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(54) Titre : UTILISATIONS DE DC-SIGN ET DE DC-SIGNR POUR INHIBER UNE INFECTION PAR LE VIRUS DE
L'HEPATITE C

(54) Title: USES OF DC-SIGN AND DC-SIGNR FOR INHIBITING HEPATITIS C VIRUS INFECTION

(57) **Abrégé/Abstract:**

This invention provides a method for identifying a compound capable of inhibiting the binding of HCV to a cell expressing a surface receptor selected from the group consisting of DC-SIGN or DC-SIGNR comprising: (a) contacting the cell expressing the surface receptor with a source of HCV in the presence or absence of a test compound for a time sufficient to allow binding of HCV to the cells; and (b) detecting the cell-bound HCV, wherein a reduction of cell-bound HCV in the presence of the test compound compared to the amount of cell-bound HCV in the absence of the test compound is indicative of a compound capable of inhibiting the binding of HVC to a cell expressing DC-SIGN or DC-SIGNR. The invention also provides methods for identifying a compound capable of inhibiting HCV infection of a cell expressing DC-SIGN or DC-SIGNR. Additionally, the invention is directed to methods for determining HCV binding to a cell comprising and methods for detecting the presence of HCV in a biological source.



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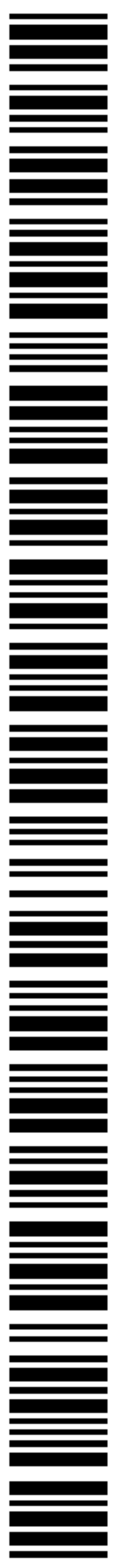
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(54) Title: USES OF DC-SIGN AND DC-SIGNR FOR INHIBITING HEPATITIS C VIRUS INFECTION

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WO 2004/058953 A1

**USES OF DC-SIGN AND DC-SIGNR FOR
INHIBITING HEPATITIS C VIRUS INFECTION**

5

The invention disclosed herein was made with United States Government support under grant number AI051134 from the National Institutes of Health, U.S. Department of Health and Human Services. Accordingly, the United States Government
10 has certain rights in this invention.

This application claims the priority of U.S. Serial No. 10/328,997, which is a continuation-in-part of U.S. Serial No. 10/184,150, filed June 26, 2002, which is a
15 continuation-in-part of, and claims the priority of U.S. Provisional Application No. 60/300,971, filed June 26, 2001, the contents of which are hereby incorporated by reference.

Throughout this application, various publications are
20 referenced by Arabic numerals. Full citations for these publications may be found at the end of the specification immediately preceding the claims. The disclosure of these publications is hereby incorporated by reference into this application to describe more fully the art to which this
25 invention pertains.

Background of the Invention

Hepatitis C virus was first recognized in 1989 and is
30 responsible for the majority of cases of non-A, non-B hepatitis [1]. Infections are typically chronic and lifelong; many infected individuals are healthy and unaffected for decades, whereas others develop chronic hepatitis or cirrhosis, the latter often leading to
35 hepatocellular carcinoma [16]. Whereas screening of the blood supply has drastically reduced new transmissions of the virus, there exists a large cohort of infected individuals who will require treatment in the coming

decades. Some reports estimate that nearly 3% of the world's population (including about 4 million people in the U.S.) is infected with HCV [2]. It is estimated that 170 million people worldwide, including about 4 million people
5 in the US, are infected with HCV.

Infected individuals have or will develop liver disease with clinical outcomes ranging from an asymptomatic carrier state to active hepatitis and cirrhosis. Chronic infection is
10 also strongly associated with the development of hepatocellular carcinoma. HCV infection and its clinical sequelae are the leading causes of liver transplantation in the US. No vaccine is currently available. Several preparations of interferon alpha and interferon alpha-2b
15 plus ribavirin are presently used for the treatment of chronic hepatitis C [32]. The best long-term response rates are obtained with a combination of interferon alpha-2b and ribavirin. However, only a minority of subjects treated with this combination achieves the desired result of no
20 detectable serum HCV RNA 6 months after stopping treatment [32]. The optimal treatment with these drugs for all infected individuals, including those co-infected with HIV-1, has not been established because data on viral dynamics in response to treatment are scarce. Interferon alpha and
25 ribavirin are non-specific anti-viral agents with incompletely understood mechanisms of action. They also are associated with severe and life-threatening toxicities, including neutropenia, hemolytic anemia and severe depression.

30

There is an urgent need for new therapeutic agents to combat HCV infection. A particularly attractive target for antiviral therapy is HCV entry into target cells because such inhibitors do not need to cross the plasma membrane nor
35 be modified intracellularly. In addition, viral entry is generally a rate-limiting step that is mediated by conserved structures on the virus and cell membrane. Consequently,

inhibitors of viral entry can provide potent and durable suppression of viral replication.

The HCV genome is a 9.6 kb positive-sense, single-stranded
5 RNA molecule that encodes a single polyprotein of ~3000
amino acids [42]. A number of isolates have been
characterized and found to exhibit considerable sequence
diversity. Virus sequences can be divided into major
genotypes (exhibiting <70% sequence identity), and further
10 into subtypes (exhibiting 80-90% identity) [53]. Genotype 1
(subtypes 1a and 1b) predominates in North America, Europe,
and Japan [46]. There are no clear differences in pathology
associated with the different genotypes.

15 Despite the sequence diversity among isolates, many features
are held in common. The genomic RNA contains a long 5' non-
translated region (NTR) of about 340 nucleotides, followed
by a single long open reading frame (ORF) encoding a
polyprotein of about 3000 amino acids [42]. A short 3' NTR
20 is followed by a poly(A) sequence and 98 highly conserved
nucleotides (the "X" region). Translation of the RNA is
mediated by an IRES element in the 5' NTR. The polyprotein
precursor is processed to generate at least ten proteins:
from amino- to carboxy-terminus these are termed C, E1, E2,
25 p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B [19]. The C
protein constitutes the nucleocapsid; E1 and E2 are
transmembrane envelope glycoproteins; p7 is of unknown
function; the various NS proteins are nonstructural proteins
with replication functions. Polyprotein cleavage in the
30 structural region (C-p7) is catalyzed in the endoplasmic
reticulum (ER) by cellular signal peptidases. Cleavage of
the polyprotein in the nonstructural region (NS2-NS5B) is
mediated by HCV encoded proteinases. NS2 and NS3 constitute
a protease that cleaves the NS2-NS3 junction. NS3 is a dual
35 function protein, containing at its amino-terminus a serine
protease domain responsible for cleavage at the remaining
sites in the precursor, and an RNA helicase/NTPase domain at

its carboxy-terminus. NS4A is thought to enhance or direct the protease activity of NS3, while the functions of NS4B and NS5A are unclear. NS5B is an RNA-dependent RNA polymerase (RdRp) and the catalytic subunit of the replicase
5 for the virus. This enzyme recognizes the 3' end of the RNA and carries out RNA synthesis to create a minus-strand RNA. The 3' end of the minus strand is then similarly recognized by the RdRp to initiate synthesis of plus strand RNAs. As these progeny viral RNAs are made they are packaged into
10 assembling virions. HCV particles bud into the ER and are transported out of the cell by microsomal vesicles [42].

There are few animal models for HCV infection. These include the chimpanzee [22, 27, 45] which is an endangered
15 species. Another model is the SCID-BNX model, whereby immunodeficient mice are implanted with human liver tissue that is infected with HCV as described [54]. Studies of viral replication *in vitro* have largely depended on infection of cell lines or primary hepatic cultures with
20 sera of HCV-infected patients [4, 5, 23, 24, 26, 29, 44, 51]. However, the levels of viral RNA in these infected cultures are very low and can only be detected by PCR [4, 5, 23, 24, 26, 29, 44, 51]. In an important recent advance, Lohmann et al. [30] replaced the structural genes in a
25 complete subtype 1b genome with the neomycin phosphotransferase gene followed by the IRES of the encephalomyocarditis virus. In the resulting construct, the phosphotransferase gene was downstream of the HCV 5' NTR (containing the HCV IRES), while the HCV nonstructural genes
30 were downstream of the encephalomyocarditis virus IRES. RNA was transcribed from this construct and transfected into the human hepatoma cell line, Huh-7. After selection in neomycin, cell lines were obtained which showed robust replication of the transfected mini-genome; viral RNA could
35 be detected by northern blot analysis and viral proteins could be detected by immunoprecipitation. There is an urgent need for additional animal models of HCV infection.

HCV entry into host cells requires attachment of the viral particle to the cell surface, followed by fusion of the viral envelope with the cellular membrane. This process is mediated by the viral envelope glycoproteins, E1 and E2.

5 Two proteins, named E1 and E2 (corresponding to amino acids 192-383 and 384-750 of the HCV polyprotein respectively), have been suggested to be external proteins of the viral envelope which are responsible for the binding of virus to target cells. HCV E1 and E2 have been expressed

10 recombinantly in a number of forms and using a variety of expression systems. Two recent reports have described fusion and entry mediated by E1 and E2 ectodomains fused to the TM domain of the VSV G envelope glycoprotein [28, 49].

15 In mammalian cell-based expression systems, the molecular weight of mature, full length E1 is ~35kD and that of E2 is ~72kD [19, 31, 48]. The amino-terminal residues of mature E1 and E2 were determined experimentally [21]. Endoproteolytic processing of the HCV polyprotein converts

20 E1 and E2 into type-1 membrane-anchored proteins [19, 48]. Furthermore, E1 and E2 form non-covalently associated heterodimers, from hereon referred to as E1/E2 [8, 19, 37, 41]. Fully processed E1/E2 heterodimers are not exported to the cell surface, but are retained in the ER, where HCV

25 budding occurs [9, 10, 11, 12, 43]. Analyses of E1 and E2 N-linked glycosylation patterns further showed that these proteins are retained in the ER without cycling through the Golgi [12, 34]. The ER retention signals are located in the TM domains of E1 and E2 [6, 7, 14]. Replacing the TM

30 domains of E1 and E2 by the TM domains of plasma membrane-associated proteins, or mutating charged residues in the TM domains of E1 and E2, results in cell surface expression of the envelope glycoproteins [6, 7, 8, 14]. Such TM domain modifications, however, also abrogate E1/E2 hetero-

35 dimerization [7, 36]. The dimerization and ER retention signals of E1 and E2 therefore cannot be dissociated. Deletion of the entire TM domain of E1 and E2 results in the

secretion of soluble, monomeric ectodomains of the envelope glycoproteins [12, 13, 35].

