# A POTENTIAL ROLE OF THE HEPARAN SULFATE IN THE HEPATITIS C VIRUS ATTACHMENT

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**Summary. –** Several putative cell surface receptors have been identified in the Hepatitis C virus (HCV) infection including CD81, low-density lipoprotein (LDL) receptor, scavenger receptor class B type I (SR-BI), and highly sulfated heparan sulfate (HS). In this study, we showed that the binding of soluble heparin to the envelope glycoprotein E2 (E2) of HCV was dependent on the dose and conformation of E2. On the other hand, the binding of E2 to the cell surface after treatment with soluble heparin was not markedly inhibited, what was different from other viruses utilizing cellular HS as the primary receptor. However, the enzymatic removal of HS from the cell surface led to a significant reduction in the binding of E2 to the cells. These facts imply that E2 was bound to cellular HS but might also have another route for cell attachment**.** Monoclonal antibodies with neutralizing activity against E2 did not completely block the binding of E2 to the cell surface, but their neutralization activity was greatly enhanced in the presence of soluble heparin. Taken together, the cellular HS could act as an alternative receptor for HCV and the interaction of E2 with HS could play a distinct role in escaping of HCV from the humoral immunity.

**Key words:** Hepatitis C virus; E2 glycoprotein; heparan sulfate; receptor; humoral immune response

## Introduction

HCV belongs to the genus *Hepacivirus,* the family *Flaviviridae.* It is an enveloped virus containing a single positive-sense RNA. The virion consists of at least 3 structural proteins: the core (nucleocapsid) protein C and two envelope proteins, E1 and E2. HCV is transmitted almost exclusively by parental exposure to the blood and blood products. Nearly 170 million people worldwide are infected with HCV. The chronic HCV infection can lead to a liver cirrhosis and hepatocellular carcinoma (Lauer and Walker, 2001). There is no specific antiviral drug available for the treatment of HCV infection. Therefore, the development of an effective anti-HCV drug and the understanding of a mechanism of HCV infection is a high priority task in medical and pharmaceutical communities. Although the tissue tropism is a critical determinant of HCV infection, a little is known about the molecular basis of HCV binding to their target cells.

The glycoproteins E1 and E2 are supposed to initiate infection of the target cells by binding to the receptors on the cellular plasma membrane. The E2 is believed to play a major role in the virus attachment (Rosa *et al.*, 1996). There are several candidate receptors for HCV attachment utilized by E2. When the soluble form of E2 was used as a probe, CD81 has been identified as a putative receptor for HCV (Pileri *et al.*, 1998). By a very similar approach, SR-BI has been identified as the other candidate receptor (Scarselli *et al.*, 2002). Additionally, since HCV is associated with LDL in serum, the LDL receptor has been proposed as a candidate receptor for HCV (Agnello *et al.*, 1999; Monazahian *et al.*, 1999; Wunschmann *et al.*, 2000). Liver-specific intercellular adhesion molecule-3-grabbing integrin (L-SIGN), dendritic cell-specific intercellular adhesion molecule-3-grabbing

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**Abbreviations:** CD81-EC2 = extracellular domain of CD81 human receptor; E2 = E2 glycoprotein; HCV = Hepatitis C virus; HS = heparan sulfate; LDL = low-density lipoprotein; MAb(s) = monoclonal antibody(ies); MFI = mean fluorescence intensity; SR-BI = scavenger receptor class B type I; RT = room temperature

nonintergrin (DC-SIGN) (Gardner *et al.*, 2003; Lozach *et al.*, 2003; Pohlmann *et al.*, 2003) and asialoglycoprotein receptor (ASPGR) (Saunier *et al.*, 2003) have been also identified as HCV receptor candidates. The involvement of such proteins in HCV cell entry implies that HCV enters the target cells via either a complex entry pathway including many sequential steps or more than one entry pathway. The complex interactions between E2 and putative multiple receptors require further investigation. Among putative receptors, CD81 and SR-BI play a direct role in HCV entry. However, co-expression of CD81 and SR-BI in the non-hepatic cell lines does not lead to the entry of HCV pseudotype particles, indicating that other molecule(s) expressed only in the hepatic cells are necessary for HCV entry (Cocquerel *et al.*, 2006). Verifying of the complex interactions between E2 and a set of the receptor candidates would lead to a better understanding of the HCV infection process.

