the differences in cell tropism of infection among HCV pseudotype viruses produced in different cell lines, and in binding profiles among the purified envelope proteins and HCV-LP produced in various cell lines. Further studies are needed to identify host factor(s) other than hCD81 involved in the binding of HCV-LP.

Acknowledgments

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