To date, two human cellular proteins, CD81 and low-density lipoprotein (LDL) receptors, have been implicated as putative receptors that mediate HCV entry [25], and glycosaminoglycans have been suggested to play a role in the nonspecific attachment of HCV to cell [52]. Uses of the CD81 in the treatment and diagnosis of HCV infection are disclosed by Abrignani et al. in the international patent application WO 99/18198. Studies have demonstrated that the recombinant soluble E2 ectodomain binds specifically and with high affinity to human and chimpanzee CD81, but not to CD81 from other species [15, 20, 38, 39]. However, these results have come into question in light of recent studies, including one showing that CD81 in the tamarin, a species that is refractive to HCV infection, also binds soluble E2 with high affinity [33]. Even though a number of studies have defined the structural determinants of the human CD81/E2 interaction, direct functional proof of CD81-mediated HCV fusion and entry is still lacking. Moreover, CD81 is expressed on numerous tissues outside of the liver, and thus CD81 tissue distribution fails to explain the cellular tropism of HCV. Similarly, studies to date have failed to demonstrate a direct interaction between LDL receptors and the HCV envelope glycoproteins [52]. In addition, LDL receptors are widely expressed on tissues other than liver, and thus its expression does not explain the tropism of HCV.

30

DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule 3-Grabbing Nonintegrin, Genbank accession number AF209479) and DC-SIGNR (DC-SIGN Related, Genbank accession number AF245219) are type II membrane proteins with close sequence homologies (77% identity in amino acids). DC-SIGN is expressed at high levels on dendritic cells; DC-SIGNR is expressed at high levels in liver and lymph nodes but not on

dendritic cells; and both molecules are expressed on the endometrium and placenta [40, 47, 3, 17].

The proteins are C-type (calcium-dependent) lectins that possess all of the residues known to be required for binding of mannose. DC-SIGN and DC-SIGNR bind the HIV-1 surface envelope glycoprotein gp120, which possesses high-mannose sugars, and this binding is inhibited by mannan [47, 3, 17]. Both DC-SIGN and DC-SIGNR bind infectious HIV-1 particles and promote infection of susceptible T cells *in trans* [40, 47, 3]. European patent applications EP 1046651A1 and EP 1086137 A1 describe the use of DC-SIGN in compositions and methods for inhibiting HIV-1 infection. The entire contents of these applications are incorporated herein by reference.

Like DC-SIGN and DC-SIGNR, the lectin *Galanthus nivalis* (GNA lectin) from snowdrop bulbs avidly binds carbohydrates and glycoproteins possessing high-mannose structures. Notably, GNA lectin avidly binds HIV-1 envelope glycoproteins [18, 50]. In addition, GNA captures the HCV envelope glycoproteins [13], which contain high-mannose carbohydrates. Based on these findings, we have discerned that DC-SIGN and DC-SIGNR avidly bind HCV envelope glycoproteins and thus serve as receptors for the virus.

To our knowledge, no association has been made between DC-SIGN, DC-SIGNR and HCV infection. DC-SIGN and DC-SIGNR are also able to mediate internalization, as required for cellular entry and infection by HCV but not HIV-1. In addition, DC-SIGNR in particular is expressed at high levels in liver, the primary target organ for HCV infection. Since the ability of DC-SIGN and particularly DC-SIGNR to serve as receptors for HCV has not been previously appreciated, this discovery affords the opportunity to treat or prevent HCV infection through therapies or vaccines that block the specific interaction between HCV and these receptors.

Summary of the Invention

This invention provides a method for determining HCV binding to a cell comprising: (a) contacting a cell expressing DC-SIGN or DC-SIGNR with a source of HCV for a time sufficient to allow binding of HCV to the cell; and (b) detecting the cell-bound HCV. In one embodiment, the cell-bound HCV is detected by RT-PCR, followed by Southern blot. In another embodiment, the cell-bound HCV is detected by real-time PCR. In a further embodiment, the cell-bound HCV is detected using an immunoassay. In still another embodiment, the cell-bound HCV is detected using an HCV-specific detection reagent. Particular examples of the HCV-specific detection reagent include an antibody and an oligonucleotide probe or primer.

This invention also provides a method for detecting the presence of HCV in a biological source comprising: (a) contacting the source suspected to contain HCV with a cell expressing DC-SIGN or DC-SIGNR for a time sufficient to allow binding of HCV to the cell; and (b) detecting the cell-bound HCV. In one embodiment, the cell-bound HCV is detected by RT-PCR followed by Southern blot. In another embodiment, the cell-bound HCV is detected by real-time PCR. In still another embodiment, the cell bound HCV is detected using an immunoassay.

This invention further provides a method for identifying a compound capable of inhibiting the binding of HCV to a cell expressing DC-SIGN comprising: (a) contacting the cell expressing DC-SIGN with a source of HCV in the presence or absence of a test compound for a time sufficient to allow binding of HCV to the cells; and (b) detecting the cell-bound HCV, wherein a reduction of cell-bound HCV in the presence of the test compound compared to the amount of cell-bound HCV in the absence of the test compound is indicative of a compound capable of inhibiting the binding

of HCV to a cell expressing DC-SIGN. In one embodiment, the cell-bound HCV is detected by RT-PCR followed by Southern blot. In another embodiment, the cell-bound HCV is detected by real-time PCR. In a further embodiment, the cell-bound HCV is detected using an immunoassay. In a still further embodiment, the cell-bound HCV is detected using an HCV-specific detection reagent. Particular examples of the HCV-specific detection reagent may include an antibody and an oligonucleotide probe or primer. In another embodiment, the oligonucleotide probe or primer specifically hybridizes to an HCV genome or a portion thereof. In a further embodiment, the source of the HCV is a biological fluid, a tissue or a cell. In yet another embodiment, the biological fluid is blood, serum, plasma or amniotic fluid. In an additional embodiment, the test compound is an antibody, a non-antibody polypeptide or a nonpeptidyl agent.

This invention still further provides a method for identifying a compound capable of inhibiting the binding of HCV to a cell expressing DC-SIGNR comprising: (a) contacting the cell expressing DC-SIGNR with a source of HCV in the presence or absence of a test compound for a time sufficient to allow binding of HCV to the cells; and (b) detecting the cell-bound HCV, wherein a reduction of cell-bound HCV in the presence of the test compound compared to the amount of cell-bound HCV in the absence of the test compound is indicative of a compound capable of inhibiting the binding of HCV to a cell expressing DC-SIGNR. In one embodiment, the cell-bound HCV is detected by RT-PCR followed by Southern blot. In a further embodiment, the cell-bound HCV is detected by real-time PCR. In another embodiment, the cell-bound HCV is detected using an immunoassay. In still another embodiment, the cell-bound HCV is detected using an HCV-specific detection reagent. Particular examples of the HCV-specific detection reagent may include an antibody and an oligonucleotide probe or primer. In another embodiment, the oligonucleotide probe or primer specifically hybridizes

to an HCV genome or a portion thereof. In a further embodiment, the source of the HCV is a biological fluid, a tissue or a cell. In yet another embodiment, the biological fluid is blood, serum, plasma or amniotic fluid. In an additional embodiment, the test compound is an antibody, a non-antibody polypeptide or a nonpeptidyl agent.

This invention additionally provides a method for identifying a compound capable of inhibiting an HCV infection of a cell expressing DC-SIGN comprising: (a) contacting the cell expressing DC-SIGN with a source of HCV in the presence or absence of a test compound for a time sufficient to allow infection of the cell expressing DC-SIGN by HCV; and (b) detecting the HCV in HCV-infected cells, wherein a reduction of HCV in the presence of the test compound compared to the amount of HCV in the absence of the test compound is indicative of a compound capable of inhibiting the infection of the cell expressing DC-SIGN by the HCV. In one embodiment, the HCV is detected by RT-PCR followed by Southern blot. In a further embodiment, the HCV is detected by real-time PCR. In another embodiment, the HCV is detected using an immunoassay. In still another embodiment, the HCV is detected using an HCV-specific detection reagent. Particular examples of the HCV-specific detection reagent may include an antibody and an oligonucleotide probe or primer. In another embodiment, the oligonucleotide probe or primer specifically hybridizes to an HCV genome or a portion thereof. In a further embodiment, the source of the HCV is a biological fluid, tissue or a cell. In yet another embodiment, the biological fluid is blood, serum, plasma or amniotic fluid. In an additional embodiment, the test compound is an antibody, a non-antibody polypeptide or a nonpeptidyl agent.

The present invention also provides a method for identifying a compound capable of inhibiting an HCV infection of a cell expressing DC-SIGNR comprising: (a) contacting the cell

expressing DC-SIGNR with a source of HCV in the presence or absence of a test compound for a time sufficient to allow infection of the cell expressing DC-SIGNR by HCV; and (b) detecting the HCV in the HCV-infected cell, wherein a reduction of HCV in the presence of the test compound compared to the amount of HCV in the absence of the test compound is indicative of a compound capable of inhibiting the infection of the cell expressing DC-SIGNR by the HCV. In one embodiment, HCV is detected by RT-PCR followed by Southern blot. In a further embodiment, the HCV is detected by real-time PCR. In another embodiment, the HCV is detected using an immunoassay. In still another embodiment, the HCV is detected using an HCV-specific detection reagent. Particular examples of the HCV-specific detection reagent may include an antibody and an oligonucleotide probe or primer. In another embodiment, the oligonucleotide probe or primer specifically hybridizes to an HCV genome or a portion thereof. In a further embodiment, the source of the HCV is a biological fluid, a tissue or a cell. In yet another embodiment, the biological fluid is blood, serum, plasma or amniotic fluid. In an additional embodiment, the test compound is an antibody, a non-antibody polypeptide or a nonpeptidyl agent.