It was demonstrated that an attachment of Dengue virus to the target cell receptor was effectively blocked by the soluble heparin and cellular HS. The Dengue virus and HCV belong to the family *Flaviviridae* and E2 is predicted to possess a heparin-binding motif. Some studies have focused on the role for glycosaminoglycans in the HCV adsorption and E2 binding (Barth *et al.*, 2003; Garson *et al.*, 1999; Yagnik *et al.*, 2000). The low-molecular weight heparin, suramin, blocks the binding of HCV to human hepatoma cells *in vitro* (Garson *et al.*, 1999). Glycoprotein E2 was also found to bind to a heparin-column and the heparin-binding peptide sequence was found in E2 (Yagnik *et al.*, 2000). Recently, it was shown that E2 interacts with highly sulfated cellular HS and this interaction plays a major role in mediating of E2 attachment to the target cells (Barth *et al.*, 2003). The same investigators also demonstrated that the interaction of the viral envelope with plasma membrane is mediated by the binding of both E1 and E2 glycoproteins to the highly sulfated HS (Barth *et al.*, 2006).

Cellular HS is a repeating highly negative-charged linear copolymer of variably sulfated uronic acid and glucosamine residues (Capila and Linhardt, 2002). The cell surface HS is attached to the core transmembrane protein and is ubiquitously distributed in the matrix and cell membrane of most tissues. A distinct cell- and tissue-specific expression pattern may contribute to the differential targeting of viruses (Kim *et al.*, 1994). An increasing number of structurally different viruses and other microbial agents utilize cellular HS in the initial attachment to target cells (Boyle and Compton, 1998; Chen *et al.*, 1997; Chung *et al.*, 1998; Dechecchi *et al.*, 2001; Feldman *et al.*, 1999; Giroglou *et al.*, 2001; Goodfellow *et al.*, 2001; Kern *et al.*, 2003; Pinon *et al.*, 2003; Reddi and Lipton, 2002; Roderiquez *et al.*, 1995; Shukla and Spear, 2001). Numerous reports suggested that cell entry of many viruses might be a two- or multi-step process with initial contact being a low-affinity HS receptor followed by a transfer to the high-affinity receptor for internalization (Fry *et al.*, 1999). Heparin, a highly sulfated analog of HS, is the most commonly used anticoagulant drug. This is largely due to the facts that heparin is the only drug that inhibits the activities of both factor Xa and thrombin.

In our work we tried to understand of the molecular mechanisms governing the E2 binding to the human cells and to the several putative receptors especially to the cellular HS. In addition, we investigated the characteristics and humoral immune responses of E2 domain accountable for binding to HS. Unlike other cellular HS-utilizing viruses, we found that E2 did not utilize HS as a primary receptor. However, E2 indeed bound to cells via HS and the corresponding domain on E2 did not elicit humoral immune response efficiently. HCV may use the interaction of E2 with cellular HS as a novel strategy to escape humoral immunity.

## Materials and Methods

*Recombinant proteins, heparin-BSA complex.* HCV E2 recombinant protein was expressed in CHO cells and purified as previously described (Heo *et al.*, 2004). The recombinant E2 protein containing 6'His tag is made up of the 278 amino acid (384–661 aa ectodomain) originated from HCV infectious clone H77, a gift of J. Bukh, NIH, USA (Yanagi *et al.*, 1997). The extracellular domain of human CD81 (CD81-EC2) protein was previously described using pGEX-EC2 vector (Seong *et al.*, 2001) (a gift of D.S. Im, KRIBB, Daejeon, Korea). Soluble heparin-BSA complex was generously provided by R.J. Linhardt, Rensselaer Polytechnic Institute, Troy, New York, USA.

*Monoclonal and polyclonal antibodies*. MAb HS4C3V against highly sulfated HS single chain and MAb MPB49V against control single chain were generously provided by T. van Kuppevelt, University of Nijmegen, Nijmegen, Netherlands. Mouse MAb H33 (a gift of J. Dubuisson, Institut Pasteur de Lille, Lille, France) and human MAbs CBH-5 and CBH-7 (a gift of S.K.H. Foung, Department of Pathology, Stanford University, Stanford, USA) were used as E2 specific MAbs. HCV patients' sera (gifts from Y. Lee, Hangang Sacred Hospital, Seoul, Korea, and J. Cho, Dankook University, Cheonan, Korea) signed as SSKOD, SSKYW, SSSHS, SSWJD, DK1, DK2, DK3, DK4, DK5, and normal human serum (control) were used for inhibition of the E2 binding to CD81-EC2, SR-BI, and heparin-BSA.

*Cell lines*. Molt-4, Huh-7, HepG2, CHO-K1 were purchased and grown as recommended by the American Type Culture Collection.

*ELISA*. The plates (Corning) were coated with 10 μg/ml of soluble heparin-BSA or BSA in PBSN (PBS containing 0.02% $NaN_3$). After incubation overnight at 4°C, the plates were blocked with PBS containing 1% BSA (Sigma) for 1 hr at room temperature (RT). Purified native E2, heat inactivated E2 (for 5 mins at 100°C), and reduced E2 (treated with 5 mmol/l dithiothreitol for 15 mins at 56°C) were serially diluted and added to each well and incubated for 1 hr at RT. Bound E2 was detected with MAb anti-

penta-His (Qiagen), followed by the AP-conjugated anti-mouse IgG (Pierce). The absorbances $A_{405,}$ $A_{490}$ were measured; the difference $A_{405} - A_{490}$ calculated and the mean values of duplicate measurements were taken into account.

For inhibition of the binding of HCV E2 to CD81-EC2, SR-BI, and heparin-BSA, the sub-saturating concentration of E2 was mixed with serially diluted patient's serum and added to wells of ELISA plates coated with 1 μg/ml of CD81-EC2 and SR-BI, or with 10 μg/ml of soluble heparin-BSA. Bound E2 was detected with biotinylated MAb anti-penta-His (Qiagen), followed by the streptavidin-AP (Pierce).

*Flow cytometry.* Molt-4, Huh-7, and HepG2 cells were washed in FACS buffer (PBS, 1% BSA, 0.1% $NaN_3$), and $2 \times 10^5$ cells were allowed to react with sub-saturating concentration of E2 protein premixed with serially diluted inhibitors for 1 hr at RT. The inhibitors used were: CD81-EC2 (serially diluted from 200 μg/ml concentration), BSA (from 5,000 μg/ml), MAb H33 (from 200 μg/ml), the mixture of 100 μg/ml MAb H33 and increasing concentration of 78, 156, 312, 625, 1,250, 2,500, and 5,000 μg/ml of heparin-BSA, patient serum DK1 (from 1:5 dilution), the mixture of DK1 serum diluted in 1:20 and heparin-BSA in concentrations 31.3, 15.6, 7.8, and 3.6 μg/ml. After washing, MAb anti-penta-His was added to the cells and incubated for 1 hr at RT. After washing, cell-bound antibodies were detected by anti-mouse IgG-RPE (R-Phycoerythrin, Southern Biotech.). Analyses were performed by using a FACScalibur flow cytometer and CELLQUEST software (Becton Dickinson). The results were determined as % inhibition calculated with the following formula: % inhibition = [(mean fluorescence intensity (MFI) of E2 alone – MFI of E2 with inhibitor)/(MFI of E2 alone – MFI of blank)] × 100.

For cellular HS expression, Molt-4, Huh-7, HepG2, and CHO-K1 cells were incubated with Vesicular stomatitis virus (VSV) tagged single chain anti-highly sulfated HS MAb HS4C3V or control MAb MPB49V. Detection and analyses by FACS were done as described above.

To detect the effect of heparinase I for the binding of E2 to cells, Molt-4, Huh-7, HepG2, and CHO-K1 cells were removed from cultivation surface without proteases and resuspended in 10 mmol/l phosphate buffer pH 7.4 with 0.14 mol/l NaCl, 3 mmol/l KCl, 0.5 mmol/l $MgCl_2$, 1 mmol/l $CaCl_2$, 0.1% glucose, 1% fetal bovine serum (Cambrex), and 0.5% BSA (Sigma) containing 10 U/ml of heparinase I or a buffer only. After incubation for 1 hr at 37°C, the cells were washed once with FACS buffer. Serially diluted E2 protein was added to the heparinase-treated or buffer-treated cells and E2 binding was analyzed as described above. The data are expressed as net MFI (MFI in the presence of E2 – MFI in the absence of E2) of the mean values of duplicate measurements.

## Results

### *E2 binds directly to the soluble heparin in dose- and conformation-dependent manner*

First, we examined the direct interaction between purified E2 and soluble heparin-BSA that is similar to the physiological cellular HS. E2 protein showed a dose-dependent binding to the soluble heparin-BSA complex in ELISA (Fig. 1). A reduction of E2 did not change its reactivity to the soluble heparin-BSA. In contrast, the heat inactivation of E2 resulted in the substantial reduction of the reactivity to the soluble heparin-BSA. The specificity of E2 binding to the soluble heparin-BSA complex was already described in our previous studies (Heo *et al.*, 2004).

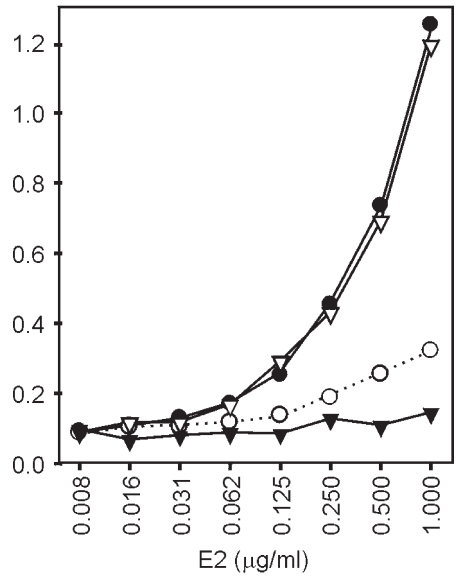


**Fig. 1**

**Binding of E2 to the soluble heparin-BSA detected by ELISA**

Native E2 (●), heat-inactivated E2 (○), reduced E2 (▽), and native E2 bound to control BSA (▼).