This invention further provides a method for identifying a compound capable of inhibiting the infection of a cell by HCV, this cell being susceptible to infection by HCV, the method comprising: (a) contacting a cell expressing DC-SIGN with a source of HCV for a time sufficient to allow binding of HCV to the cell expressing DC-SIGN; (b) contacting the cell-bound HCV with a cell susceptible to infection by HCV in the presence or absence of a test compound for a time sufficient for infection in the absence of the test compound; and (c) detecting infection of the cell susceptible to infection by HCV, wherein the absence of infection or the reduction of infection in the presence of the test compound compared to the infection in the absence

of the test compound is indicative of a compound capable of inhibiting infection.

This invention still further provides a method for
5 identifying a compound capable of inhibiting the infection
of a cell by HCV, this cell being susceptible to infection
by HCV, the method comprising: (a) contacting a cell
expressing DC-SIGNR with a source of HCV for a time
sufficient to allow binding of HCV to the cell expressing
10 DC-SIGNR; (b) contacting the cell-bound HCV with a cell
susceptible to infection by HCV in the presence or absence
of a test compound for a time sufficient for infection in
the absence of the test compound; and (c) detecting
infection of the cell susceptible to infection by HCV,
15 wherein the absence of infection or the reduction of
infection in the presence of the test compound compared to
the infection in the absence of the test compound is
indicative of a compound capable of inhibiting infection.

Brief Description of the Figures

Figure 1. Amino acid sequence for *Homo sapiens* DC-SIGN as set forth in Genbank No. AAK20997 (SEQ ID NO:1).

5

Figure 2. Amino acid sequence for homo sapien DC-SIGNR as set forth in Genbank No. AAG13848 (SEQ ID NO:2).

10 Figure 3. Amino acid sequence for Hepatitis C Virus polyprotein gene as set forth in Genbank No. AF009606 (SEQ ID NO:3).

15 Figure 4. Characterization of HeLa-DC-SIGN and HeLa-DC-SIGN-R cell lines using antibodies specific for DC-SIGN (507(D)), DC-SIGN-R (604(L)), or both molecules (612(X)).

20 Figure 5. DC-SIGN and DC-SIGNR transfectants bind HCV-E2. (A) HeLa-DC-SIGN, (B) HeLa-DC-SIGNR and (C) parental HeLa cells were allowed to bind to HCV-E2-coated beads that were prepared by conjugation with a panel of anti-E2 mAbs. Adhesion was quantified by FACS analysis in the presence of adherence buffer (black shading), and was blocked by mannan (20 µg/ml) (no shading). Different anti-E2 mAbs are indicated on the X-axis and the Y-axis represents the percentage of cells that have bound beads as determined by histogram analysis. One representative experiment out of three is shown.

25 Figure 6. Effect of mAbs or soluble ICAMs on adhesion of HCV-E2 to DC-SIGN-R or DC-SIGN. HeLa cells expressing DC-SIGN-R or DC-SIGN were incubated with individual mAbs that bind the repeat region (DC6 and DC28) or the lectin-binding domain (612X, 604L and 507D) or soluble ICAM-Fc conjugates as described, and E2 beads added. Binding was quantified by fluorescence using a FACScan machine and results normalized to isotype control (mIgG) levels. One representative data set from three experiments is shown.

30

35

Figure 7. DC-SIGN-R and DC-SIGN bind to HCV virions from infected patients. HeLa transfectants or control cells were incubated with sera from three HCV RNA+ patients. HCV sera RNA titers (copies/ml) were: #1: 850,000, #2: 242,000 and
5 #3: 161,000 by COBAS MONITOR assay (Roche Molecular Systems). After washing, cells were lysed and RNA was extracted. HCV-RNA was measured by a qualitative RT-PCR and Southern blot assay 7(a) or by a quantitative real-time PCR assay 7(b). Data are presented as fold increase above HeLa
10 control cell binding for each matched sera, and absolute values (IU/ml) are depicted for each sample. Binding to DC-SIGN-R was inhibited by mannan. The cells were pre-incubated with mannan prior to addition of serum #2 as described above. Bound HCV RNA was extracted and analyzed
15 either by Southern blot 7(c) or by quantitative real-time PCR 7(d).

Figure 8. Inhibition of HCV virion binding to DC-SIGN-R and DC-SIGN by mannan. Cells were incubated with sera from
20 three HCV RNA+ patients as described in Fig. 5. HCV sera RNA titers (copies/ml) were: #4: 2,150,000, #5: 1,860,000 and #6: 1,160,000. Each serum was incubated with cells that had been pretreated with adherence buffer (black shading) or mannan (no shading), and bound RNA analyzed by real-time PCR
25 as described. Fold-increase above HeLa cell binding and absolute levels (IU/ml) are depicted.

Detailed Description of the Invention

This invention provides a method of inhibiting HCV infection of a cell susceptible to HCV infection which comprises
5 contacting the cell with an amount of a compound effective to inhibit binding of an HCV envelope glycoprotein to a DC-SIGN protein present on the surface of the cell, so as to thereby inhibit HCV infection of the cell susceptible to HCV infection. This invention provides a method of inhibiting
10 HCV infection of a cell susceptible to HCV infection which comprises contacting the cell with an amount of a compound effective to inhibit binding of an HCV envelope glycoprotein to a DC-SIGNR protein present on the surface of the cell, so as to thereby inhibit HCV infection of the cell susceptible
15 to HCV infection.

Cells which are susceptible to HCV infection may bind virus through DC-SIGN and/or DC-SIGNR molecules. In addition, cells which are not susceptible to HCV infection may bind
20 virus through DC-SIGN and/or DC-SIGNR molecules. Bound virus is then transmitted to a second susceptible target cell *in trans*. Accordingly, this invention provides a method of inhibiting the initial attachment of virus to a DC-SIGN and/or DC-SIGNR expressing, non-susceptible cell,
25 and then this results in the prevention of subsequent infection of the susceptible target cell. This invention provides a method of inhibiting HCV infection of a target cell whose susceptibility to HCV infection is increased when HCV binds to a second cell which is DC-SIGN protein
30 expressing cell, which method comprises contacting the DC-SIGN protein expressing cell with an amount of a compound effective to inhibit binding of an HCV envelope glycoprotein to a DC-SIGN protein, so as to thereby inhibiting HCV infection of the target cell. This invention provides a
35 method of inhibiting HCV infection of a target cell whose susceptibility to HCV infection is increased when HCV binds to a second cell which is a DC-SIGNR protein expressing

cell, which method comprises contacting the DC-SIGNR protein
expressing cell with an amount of a compound effective to
inhibit binding of an HCV envelope glycoprotein to a DC-SIGN
protein, so as to thereby inhibiting HCV infection of the
5 target cell.

This invention provides a method of inhibiting HCV infection
of a target cell which does not express a DC-SIGN and/or DC-
SIGNR receptor on its surface which comprises contacting a
10 second cell that does express a DC-SIGN and/or DC-SIGNR
receptor on its surface with an amount of a compound
described herein effective to inhibit binding of HCV to the
DC-SIGN and/or DC-SIGNR receptor so as to thereby inhibit
HCV infection of the first target cell in trans. In one
15 embodiment of this method, the target cell is present in a
subject and the contacting is effected by administering the
compound to the subject. In one embodiment, the target cell
which does not express the DC-SIGN and/or DC-SIGNR receptor
and the second cell which does express the DC-SIGN and/or
20 DC-SIGNR receptor are neighboring. In one embodiment, the
target cell and the second cell are adjacent. In another
embodiment, the target cell and the second cell are not
neighboring. In various embodiments, the target cell and
the second cell are less than 1 Å apart, at least 1 Å apart,
25 at least 10 Å apart, at least 100 Å apart, at least 1 nm
apart, at least 10 nm apart, at least 100 nm apart, at least
1 µm apart, at least 10 µm apart, at least 100 µm apart, at
least 1 mm apart, at least 1 cm apart, at least 10 cm apart,
and at least 1 m apart.

30

As used herein, "HCV" means the Hepatitis C Virus. HCV
includes but is not limited to extracellular virus particles
and the forms of HCV associated with and/or found in HCV
infected cells. As used herein, a "cell expressing an HCV
35 envelope glycoprotein on its surface" may also be denoted as
an "HCV envelope glycoprotein⁺ cell". As used herein, "HCV
infection" means the introduction of HCV genetic information

into a target cell, such as by fusion of the target cell membrane with HCV or an HCV envelope glycoprotein⁺ cell. The target cell may be a bodily cell of a subject. In one embodiment, the target cell is a bodily cell from a subject, 5 such as from a human subject. As used herein, "inhibiting HCV infection" means reducing the amount of HCV genetic information introduced into a target cell population as compared to the amount that would be introduced without, for example, an inhibiting agent. As used herein, "inhibits" 10 means that the amount is reduced as compared with the amount that would occur in a control sample. For example, a control sample may be one which does not contain the inhibiting agent and therefore, there would be no inhibition of HCV infection. In a preferred embodiment, inhibits means 15 that the amount is reduced 100%. As used herein, "fusion" means the joining or union of the lipid bilayer membranes found on mammalian cells or viruses such as HCV. This process is distinguished from the attachment of HCV to a target cell. Attachment is mediated by the binding of the 20 HCV exterior glycoprotein to a ligand present on the surface of a cell susceptible to HCV infection. As used herein, such ligand includes DC-SIGN and/or DC-SIGNR. As used herein, the fusion of cell membrane of the cell susceptible to HCV infection with HCV envelope glycoprotein⁺ cell 25 membrane means the hydrophobic joining and integration of the cell membrane of the infection susceptible cell with HCV envelope glycoprotein⁺ cell to form a hybrid membrane comprising components of both cell membranes. As used herein, "attachment" means the process that is mediated by 30 the binding of the HCV envelope glycoprotein to a ligand present on the surface of a cell susceptible to HCV infection. As used herein, "inhibiting fusion of an HCV envelope glycoprotein⁺ cell with a cell susceptible to HCV infection" means (a) reducing the rate of fusion of a cell 35 membrane of a cell susceptible to HCV infection with a cell membrane of an HCV envelope glycoprotein⁺ cell by at least 5%, or (b) reducing by at least 5% the total amount of

fusion of a cell membrane of a cell susceptible to HCV infection with an HCV envelope glycoprotein⁺ cell membrane occurring by the endpoint of fusion. As used herein, the rate of cell membrane fusion means the total quantity of cell membrane fused per unit of time. As used herein, the "endpoint of fusion" means the point in time at which all fusion of cell membranes of cells susceptible to HCV infection with HCV envelope glycoprotein⁺ cell membrane capable of occurring has occurred. As used herein, a "cell susceptible to HCV infection" may also be referred to as a "target cell" and includes cells capable of being infected by or fusing with HCV or HCV infected cells. As used herein, the word "cell" includes a biological cell, e.g., a HeLa cell, and a non-biological cell, e.g., a lipid vesicle (e.g., a phospholipid vesicle) or virion.