### *Cellular HS plays a minor role in the binding of E2 to cells*

As described previously (Heo *et al.*, 2004), the majority of E2 did not co-localize with the cellular HS on Huh-7 cells and co-localized only partially with HS on HepG2 cells. These data indicated that E2 was attached probably through other receptors such as CD81 or SR-BI, and HS was not a major factor for the initial binding of E2 to the cell surface. To confirm the biological relevance of the results obtained from co-localization assay, the subsequent experiments were performed to find out, whether soluble heparin-BSA could competitively inhibit the binding of E2 to cell surface.

The E2 protein was pre-incubated with the soluble heparin-BSA, CD81-EC2, and BSA as a control inhibitor and the mixtures were added to the Molt-4, Huh-7, and HepG2 cells. The E2 bound to the cells was detected by flow cytometry (Fig. 2A,B,C). Soluble heparin-BSA did not inhibit significantly the binding of E2 to the cell surface

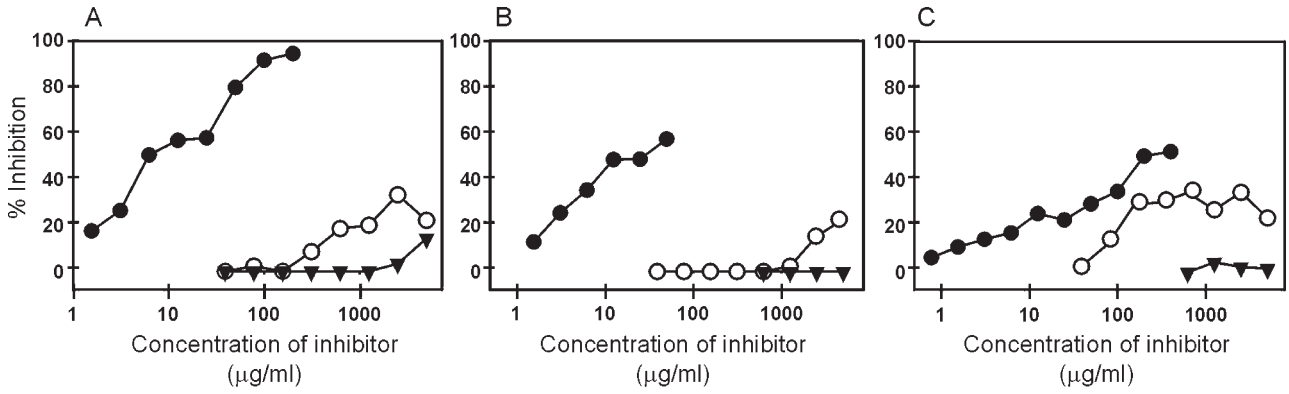


**Fig. 2**

**Inhibition of the E2 binding to Molt-4 (A), Huh-7 (B), and HepG2 (C) cells by CD81-EC2 (●), heparin-BSA (○), and BSA (▼) detected by flow cytometry**

even at the concentration as high as 5,000 μg/ml. The maximum inhibition for heparin-BSA was 33% at Molt-4, 23% at Huh-7, and 35% at HepG2 cells. The fact that the soluble heparin, an analog of cellular HS, was not able to inhibit the binding of E2 to the cell surface suggested that other receptor such as CD81 and SR-BI could play a more important role in mediating the attachment of E2 to the cell surface. We also found that heparin-bound E2 could interact well with other receptors on Molt-4, Huh-7, and HepG2 cells (Fig. 2).

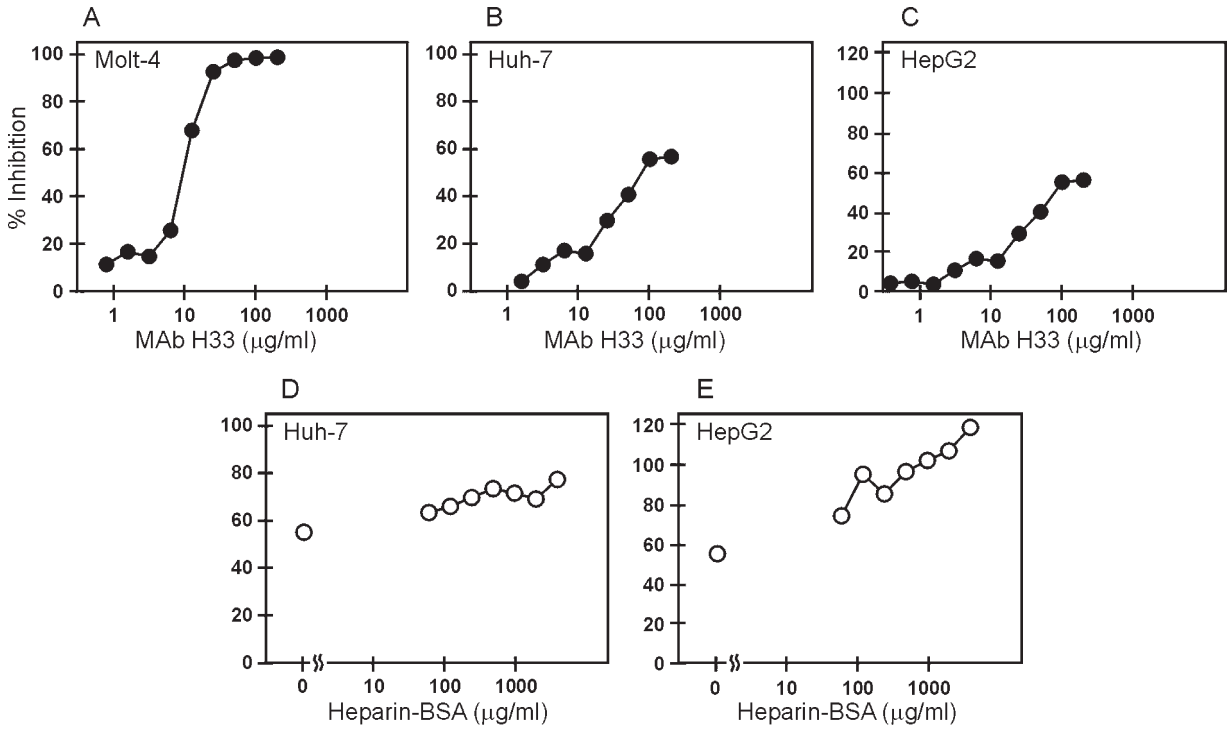


**Fig. 3**

**Inhibition of the E2 binding to Molt-4 (A), Huh-7 (B, D), and HepG2 (C, E) cells by MAb H33 alone (●) or in the mixture of 100 μg/ml MAb H33 and increasing concentration of 78, 156, 312, 625, 1,250, 2,500, and 5,000 μg/ml of heparin-BSA (○) detected by flow cytometry**