In one embodiment of the methods described herein, the compound is an antibody or portion of an antibody. In one embodiment, the antibody is a monoclonal antibody. In one embodiment, the antibody is a polyclonal antibody. In one embodiment, the antibody is a humanized antibody. In one embodiment, the antibody is a chimeric antibody. In one embodiment, the portion of the antibody comprises a light chain of the antibody. In one embodiment, the portion of the antibody comprises a heavy chain of the antibody. In one embodiment, the portion of the antibody comprises a Fab portion of the antibody. In one embodiment, the portion of the antibody comprises a F(ab')₂ portion of the antibody. In one embodiment, the portion of the antibody comprises an Fd portion of the antibody. In one embodiment, the portion of the antibody comprises an Fv portion of the antibody. In one embodiment, the portion of the antibody comprises a variable domain of the antibody. In one embodiment, the portion of the antibody comprises one or more CDR domains of the antibody.

In one embodiment of the methods described herein, the

compound is a polypeptide. In one embodiment, the compound is a peptide. In one embodiment, the compound is an oligopeptide.

- 5 In one embodiment of the methods described herein, the compound is a nonpeptidyl agent. In one embodiment, the nonpeptidyl agent is a carbohydrate. Such carbohydrate may be any carbohydrate known to one skilled in the art including but not limited to mannose, mannan or methyl- α -D-
- 10 mannopyranoside. In one embodiment of the methods described herein, the compound is a small molecule or small molecular weight molecule. In one embodiment, the compound has a molecular weight less than 500 daltons.
- 15 In one embodiment of the methods described herein, the HCV envelope glycoprotein is an HCV E1 envelope glycoprotein. In one embodiment of the methods described herein, the HCV envelope glycoprotein is an HCV E2 envelope glycoprotein.
- 20 In one embodiment of the methods described herein, the cell is present in a subject and the contacting is effected by administering the agent to the subject. Accordingly, the subject invention has various applications which include HCV treatment such as treating a subject who has become
- 25 afflicted with HCV. As used herein, "afflicted with HCV" means that the subject has at least one cell which has been infected by HCV. As used herein, "treating" means either slowing, stopping or reversing the progression of an HCV disorder. In the preferred embodiment, "treating" means
- 30 reversing the progression to the point of eliminating the disorder. As used herein, "treating" also means reducing the number of viral infections, reducing the number of infectious viral particles, reducing the number of virally infected cells, or ameliorating symptoms associated with
- 35 HCV. Another application of the subject invention is to prevent a subject from contracting HCV. As used herein, "contracting HCV" means becoming infected with HCV, whose

genetic information replicates in and/or incorporates into the host cells. Another application of the subject invention is to treat a subject who has become infected with HCV. As used herein, "HCV infection" means the introduction
5 of HCV genetic information into a target cell, such as by fusion of the target cell membrane with HCV or an HCV envelope glycoprotein⁺ cell. The target cell may be a bodily cell of a subject. In the preferred embodiment, the target cell is a bodily cell from a human subject. Another
10 application of the subject invention is to inhibit HCV infection. As used herein, "inhibiting HCV infection" means reducing the amount of HCV genetic information introduced into a target cell population as compared to the amount that would be introduced without said composition.

15 As for the amount of the compound and/or agent for administration to the subject, one skilled in the art would know how to determine the appropriate amount. As used herein, a dose or amount would be one in sufficient
20 quantities to either inhibit HCV infection, treat HCV infection, treat the subject or prevent the subject from becoming infected with HCV. This amount may be considered an effective amount. A person of ordinary skill in the art can perform simple titration experiments to determine what
25 amount is required to treat the subject. The dose of the composition of the invention will vary depending on the subject and upon the particular route of administration used. In one embodiment, the dosage can range from about 0.1 to about 100,000 µg/kg body weight of the subject.
30 Based upon the composition, the dose can be delivered continuously, such as by continuous pump, or at periodic intervals, for example, on one or more separate occasions. Desired time intervals of multiple doses of a particular composition can be determined without undue experimentation
35 by one skilled in the art.

In one embodiment of the methods described herein, the

effective amount of the compound is between about 1mg and about 50 mg per kg body weight of the subject. In one embodiment, the effective amount of the compound is between about 2 mg and about 40 mg per kg body weight of the subject. In one embodiment, the effective amount of the compound is between about 3 mg and about 30 mg per kg body weight of the subject. In one embodiment, the effective amount of the compound is between about 4 mg and about 20 mg per kg body weight of the subject. In one embodiment, the effective amount of the compound is between about 5 mg and about 10 mg per kg body weight of the subject. The effective amount of the compound may comprise from about 0.000001 mg/kg body weight to about 100 mg/kg body weight. In one embodiment, the effective amount may comprise from about 0.001 mg/kg body weight to about 50 mg/kg body weight. In another embodiment, the effective amount may range from about 0.01 mg/kg body weight to about 10 mg/kg body weight. The effective amount may be based upon, among other things, the size of the compound, the biodegradability of the compound, the bioactivity of the compound and the bioavailability of the compound. If the compound does not degrade quickly, is bioavailable and highly active, a smaller amount will be required to be effective. The effective amount will be known to one of skill in the art; it will also be dependent upon the form of the compound, the size of the compound and the bioactivity of the compound. One of skill in the art could routinely perform empirical activity tests for a compound to determine the bioactivity in bioassays and thus determine the effective amount. In one embodiment of the above methods, the effective amount of the compound comprises from about 1.0 ng/kg to about 100 mg/kg body weight of the subject. In another embodiment of the above methods, the effective amount of the compound comprises from about 100 ng/kg to about 50 mg/kg body weight of the subject. In another embodiment of the above methods, the effective amount of the compound comprises from about 1 µg/kg to about 10 mg/kg body weight of the subject. In

another embodiment of the above methods, the effective amount of the compound comprises from about 100 µg/kg to about 1 mg/kg body weight of the subject.

5 As for when the compound and/or agent is to be administered, one skilled in the art can determine when to administer such compound and/or agent. The administration may be constant for a certain period of time or periodic and at specific intervals. The compound may be delivered hourly, daily,
10 weekly, monthly, yearly (e.g., in a time release form) or as a one time delivery. The delivery may be continuous delivery for a period of time, e.g., intravenous delivery. In one embodiment of the methods described herein, the agent is administered at least once per day. In one embodiment of
15 the methods described herein, the agent is administered daily. In one embodiment of the methods described herein, the agent is administered every other day. In one embodiment of the methods described herein, the agent is administered every 6 to 8 days. In one embodiment of the
20 methods described herein, the agent is administered weekly.

As used herein, "subject" means any animal or artificially modified animal capable of becoming HCV-infected. The subjects include but are not limited to a human being, a
25 primate, an equine, an opine, an avian, a bovine, a porcine, a canine, a feline or a mouse. Artificially modified animals include, but are not limited to, SCID mice with human immune systems. The animals include but are not limited to mice, rats, dogs, guinea pigs, ferrets, rabbits,
30 and primates. In the preferred embodiment, the subject is a human being. The subject may be an "HCV-infected subject" which is a subject having at least one of his or her own cells invaded by HCV. In the preferred embodiment, the HCV infected subject is a human being. The subject may be a
35 "non-HCV-infected subject" which is a subject not having any of his own cells invaded by HCV. In the preferred embodiment, the non-HCV infected subject is a human being.

As used herein, "administering" may be effected or performed using any of the methods known to one skilled in the art. The compound may be administered by various routes including but not limited to aerosol, intravenous, oral or topical route. The administration may comprise intralesional, intraperitoneal, subcutaneous, intramuscular or intravenous injection; infusion; liposome-mediated delivery; topical, intrathecal, gingival pocket, per rectum, intrabronchial, nasal, transmucosal, intestinal, oral, ocular or otic delivery. In a further embodiment, the administration includes intrabronchial administration, anal, intrathecal administration or transdermal delivery. The compounds and/or agents of the subject invention may be delivered locally via a capsule which allows sustained release of the agent or the peptide over a period of time. Controlled or sustained release compositions include formulation in lipophilic depots (e.g., fatty acids, waxes, oils). Also comprehended by the invention are particulate compositions coated with polymers (e.g., poloxamers or poloxamines) and the agent coupled to antibodies directed against tissue-specific receptors, ligands or antigens or coupled to ligands of tissue-specific receptors. Other embodiments of the compositions of the invention incorporate particulate forms, protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including parenteral, pulmonary, nasal and oral.

The carrier may be a diluent, an aerosol, a topical carrier, an aqueous solution, a nonaqueous solution or a solid carrier.

This invention provides a method of treating HCV infection in a subject which comprises inhibiting HCV infection of the subject's cells susceptible to HCV infection by a method described herein, wherein the contacting is effected by administering the compound to the subject. This invention provides a method of preventing HCV infection of a subject

which comprises inhibiting HCV infection of the subject's cells susceptible to HCV infection by a method described herein, wherein the contacting is effected by administering the compound to the subject. This invention provides a method of preventing a cell or cells of a subject from becoming infected with HCV which comprises administering to the subject an amount of one of the compounds described herein effective to inhibit binding of HCV to DC-SIGN and/or DC-SIGNR receptors on the surface of the subject's cells so as to thereby prevent the subject's cell or cells from becoming infected with HCV. This invention provides a method of treating a subject whose cells are infected with HCV which comprises administering to the subject an amount of one of the compounds described herein effective to inhibit binding of HCV to DC-SIGN and/or DC-SIGNR receptors on the surface of the subject's cells so as to thereby treat the subject. In a preferred embodiment, the subject is a human. In another embodiment, the subject is a SCID-BNX mouse (Galun et al., J. Inf. Dis. 172: 25-34, 1995).