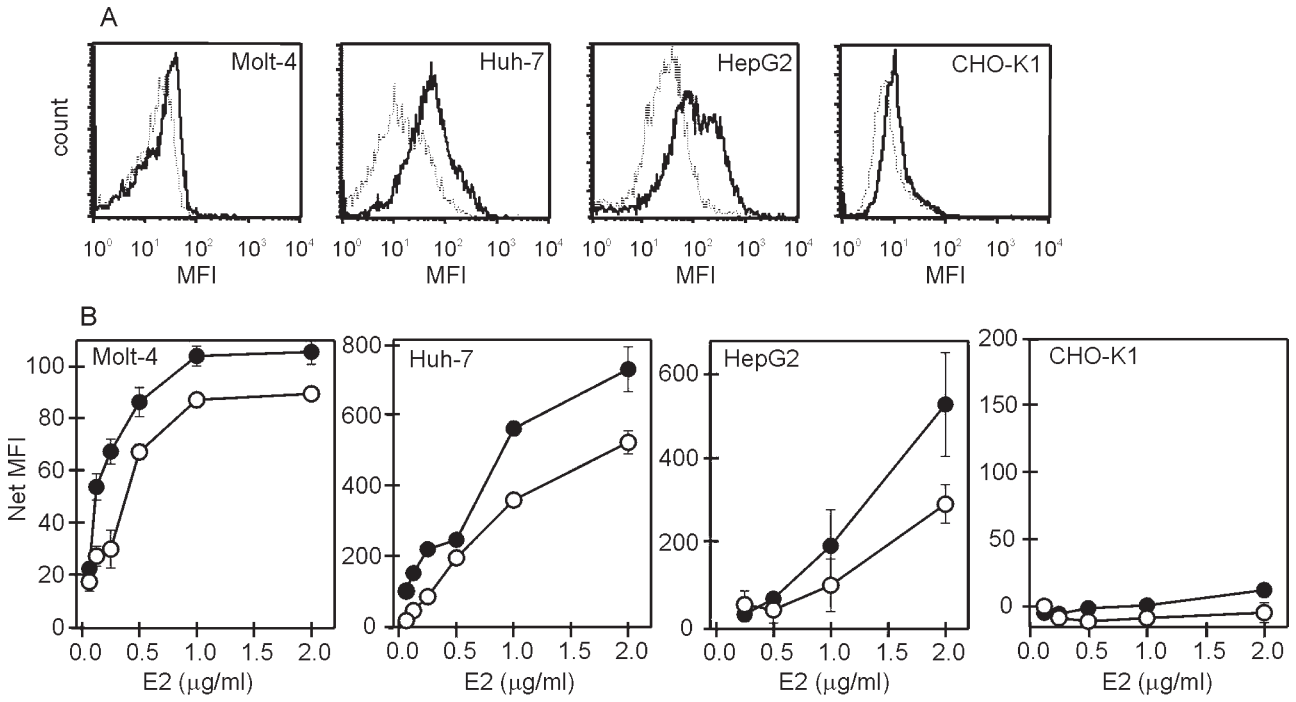


**Fig. 4**

**The effect of heparinase I on the binding of E2 to Molt-4, Huh-7, HepG2, and CHO-K1 cells detected by flow cytometry**

A: Presence of HS on the cell surface. Cells were stained with anti-HS MAb HS4C3 (black lines) or with control MAb MPB49V (grey lines). B: Binding of E2 to the cells treated with heparinase I (○) or to non-treated cells (●).

While CD81 and SR-BI binding domains on E2 were blocked by corresponding MAbs, some portion of E2 was still able to bind to the target cells (Fig. 3B,C). We tested whether the soluble heparin could combine with the unblocked portion of E2 or not. In order to test this assumption, we pre-incubated E2 protein with serially diluted MAb H33 (double blocker of CD81 and SR-BI) alone or with saturating concentration 100 μg/ml of MAb H33 mixed with various dilutions of soluble heparin-BSA (starting from 5,000 μg/ml). Then, the mixture was added to Huh-7 and HepG2 cells. In the presence of MAb H33 and heparin-BSA, the residual unblocked portion of E2 was less inhibited in binding to Huh-7 cells, but it was completely inhibited in binding to HepG2 cells (Fig. 3B,C). The binding of E2 to Molt-4 cells was completely inhibited by MAb H33 only (Fig. 3A). We postulated that high level of CD81 expression on Huh-7 cells caused the incomplete inhibition of E2 binding by soluble heparin in spite of pretreatment with H33 (Fig. 3B). From these results followed that E2 utilized cellular HS as well as other receptors for binding to Huh-7 cells.

Next, we focused on the interaction between E2 and native cellular HS expressed on the target cells. We tested the effects of heparinase I on the expression of cellular HS on the surface of various cell lines. Heparinase I degrades highly sulfated domains of cellular HS. The presence of HS on the cell surface was confirmed by flow cytometry with MAb specific for single chain HS (Fig. 4A). Although there were differences in cellular HS expression levels, all tested cell lines expressed HS. All cell lines except CHO-K1 cells could bind the increasing amount of E2 protein. However, the binding of E2 was significantly reduced by the treatment of cells with heparinase I (Fig. 4B). These data implied that cellular highly sulfated HS might play a minor, but contributive role in E2 binding. The cell line CHO-K1 almost did not bind E2, but these cells expressed only HS and not human CD81 and SR-BI. A rational explanation might be the supporting role of cellular HS in E2 binding to cells. Based on the above data, we concluded that cellular HS plays a minor but distinct role in E2 binding.

*Humoral immune response to HCV does not block the binding of E2 to soluble heparin-BSA*

The MAbs CBH-5, CBH-7, and H33 (all with neutralizing activity) were able to block the binding of E2 to CD81-EC2 (Heo *et al.*, 2004). However, these MAbs were unable to inhibit the binding of E2 to the soluble heparin. This finding