In one embodiment of the above methods, the subject is infected with HCV prior to administering the compound to the subject. In one embodiment of the above methods, the subject is not infected with HCV prior to administering the compound to the subject. In one embodiment of the above methods, the subject is not infected with, but has been exposed to, HCV.

In one embodiment of the methods described herein, the cell susceptible to HCV infection is a primary cell. In one embodiment, the cell is a dendritic cell, placental cell or endometrial cell. In one embodiment, the cell is a liver cell, lymph node cell, endometrial cell in liver or placenta cell. In one embodiment of the methods described herein, the cell susceptible to HCV infection is a eukaryotic cell. In one embodiment of the methods described herein, the cell susceptible to HCV infection is a human cell. In one

embodiment of the methods described herein, the cell susceptible to HCV infection is a peripheral blood mononuclear cell. In one embodiment of the methods described herein, the cell susceptible to HCV infection is a
5 HeLa cell. In one embodiment of the methods described herein, the cell susceptible to HCV infection is a hepatic cell. A hepatic cell may include but is not limited to a HepG2 cell, SK-HEP1 cell, C3A cell or an Huh-7 cell. In one embodiment, the hepatic cell is a primary hepatic cell.

10

This invention provides a method of treating a subject afflicted with HCV which comprises administering to the subject an effective dose of an agent or composition described herein. In one embodiment, the agent or
15 composition may be enough to decrease the subject's viral load. As used herein, "treating" means either slowing, stopping or reversing the progression of an HCV disorder. In the preferred embodiment, "treating" means reversing the progression to the point of eliminating the disorder. As
20 used herein, "treating" also means reducing the number of viral infections, reducing the number of infectious viral particles, reducing the number of virally infected cells, or ameliorating symptoms associated with HCV. As used herein, "afflicted with HCV" means that the subject has at least one
25 cell which has been infected by HCV.

This invention provides a method of preventing a subject from contracting HCV which comprises administering to the subject an effective dose of an agent or composition
30 described herein.

This invention provides a use of a compound and/or agent described herein, such as an antibody or portion thereof, peptide, polypeptide or oligopeptide, or nonpeptidyl agent
35 for the preparation of a pharmaceutical composition for inhibiting HCV infection of a cell susceptible to HCV infection. This invention provides a use of a compound

and/or agent described herein, such as an antibody or portion thereof, peptide, polypeptide or oligopeptide, or nonpeptidyl agent for the preparation of a pharmaceutical composition for treating HCV infection in a subject. This invention provides a use of a compound and/or agent described herein, such as an antibody or portion thereof, peptide, polypeptide or oligopeptide, or nonpeptidyl agent for the preparation of a pharmaceutical composition for preventing HCV infection in a subject.

10

This invention provides a method of determining whether a compound is capable of inhibiting HCV infection of a cell which comprises: (a) immobilizing an HCV envelope glycoprotein on a solid support; (b) contacting the immobilized HCV envelope glycoprotein with sufficient detectable DC-SIGN protein to saturate all binding sites for the DC-SIGN protein on the immobilized HCV envelope glycoprotein under conditions permitting binding of the DC-SIGN protein to the immobilized HCV envelope glycoprotein so as to form a complex; (c) removing unbound DC-SIGN protein; (d) contacting the complex with the compound; and (e) determining whether any DC-SIGN protein is displaced from the complex, wherein displacement of DC-SIGN protein from the complex indicates that the compound binds to the HCV envelope glycoprotein, so as to thereby determine that the compound is one which is capable of inhibiting HCV infection of the cell.

This invention provides a method of determining whether a compound is capable of inhibiting HCV infection of a cell which comprises: (a) immobilizing an HCV envelope glycoprotein on a solid support; (b) contacting the immobilized HCV envelope glycoprotein with sufficient detectable DC-SIGNR protein to saturate all binding sites for the DC-SIGNR protein on the immobilized HCV envelope glycoprotein under conditions permitting binding of the DC-SIGNR protein to the immobilized HCV envelope glycoprotein

so as to form a complex; (c) removing unbound DC-SIGNR protein; (d) contacting the complex with the compound; (e) determining whether any DC-SIGNR protein is displaced from the complex, wherein displacement of DC-SIGNR protein from the complex indicates that the compound binds to the HCV envelope glycoprotein, so as to thereby determine that the compound is one which is capable of inhibiting HCV infection of the cell.

10 This invention provides a method of determining whether a compound is capable of inhibiting HCV infection of a cell which comprises: (a) immobilizing a DC-SIGN protein on a solid support; (b) contacting the immobilized DC-SIGN protein with sufficient detectable HCV envelope glycoprotein to saturate all binding sites for the HCV envelope glycoprotein on the immobilized DC-SIGN protein under conditions permitting binding of the immobilized DC-SIGN protein to the HCV envelope glycoprotein so as to form a complex; (c) removing unbound HCV envelope glycoprotein; (d) contacting the complex with the compound; (e) determining whether any HCV envelope glycoprotein is displaced from the complex, wherein displacement of HCV envelope glycoprotein from the complex indicates that the compound binds to the DC-SIGN protein, so as to thereby determine that the compound is one which is capable of inhibiting HCV infection of the cell.

This invention provides a method of determining whether a compound is capable of inhibiting HCV infection of a cell which comprises: (a) immobilizing a DC-SIGNR protein on a solid support; (b) contacting the immobilized DC-SIGNR protein with sufficient detectable HCV envelope glycoprotein to saturate all binding sites for the HCV envelope glycoprotein on the immobilized DC-SIGNR protein under conditions permitting binding of the immobilized DC-SIGNR protein to the HCV envelope glycoprotein so as to form a complex; (c) removing unbound HCV envelope glycoprotein; (d)

contacting the complex with the compound; (e) determining whether any HCV envelope glycoprotein is displaced from the complex, wherein displacement of HCV envelope glycoprotein from the complex indicates that the compound binds to the DC-SIGNR protein, so as to thereby determine that the compound is one which is capable of inhibiting HCV infection of the cell.

This invention provides a method of determining whether a compound is capable of inhibiting HCV infection of a cell which comprises: (a) contacting an HCV envelope glycoprotein with sufficient detectable DC-SIGN protein to saturate all binding sites for the DC-SIGN protein on the HCV envelope glycoprotein under conditions permitting binding of the DC-SIGN protein to the HCV envelope glycoprotein so as to form a complex; (b) removing unbound DC-SIGN protein; (c) measuring the amount of DC-SIGN protein which is bound to the HCV envelope glycoprotein in the complex; (d) contacting the complex with the compound so as to displace DC-SIGN protein from the complex; (e) measuring the amount of DC-SIGN protein which is bound to the compound in the presence of the compound; and (f) comparing the amount of DC-SIGN protein bound to the HCV envelope glycoprotein in step (e) with the amount measured in step (c), wherein a reduced amount measured in step (e) indicates that the compound binds to the HCV envelope glycoprotein, so as to thereby determine that the compound is one which is capable of inhibiting HCV infection of the cell.

This invention provides a method of determining whether a compound is capable of inhibiting HCV infection of a cell which comprises: (a) contacting an HCV envelope glycoprotein with sufficient detectable DC-SIGNR protein to saturate all binding sites for the DC-SIGNR protein on the HCV envelope glycoprotein under conditions permitting binding of the DC-SIGNR protein to the HCV envelope glycoprotein so as to form a complex; (b) removing unbound DC-SIGNR protein; (c)

measuring the amount of DC-SIGNR protein which is bound to the HCV envelope glycoprotein in the complex; (d) contacting the complex with the compound so as to displace DC-SIGNR protein from the complex; (e) measuring the amount of DC-SIGNR protein which is bound to the compound in the presence of the compound; and (f) comparing the amount of DC-SIGNR protein bound to the HCV envelope glycoprotein in step (e) with the amount measured in step (c), wherein a reduced amount measured in step (e) indicates that the compound binds to the HCV envelope glycoprotein so as to thereby identify the compound as one which is capable of inhibiting HCV infection of a cell.

This invention provides a method of determining whether a compound is capable of inhibiting HCV infection of a cell which comprises: (a) immobilizing an HCV envelope glycoprotein on a solid support; (b) contacting the immobilized HCV envelope glycoprotein with the compound and detectable DC-SIGN protein under conditions permitting binding of the DC-SIGN protein to the immobilized HCV envelope glycoprotein so as to form a complex; (c) removing unbound DC-SIGN protein; (d) comparing the amount of detectable DC-SIGN protein which is bound to the immobilized HCV envelope glycoprotein in the complex in the presence of the compound with the amount of detectable DC-SIGN protein which binds to the immobilized HCV envelope glycoprotein in the absence of the compound; (e) wherein a reduced amount of DC-SIGN protein measured in the presence of the compound indicates that the compound binds to the HCV envelope glycoprotein or the DC-SIGN protein, so as to thereby determine that the compound is one which is capable of inhibiting HCV infection of the cell.

In one embodiment of the methods described herein, the amount of the detectable DC-SIGN is sufficient to saturate all binding sites for the DC-SIGN protein on the HCV envelope glycoprotein.

This invention provides a method of determining whether a compound is capable of inhibiting HCV infection of a cell which comprises: (a) immobilizing an HCV envelope glycoprotein on a solid support; (b) contacting the
5 immobilized HCV envelope glycoprotein with the compound and detectable DC-SIGNR protein under conditions permitting binding of the DC-SIGNR protein to the immobilized HCV envelope glycoprotein so as to form a complex; (c) removing unbound DC-SIGNR protein; (d) comparing the amount of
10 detectable DC-SIGNR protein which is bound to the immobilized HCV envelope glycoprotein in the complex in the presence of the compound with the amount of detectable DC-SIGNR protein which binds to the immobilized HCV envelope glycoprotein in the absence of the compound; (e) wherein a
15 reduced amount of DC-SIGNR protein measured in the presence of the compound indicates that the compound binds to the HCV envelope glycoprotein or the DC-SIGNR protein, so as to thereby determine that the compound is one which is capable of inhibiting HCV infection of the cell.