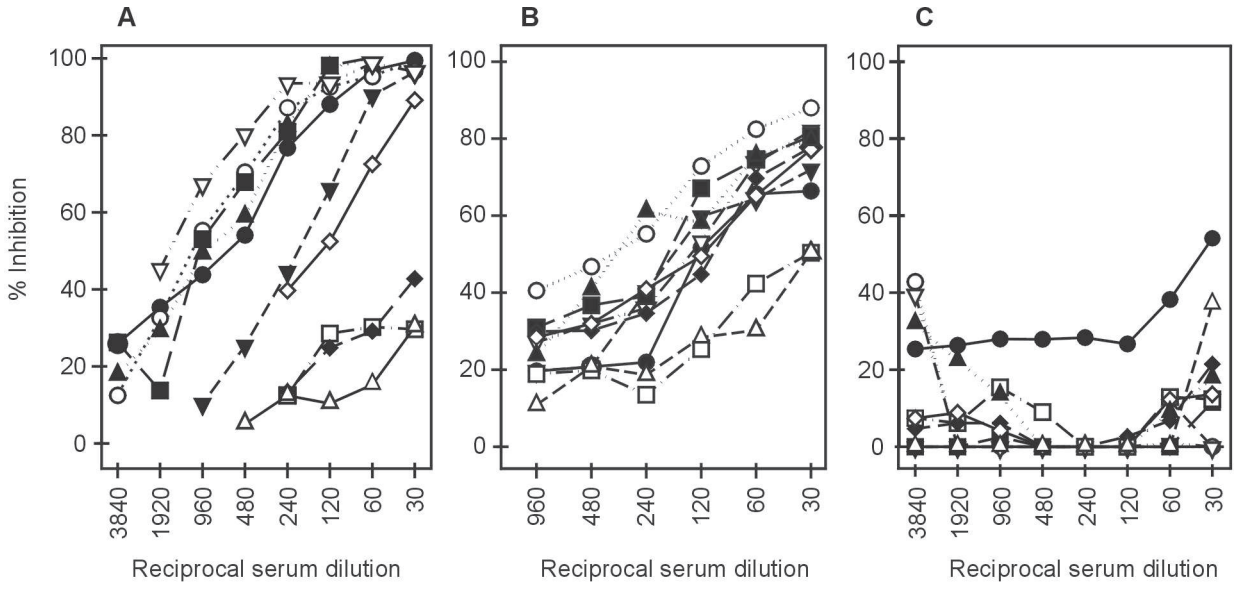


**Fig. 5**

**Inhibition of the E2 binding to CD81-EC2 (A), SR-BI (B), and heparin-BSA (C) by human sera detected in ELISA**

The human sera from HCV patients signed as SSKOD(●), SSKYW(○), SSSHS (▼), SSWJD (▽), DK1 (■), DK2 (□), DK3 (♦), DK4 (◇), DK5 (▲) and normal human serum (NP) (△) were used for the inhibition of E2 binding.

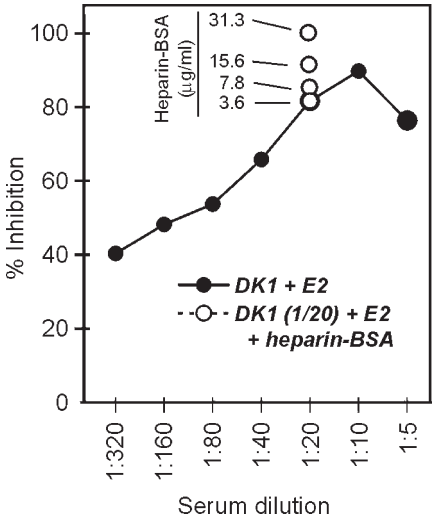


**Fig. 6**

**Inhibition of the E2 binding to HepG2 cells in the presence of patient serum DK1 alone or the mixture of DK1 and heparin-BSA detected by flow cytometry**

HepG2 cells were treated with various dilutions of patient serum DK1 with E2 (●) or with DK1 serum diluted 1:20 with E2 and heparin-BSA in concentrations 31.3, 15.6, 7.8, and 3.6 μg/ml (○).

suggested the presence of at least two epitopes that are involved in the E2 attachment to cells. One of the epitope seemed to be specific for CD81 binding and another epitope for the HS binding.

Furthermore, we tested a number of MAbs of human and mouse origin to find any MAb able to block the binding of E2 to soluble heparin. Unfortunately, we failed to find a MAb with this activity. Thus, we supposed that E2 might elicit very low levels of neutralizing antibodies against the domain of E2 responsible for soluble heparin binding during the infection. To examine the accessibility of heparin-binding domain on E2 to the humoral immune response, HCV patients' sera were tested for the presence of antibodies against the domain of E2 responsible for binding to heparin-BSA. Interestingly, different sera showed different binding activities depending on the target receptor proteins. Generally, the sera neutralized E2-CD81 binding significantly (Fig. 5A), E2-SR-BI binding moderately (Fig. 5B), and E2-soluble heparin binding very poorly (Fig. 5C). Thus, it appeared that there were low levels of neutralizing antibodies for E2-soluble heparin binding in sera of chronically HCV-infected patients.

To confirm these observations, we performed inhibition of E2 binding assay with human patient's serum DK1 and soluble heparin-BSA (Fig. 6). HepG2 cells were incubated

with sub-saturating concentration of E2 protein premixed with serially diluted DK1 serum alone or with DK1 serum diluted 1:20 mixed with serially diluted soluble heparin-BSA. The binding of E2 to HepG2 cells was inhibited in proportion to the serum dilution, but the binding could not be completely blocked even with the lowest serum dilution. However, the residual non-neutralized E2 was completely blocked by approximately 30 μg/ml of soluble heparin-BSA in the presence of diluted DK1 serum (Fig. 6). This observation confirmed previous finding that human HCV patients' sera were deficient of antibodies that were able to neutralize E2.