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In one embodiment of the methods described herein, the amount of the detectable DC-SIGNR is sufficient to saturate all binding sites for the DC-SIGNR protein on the HCV envelope glycoprotein.

25

This invention provides a method of determining whether a compound is capable of inhibiting HCV infection of a cell which comprises: (a) immobilizing a DC-SIGN protein on a solid support; (b) contacting the immobilized DC-SIGN
30 protein with the compound and detectable HCV envelope glycoprotein under conditions permitting binding of the immobilized DC-SIGN protein to the HCV envelope glycoprotein so as to form a complex; (c) removing unbound HCV envelope glycoprotein; (d) comparing the amount of detectable HCV
35 envelope glycoprotein which is bound to the immobilized DC-SIGN protein in the complex in the presence of the compound with the amount of detectable HCV envelope glycoprotein

which binds to the immobilized DC-SIGN protein in the absence of the compound; (e) wherein a reduced amount of HCV envelope glycoprotein measured in the presence of the compound indicates that the compound binds to the HCV envelope glycoprotein or the DC-SIGN protein, so as to thereby determine that the compound is one which is capable of inhibiting HCV infection of the cell.

In one embodiment of the methods described herein, the amount of the detectable HCV envelope glycoprotein is sufficient to saturate all binding sites for the HCV envelope glycoprotein on the DC-SIGN protein.

This invention provides a method of determining whether a compound is capable of inhibiting HCV infection of a cell which comprises: (a) immobilizing a DC-SIGNR protein on a solid support; (b) contacting the immobilized DC-SIGNR protein with the compound and detectable HCV envelope glycoprotein under conditions permitting binding of the immobilized DC-SIGNR protein to the HCV envelope glycoprotein so as to form a complex; (c) removing unbound HCV envelope glycoprotein; (d) comparing the amount of detectable HCV envelope glycoprotein which is bound to the immobilized DC-SIGNR protein in the complex in the presence of the compound with the amount of detectable HCV envelope glycoprotein which binds to the immobilized DC-SIGNR protein in the absence of the compound; (e) wherein a reduced amount of HCV envelope glycoprotein measured in the presence of the compound indicates that the compound binds to the HCV envelope glycoprotein or the DC-SIGNR protein, so as to thereby determine that the compound is one which is capable of inhibiting HCV infection of the cell.

In one embodiment of the methods described herein, the amount of the detectable HCV envelope glycoprotein is sufficient to saturate all binding sites for the HCV envelope glycoprotein on the DC-SIGNR protein.

This invention provides a method of determining whether a compound is capable of inhibiting HCV infection of a cell which comprises: (a) contacting an HCV envelope glycoprotein with the compound and detectable DC-SIGN protein under
5 conditions permitting binding of the DC-SIGN protein to the HCV envelope glycoprotein so as to form a complex; (b) removing unbound DC-SIGN protein; (c) comparing the amount of detectable DC-SIGN protein which is bound to the HCV envelope glycoprotein in the complex in the presence of the
10 compound with the amount of detectable DC-SIGN protein which binds to the compound in the absence of the compound; wherein a reduced amount of DC-SIGN protein measured in presence of the compound indicates that the compound binds to the HCV envelope glycoprotein or DC-SIGN protein so as to
15 thereby determine that the compound is one which is capable of inhibiting HCV infection of the cell.

In one embodiment of the methods described herein, the amount of the detectable DC-SIGN protein is sufficient to
20 saturate all binding sites for the DC-SIGN protein on the HCV envelope glycoprotein.

This invention provides a method of determining whether a compound is capable of inhibiting HCV infection of a cell
25 which comprises: (a) contacting an HCV envelope glycoprotein with the compound and detectable DC-SIGNR protein under conditions permitting binding of the DC-SIGNR protein to the HCV envelope glycoprotein so as to form a complex; (b) removing unbound DC-SIGNR protein; (c) comparing the amount
30 of detectable DC-SIGNR protein which is bound to the HCV envelope glycoprotein in the complex in the presence of the compound with the amount of detectable DC-SIGNR protein which binds to the compound in the absence of the compound; wherein a reduced amount of DC-SIGNR protein measured in
35 presence of the compound indicates that the compound binds to the HCV envelope glycoprotein or DC-SIGNR protein so as to thereby determine that the compound is one which is

capable of inhibiting HCV infection of the cell.

In one embodiment of the methods described herein, the amount of the detectable DC-SIGNR protein is sufficient to
5 saturate all binding sites for the DC-SIGNR protein on the HCV envelope glycoprotein.

In the methods described herein, an entity may be made detectable by labeling it with a detectable marker. For
10 example, in one embodiment of the methods described herein, the detectable DC-SIGN protein is labeled with a detectable marker. In one embodiment of the methods described herein, the detectable DC-SIGNR protein is labeled with a detectable marker. In one embodiment of the methods described herein,
15 the detectable HCV envelope glycoprotein is labeled with a detectable marker. One skilled in the art would know various types of detectable markers. Such detectable markers include but are not limited to radioactive, colorimetric, luminescent and fluorescent markers.

20

This invention provides a method of identifying an agent which inhibits binding of HCV to DC-SIGN which comprises:
(a) immobilizing one or both of the HCV envelope glycoproteins on a solid support; (b) contacting the result
25 from step (a) with the agent; (c) contacting the result from step (c) with a detectable form of DC-SIGN protein under conditions that permit binding of the detectable DC-SIGN protein in the absence of the compound; (d) detecting the amount of bound detectable DC-SIGN protein, wherein a
30 reduction of the amount of bound detectable DC-SIGN protein compared to an amount bound in the absence of the agent thereby identifies the agent as one which inhibits binding of HCV to the DC-SIGN.

35 This invention provides a method of identifying an agent which inhibits binding of HCV to DC-SIGNR which comprises:
(a) immobilizing one or both of the HCV envelope

glycoproteins on a solid support; (b) contacting the result from step (a) with the agent; (c) contacting the result from step (b) with a detectable form of DC-SIGNR protein under conditions that permit binding of the detectable DC-SIGNR protein in the absence of the compound; detecting the amount of bound detectable DC-SIGNR protein, wherein a reduction of the amount of bound detectable DC-SIGNR protein compared to an amount bound in the absence of the agent thereby identifies the agent as one which inhibits binding of HCV to the DC-SIGNR.

This invention provides a method of identifying an agent which inhibits binding of HCV to DC-SIGN which comprises: (a) immobilizing a DC-SIGN protein on a solid support; (b) contacting the result from step (a) with the agent; (c) contacting the result from step (b) with a detectable form of one or more of the HCV envelope glycoproteins under conditions that permit binding of the detectable HCV envelope glycoprotein(s) in the absence of the compound; (d) detecting the amount of bound detectable HCV envelope glycoprotein(s), wherein a reduction of the amount of bound detectable HCV envelope glycoprotein(s) compared to an amount bound in the absence of the agent thereby identifies the agent as one which inhibits binding of HCV to the DC-SIGN.

This invention provides a method of identifying an agent which inhibits binding of HCV to DC-SIGNR which comprises: (a) immobilizing a DC-SIGNR protein on a solid support; (b) contacting the result from step (a) with the agent; (c) contacting the result from step (c) with a detectable form of one or more of the HCV envelope glycoproteins under conditions that permit binding of the detectable HCV envelope glycoprotein(s) in the absence of the compound; (d) detecting the amount of bound detectable HCV envelope glycoprotein(s), wherein a reduction of the amount of bound detectable HCV envelope glycoprotein(s) compared to an

amount bound in the absence of the agent thereby identifies the agent as one which inhibits binding of HCV to the DC-SIGNR.

5 In one embodiment of the method described herein, the solid support is a microtiter plate well. In another embodiment, the solid support is a bead. In a further embodiment, the solid support is a surface plasmon resonance sensor chip. The surface plasmon resonance sensor chip can have pre-immobilized streptavidin. In one embodiment, the surface
10 plasmon resonance sensor chip is a BIAcore™ chip.

In one embodiment of the above methods, the detectable molecule is labeled with a detectable marker. In another
15 embodiment of the above methods, the detectable molecule is detected by contacting it with another compound which is both capable of binding the detectable molecule and is detectable. The detectable markers include those described above.

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As used herein, the terms "agent" and "compound" include both protein and non-protein moieties. In one embodiment, the agent/compound is a small molecule. In another embodiment, the agent/compound is a protein. The protein
25 may be, by way of example, an antibody directed against a portion of an HCV envelope glycoprotein. The agent/compound may be derived from a library of low molecular weight compounds or a library of extracts from plants or other organisms. In an embodiment, the agent is known. In a
30 separate embodiment, the agent/compound is not previously known. The agents/compounds of the subject invention include but are not limited to compounds or molecular entities such as peptides, polypeptides, and other organic or inorganic molecules and combinations thereof.

35

Compounds of the present invention inhibit HCV infection of cells susceptible to HCV infection. The compounds of the

present invention preferable have specificity for preventing or inhibiting infection by HCV and do not inhibit infection by other viruses, such as HIV, that may utilize DC-SIGN or DC-SIGNR for infection. Moreover the compounds of the present invention preferably do not interfere or inhibit members of the immunoglobulin superfamily; in particular, the compounds do not interfere with ICAM-2 or ICAM-3 or with ICAM-2-like, or ICAM-3-like molecules.

As used herein, the terms "agent" and "compound" may be used interchangeably. In one embodiment of the methods described herein, the agent is an antibody or a portion of an antibody. In one embodiment of the antibody, the antibody is a monoclonal antibody. In one embodiment of the antibody, the antibody is a polyclonal antibody. In one embodiment of the antibody, the antibody is a humanized antibody. In one embodiment of the antibody, the antibody is a chimeric antibody. The portion of the antibody may comprise a light chain of the antibody. The portion of the antibody may comprise a heavy chain of the antibody. The portion of the antibody may comprise an Fab portion of the antibody. The portion of the antibody may comprise an F(ab')₂ portion of the antibody. The portion of the antibody may comprise an Fd portion of the antibody. The portion of the antibody may comprise a Fv portion of the antibody. The portion of the antibody may comprise a variable domain of the antibody. The portion of the antibody may comprise one or more CDR domains of the antibody.