## Discussion

This study provided evidence that cellular HS play a minor but significant role in the binding of E2 to the target cell lines. Our results consistently showed that in addition to cellular HS, cell surface non-HS molecules play a major role in the E2-cell binding process. Here, we suggested the non-HS molecules were human CD81 and SR-BI, which were already defined as putative cellular receptors (Pileri *et al.*, 1998; Scarselli *et al.*, 2002). This interpretation was based on the following observations. Firstly, cell-bound E2 protein did not co-localize exactly with cellular HS, while E2 co-localized with CD81 and SR-BI. Next, E2 binding to the target cells was weakly inhibited even by high concentration of soluble heparin-BSA. In contrast, the majority of E2 binding was dependent on cell surface CD81, as shown by blocking assay with soluble CD81. Conclusively, we identified the fact that soluble heparin-bound E2 was still able to interact properly with soluble CD81 and the binding affinity of the E2 to the cellular HS was lower than binding affinity of E2 to CD81 or to SR-BI (Heo *et al.*, 2004). Next, although soluble heparin alone did not significantly affect the E2-cell binding, soluble heparin in the presence of saturating concentration of neutralizing antibodies against CD81 and SR-BI showed a marked inhibitory effect on E2-HepG2 cell binding. Finally, the binding of E2 to the cell surface was significantly reduced by the heparinase pretreatment of human cells. Moreover, E2 does not bind efficiently to HS expressed on the CHO-K1 cells in the presence or absence of heparinase I pretreatment. Therefore, cellular HS indeed influenced the primary E2 binding, but it is not the sole determinant for the E2-cell interaction. Based on these observations, we concluded that although the E2-cell binding was mediated mainly by other receptor(s), cellular HS had some impact on the primary interaction of E2 with cell surface. Conversely, in the case when other receptor(s) are not available, cellular HS may be able to act as an alternative receptor.

It has been demonstrated that cellular HS is used by numerous viruses for binding to the target cells. They include Herpes simplex virus, Vaccinia virus, papillomavirus, Respiratory syncytial virus, Human immunodeficiency virus, echovirus, and Human cytomegalovirus (Boyle and Compton, 1998; Chung *et al.*, 1998; Feldman *et al.*, 2000; Giroglou *et al.*, 2001; Goodfellow *et al.*, 2001; Roderiquez *et al.*, 1995; Shukla and Spear, 2001). Dengue virus and Tick-borne encephalitis virus, the members of the family *Flaviviridae,* use cellular HS as receptor (Chen *et al.*, 1997; Kroschewski *et al.*, 2003). Many viruses that utilized cellular HS as their primary receptor are dramatically inhibited in their binding to the cell surface after treatment with soluble HS. The viruses able to bind to cellular HS, use also other cell surface molecules usually proteins as receptors. In case of Human parainfluenza virus type 3, cellular HS played secondary role during the virus entry and ongoing infection was inhibited in cells lacking cellular HS (Bose and Banerjee, 2002). For these viruses, interaction of viral membrane protein(s) with cell surface proteoglycans led to interaction with receptor(s) of high affinity, which is required for efficient virus entry. For HCV, it has already been shown that soluble heparin and suramin can inhibit HCV binding to human cells (Garson *et al.*, 1999; Germi *et al.*, 2002). Recently, by using soluble E2 and HCV-like particle model, it was demonstrated that highly sulfated cellular HS was necessary for the E2-cell binding and the E2-cell interaction was dramatically inhibited to nearly 80% by soluble heparin (Barth *et al.*, 2003). In the other hand, as mentioned above, soluble heparin showed a weak inhibitory effect on the E2-cell binding in our experiments. We attributed this discrepancy to the different conditions such as E2 concentration used in the inhibition assays. It was demonstrated consistently with our data that the amount of inhibition of HCV binding by the heparinase treatment is less extensive than the inhibition by the same enzyme of cell binding by Yellow fever virus and Dengue virus (Germi *et al.*, 2002). As a result, non-saturable binding pattern and low binding affinity of E2-HepG2 cells binding indicated the E2-cellular HS interaction was not a ligand-receptor relationship in the regular meaning. Cellular HS possibly serves for capture and concentration of HCV at the cell surface, thereby passing the virus to the high affinity receptor(s), what lead to viral internalization.

Up to the present, there is no specific treatment for HCV and no vaccine is available. Characterization of the molecular basis concerning the initial binding of virus to the target cells may facilitate the development of vaccines and pharmaceuticals for prevention and treatment of HCV-related liver diseases.

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