In one embodiment of the methods described herein, the agent is a polypeptide. In one embodiment of the methods described herein, the agent is a oligopeptide. In one embodiment of the methods described herein, the agent is a nonpeptidyl agent. In one embodiment, the nonpeptidyl agent is a compound having a molecular weight less than 500 daltons.

This invention provides a method of obtaining a composition which comprises: (a) identifying a compound which inhibits HCV infection of a cell according to a method described herein; and (b) admixing the compound so identified or a
5 homolog or derivative thereof with a carrier, so as to thereby obtain a composition.

This invention provides a method of obtaining a composition which comprises: (a) identifying a compound which inhibits
10 binding of HCV to DC-SIGN according to one of the methods described herein; and (b) admixing the compound so identified or a homolog or derivative thereof with a carrier.

15 This invention provides a method of obtaining a composition which comprises: (a) identifying a compound which inhibits binding of HCV to DC-SIGNR according to one of the above methods; and (b) admixing the compound so identified or a homolog or derivative thereof with a carrier.

20

In one embodiment of these methods of obtaining a composition, this method further comprises recovering the identified compound before it is admixed with the carrier.

25 This invention provides a method of treating or preventing a liver disease in a subject which comprises administering to the subject an effective amount of a compound capable of inhibiting binding of an HCV envelope glycoprotein to a DC-SIGN protein present on the surface of the subject's cells,
30 so as to thereby treat or prevent the liver disease in a subject. This invention provides a method of treating or preventing a liver disease in a subject which comprises administering to the subject an effective amount of a compound capable of inhibiting binding of an HCV envelope
35 glycoprotein to a DC-SIGNR protein present on the surface of the subject's cells, so as to thereby treat or prevent the liver disease in a subject. In one embodiment of the

methods described herein, the liver disease is hepatitis. In one embodiment of the methods described herein, the liver disease is cirrhosis.

5 This invention provides a method of treating or preventing hepatocellular carcinoma in a subject which comprises administering to the subject an effective amount of a compound capable of inhibiting binding of an HCV envelope glycoprotein to a DC-SIGN protein present on the surface of
10 the subject's cells, so as to thereby treat or prevent hepatocellular carcinoma in a subject. This invention provides a method of treating or preventing hepatocellular carcinoma in a subject which comprises administering to the subject an effective amount of the compound capable of
15 inhibiting binding of an HCV envelope glycoprotein to a DC-SIGNR protein present on the surface of the subject's cells, so as to thereby treat or prevent hepatocellular carcinoma in a subject.

20 This invention provides a method of diagnosing HCV infection of a subject which comprises: (a) immobilizing a DC-SIGN protein on a solid support; (b) contacting the immobilized DC-SIGN protein with sufficient detectable HCV envelope glycoprotein to saturate all binding sites for the HCV
25 envelope glycoprotein on the immobilized DC-SIGN protein so as to form a complex; (c) removing unbound HCV envelope glycoprotein; (d) contacting the complex with a suitable sample obtained from the subject; and (e) detecting whether any HCV envelope glycoprotein is displaced from the complex,
30 wherein displacement of the HCV envelope glycoprotein from the complex indicates the presence of anti-HCV antibodies present in the sample, so as to thereby diagnose HCV infection of the subject.

35 This invention provides a method of diagnosing HCV infection of a subject which comprises: (a) immobilizing a DC-SIGNR protein on a solid support; (b) contacting the immobilized

DC-SIGNR protein with sufficient detectable HCV envelope glycoprotein to saturate all binding sites for the HCV envelope glycoprotein on the immobilized DC-SIGNR protein so as to form a complex; (c) removing unbound HCV envelope glycoprotein; (d) contacting the complex with a suitable sample obtained from the subject; and (e) detecting whether any HCV envelope glycoprotein is displaced from the complex, wherein displacement of the HCV envelope glycoprotein from the complex indicates the presence of anti-HCV antibodies present in the sample, so as to thereby diagnose HCV infection of the subject.

This invention provides a method of diagnosing HCV infection of a subject which comprises: (a) contacting DC-SIGN protein with sufficient detectable HCV envelope glycoprotein to saturate all binding sites for the HCV envelope glycoprotein on the DC-SIGN protein so as to form a complex; (b) removing unbound HCV envelope glycoprotein; (c) contacting the complex with a suitable sample obtained from the subject; and (d) detecting whether any HCV envelope glycoprotein is displaced from the complex, wherein displacement of the HCV envelope glycoprotein from the complex indicates the presence of anti-HCV antibodies present in the sample, so as to thereby diagnose HCV infection of the subject.

This invention provides a method of diagnosing HCV infection of a subject which comprises: (a) contacting DC-SIGNR protein with sufficient detectable HCV envelope glycoprotein to saturate all binding sites for the HCV envelope glycoprotein on the DC-SIGNR protein so as to form a complex; (b) removing unbound HCV envelope glycoprotein; (c) contacting the complex with a suitable sample obtained from the subject; and (d) detecting whether any HCV envelope glycoprotein is displaced from the complex, wherein displacement of the HCV envelope glycoprotein from the complex indicates the presence of anti-HCV antibodies present in the sample, so as to thereby diagnose HCV

infection of the subject.

The ability of a DC-SIGN protein, a DC-SIGNR protein or functional equivalent thereof to bind to HCV permits the use
5 of the protein as a diagnostic for HCV infection, for example in an ELISA (Enzyme linked immunosorbent assay). In one embodiment, a soluble form of a DC-SIGN protein and/or a DC-SIGNR protein could be used to detect serum antibodies to HCV. In a preferred embodiment, the DC-SIGN protein and/or
10 DC-SIGNR protein or functional equivalent thereof is immobilized on a solid support and contacted with the HCV envelope glycoprotein(s), which may be an E1 HCV envelope glycoprotein, an E2 HCV envelope glycoprotein, or both. The contacting may occur in the presence or absence of serum or
15 serum antibodies. In an assay of this form, competitive binding between antibodies and the HCV glycoprotein(s) for binding to the immobilized protein thereof results in the bound HCV protein being a measure of antibodies in the serum sample, most particularly. The amount of bound HCV
20 glycoprotein(s) is then detected. The HCV glycoprotein(s) may be labeled with radioactive, enzymatic, biotin, fluorescent or other detectable marker to facilitate detection.

25 This invention provides methods of diagnosing HCV infection in a subject employing a method known to one skilled in the art, including but not limited to a sandwich assay and a competition assay. For example, one embodiment of a sandwich assay is as follows: (1) obtain a suitable sample
30 of DC-SIGN and/or DC-SIGNR protein; (2) contact the DC-SIGN and/or DC-SIGNR protein with an HCV envelope glycoprotein, so as to form a complex; (3) obtain a suitable sample from the subject and contact the HCV envelope glycoprotein with the sample, under conditions permitting formation of a
35 complex between the HCV envelope glycoprotein and any anti-HCV envelope glycoprotein antibodies present in the subject's sample; (4) contacting the bound anti-HCV envelope

glycoprotein antibodies with detectable anti-human IgG antibodies, which would bind to any bound anti-HCV envelope glycoprotein antibodies; and (5) detecting the anti-human IgG antibodies, wherein the presence of such antibodies
5 indicates that the subject is HCV infected.

For example, one embodiment of a competition assay is as follows: (1) obtaining a suitable sample of DC-SIGN and/or DC-SIGNR protein; (2) contacting the DC-SIGN and/or DC-SIGNR protein with an HCV envelope glycoprotein, so as to form a complex; (3) contacting the HCV envelope glycoprotein with a sample from the subject, under conditions permitting binding between any anti-HCV antibodies present in the sample and the HCV envelope glycoprotein; (4) also contacting the HCV
10 envelope glycoprotein with detectable anti-HCV envelope glycoprotein antibodies, under conditions permitting binding between the detectable anti-HCV envelope glycoprotein antibodies and the HCV envelope glycoprotein; and (5) determining the amount of detectable anti-HCV envelope
15 glycoprotein antibodies bound, compared with the amount bound in the absence of any sample from the subject, wherein an increased amount measured in the absence of the sample indicates that the subject is HCV infected.

25 In one embodiment of the methods and assays described herein, the sample from the subject is a serum sample. In one embodiment, the DC-SIGN and/or DC-SIGNR protein is immobilized. The above methods may include wash steps so as to wash unbound compounds including but not limited to
30 unbound HCV envelope glycoprotein, unbound sample from the subject, unbound anti-HCV envelope glycoprotein antibodies, and unbound detectable anti-human IgG antibodies. In one embodiment, the detectable anti-human IgG antibodies are labeled with a detectable marker. In one embodiment, the
35 detectable anti-HCV envelope antibodies are labeled with a detectable marker. In one embodiment, the amount of anti-human IgG antibodies detected is compared with an amount

measured in the absence of HCV envelope glycoprotein, so as to determine a baseline measurement.

5 This invention provides an article of manufacture comprising a solid support having operably affixed thereto an agent capable of specifically forming a complex with a domain present on an HCV envelope glycoprotein.

10 The solid support may be any solid support known in the art to which the agent can be operably affixed. Solid supports include, by way of example, natural or synthetic polymers. Synthetic polymers include, by way of example, polystyrene, polyethylene and polypropylene. Natural polymers include, by way of example, latex. The solid support may be
15 selected, for example, from the group consisting of a bead, a receptacle, and a filter. Solid supports in the form of beads are widely used and readily available to those skilled in the art. Beads include, for example, latex and polystyrene beads.

20

The receptacle can be any receptacle in which a bodily fluid is stored, or with which such fluid comes into contact. For example, the receptacle may be in the form of a bag or tubing. In the preferred embodiment, the receptacle is a
25 bag specifically intended for the collection and/or storage of blood or blood components.

Solid supports in the form of filters are widely used and readily available to those skilled in the art. Filters
30 include, for example, polyester filters (e.g., polyester leukofiltration devices) and cellulose acetate filters.

The agent affixed to the solid support may either be a protein or a non-protein agent. In one embodiment, the
35 agent is DC-SIGN and/or DC-SIGNR. In one embodiment, the agent is an antibody or portion. Such antibody may be one which is capable of binding to an HCV envelope glycoprotein.

As used herein, "operably affixed" means affixed in a manner permitting the formation of a complex between the affixed agent and the domain present on an HCV envelope glycoprotein. Methods of operably affixing an agent to a solid support are well known to those skilled in the art.

As used herein, "capable of specifically forming a complex with a domain present on an HCV envelope glycoprotein" means capable of forming a complex with a domain present on an HCV envelope glycoprotein but not capable of forming a complex with any other domain.

In one embodiment, the domain present on the HCV envelope glycoprotein is a conserved domain. As used herein, a "conserved domain" is an envelope glycoprotein domain which is present on, and whose structure is invariant among, at least 90% of all strains of HCV. In the preferred embodiment, the conserved domain present on the HCV envelope glycoprotein is the DC-SIGN and/or DC-SIGNR-binding domain of the HCV envelope glycoprotein. In another embodiment, the domain present on the HCV envelope glycoprotein is a non-conserved domain.

This invention further provides an article of manufacture comprising a solid support having operably affixed thereto a plurality of agents each capable of specifically forming a complex with a domain present on an HCV envelope glycoprotein.

30

As used herein, a "plurality of agents" means at least two agents. In one embodiment, the plurality of agents consists of a plurality of DC-SIGN and/or DC-SIGNR-based molecules. In another embodiment, the plurality of agents consists of a plurality of antibodies. In a further embodiment, the plurality of agents comprises an antibody and a DC-SIGN and/or DC-SIGNR-based molecule.

This invention further provides an aqueous-soluble agent which (a) is capable of specifically forming a complex with a domain present on an HCV envelope glycoprotein, and (b) comprises a moiety capable of specifically forming a complex with a known ligand, which moiety permits the removal of the agent from a sample via contact with an immobilized form of the known ligand. As used herein, "aqueous-soluble" means capable of existing in soluble form in water at 4°C at a concentration of at least 1 pM.

10

The use of a moiety capable of specifically forming a complex with a known ligand is commonly referred to in the art as "molecular tagging." The moiety may be selected, for example, from the group consisting of a small molecule and a protein. The ligand includes but is not limited to for example, a metal ion, a small molecule, a peptide or a protein. Specific examples of moiety/ligand combinations include, but are not limited to, (a) oligohistidine/nickel ion, (b) glutathione-S-transferase/glutathione, (c) biotin/streptavidin, and (d) the HA peptide YPYDVPDYA/anti-HA peptide antibody. The moiety may be attached by any means known to one skilled in the art, such as for example, chemically or genetically.

25 This invention further provides a method of treating a bodily fluid sample so as to remove therefrom HCV or HCV envelope glycoprotein if present in the sample which comprises contacting the sample under suitable conditions with an article of manufacture comprising a solid support having operably affixed thereto an agent capable of specifically forming a complex with a domain present on an HCV envelope glycoprotein, thereby removing therefrom HCV or HCV envelope glycoprotein if present in the sample.

35 As used herein, "treating a bodily fluid sample so as to remove therefrom HCV" means either (a) rendering the HCV in the bodily fluid sample unable to invade target cells, such

as those expressing DC-SIGN and/or DC-SIGNR, (b) physically separating HCV from the bodily fluid sample, or (c) a combination of (a) and (b), with the proviso that the HCV present in the resulting sample and capable of invading
5 target cells does not exceed 50% of the amount of such HCV present in the sample prior to removing HCV. As used herein, a target cell includes a cell having DC-SIGN and/or DC-SIGNR present on its surface, wherein the DC-SIGN and/or DC-SIGNR expressing cell is capable of specifically binding
10 to and fusing with HCV contacted therewith.

Suitable conditions for contacting the sample with the subject article of manufacture are conditions which would permit the formation of a complex between the agent and HCV.
15 Such conditions are known to those skilled in the art.

This invention further provides a method of treating a bodily fluid sample so as to substantially reduce the likelihood of a subject's becoming infected with HCV as a
20 result of contact with the sample which comprises contacting the sample with a suitable amount of an aqueous-soluble agent capable of specifically forming a complex with a domain present on an HCV envelope glycoprotein, so as to form a complex between the agent and HCV if present in the
25 sample and thereby reduce the likelihood of a subject's becoming infected with HCV as a result of contact with the sample.

This invention provides a method of substantially reducing
30 the amount of HCV envelope glycoprotein in a bodily fluid sample which comprises contacting the sample with a suitable amount of an aqueous-soluble agent capable of specifically forming a complex with a domain present on an HCV envelope glycoprotein, so as to form a complex between the agent and
35 HCV if present in the sample and thereby reduce the amount of HCV envelope glycoprotein in the sample.

In an embodiment, the blood of HCV-infected individuals will be passed through filters on which DC-SIGN and/or DC-SIGNR-based proteins or antibodies have been immobilized. This would allow the removal of HCV virions and/or HCV envelope glycoprotein from the blood. The presence of HCV envelope glycoprotein in the blood may be pathogenic for example by binding to DC-SIGN and/or DC-SIGNR-expressing cells and inhibiting the immune response or by initiating apoptosis of these cells.

10

In the preferred embodiment, the subject is a human. As used herein, substantially reducing the likelihood of the subject's becoming infected with HCV means reducing the likelihood of the subject's becoming infected with HCV by at least two-fold. For example, if a subject has a 1% chance of becoming infected with HCV, a two-fold reduction in the likelihood of the subject's becoming infected with HCV would result in the subject's having a 0.5% chance of becoming infected with HCV. In one embodiment, substantially reducing the likelihood of the subject's becoming infected with HCV means reducing the likelihood by at least ten-fold. In the preferred embodiment, substantially reducing the likelihood of a subject's becoming infected with HCV means reducing the likelihood by at least 100-fold.

25

As used herein, "the subject's becoming infected with HCV" means the invasion of the subject's own cells by HCV.

As used herein, contact with a bodily fluid sample is any contact sufficient to cause HCV in the sample to be transmitted to the subject's body, and thereby infect the subject with HCV.

The amount of aqueous-soluble agent suitable to substantially reduce the likelihood of a subject's becoming infected with HCV may be determined according to methods known to those skilled in the art. In one embodiment, the

suitable amount of aqueous-soluble agent is an amount between about 1 pM and about 10 mM. In the preferred embodiment, the suitable amount of aqueous-soluble agent is an amount between about 1 pM and about 10 μ M.

5

In one embodiment, the agent is an antibody. In another embodiment, the agent is a DC-SIGN and/or DC-SIGNR-based molecule.

10 This invention further provides a method of treating a bodily fluid sample so as to substantially reduce the likelihood of a subject's becoming infected with HIV-1 as a result of contact with the sample which comprises the steps of (a) contacting the sample with a suitable amount of an
15 aqueous-soluble agent capable of specifically forming a complex with a domain present on an HCV envelope glycoprotein, thereby forming a complex between the agent and HCV if present in the sample; and (b) removing any complex so formed from the resulting sample, so as to
20 thereby reduce the likelihood of a subject's becoming infected with HCV as a result of contact with the sample.

Removing complex from the resulting sample may be accomplished according to methods well known to those
25 skilled in the art. Such methods include, for example, affinity chromatography.

The subject method may further comprise the step of removing uncomplexed agent from the sample should such removal be
30 desirable (e.g., when the agent would cause undesirable effects in a subject to whom it is administered).

This invention further provides a method of treating a bodily fluid sample so as to substantially reduce the
35 likelihood of a subject's becoming infected with HCV as a result of contact with the sample which comprises the steps of (a) contacting the sample with a suitable amount of an

aqueous-soluble agent which (i) is capable of specifically forming a complex with a domain present on an HCV envelope glycoprotein, and (ii) comprises a moiety capable of specifically forming a complex with a known ligand, which moiety permits the removal of the agent from a sample via contact with an immobilized form of the known ligand, thereby forming a complex between the agent and HCV if present in the sample; and (b) removing any complex so formed from the resulting sample by contacting the resulting sample with an immobilized form of the known ligand, so as to thereby reduce the likelihood of a subject's becoming infected with HCV as a result of contact with the sample.

Methods of immobilizing a ligand are well known to those skilled in the art. As used herein, a ligand in its "immobilized form" is capable of forming a complex with the moiety specifically recognized by the ligand in its free form.

This invention further provides a method of treating a bodily fluid sample so as to substantially reduce the likelihood of a subject's becoming infected with HCV as a result of contact with the sample which comprises the steps of (a) contacting the sample under suitable conditions with an article of manufacture comprising a solid support having operably affixed thereto an agent capable of specifically forming a complex with a domain present on an HCV envelope glycoprotein; and (b) contacting the sample with a suitable amount of an aqueous-soluble agent capable of specifically forming a complex with a domain present on an HCV envelope glycoprotein, so as to form a complex between the agent and HCV if present in the sample, with the proviso that step (a) may either precede or follow step (b).

This invention further provides a method of treating a bodily fluid sample so as to substantially reduce the likelihood of a subject's becoming infected with HCV as a

result of contact with the sample which comprises the steps of (a) contacting the sample under suitable conditions with an article of manufacture comprising a solid support having operably affixed thereto an agent capable of specifically forming a complex with a domain present on an HCV envelope glycoprotein; and (b) (i) contacting the sample with a suitable amount of an aqueous-soluble agent capable of specifically forming a complex with a domain present on an HCV envelope glycoprotein, thereby forming a complex between the agent and HIV-1 if present in the sample, and (ii) removing any complex so formed from the resulting sample, with the proviso that step (a) may either precede or follow step (b).

15 This invention further provides a method of treating a bodily fluid sample so as to substantially reduce the likelihood of a subject's becoming infected with HCV as a result of contact with the sample which comprises the steps of (a) contacting the sample under suitable conditions with an article of manufacture comprising a solid support having operably affixed thereto an agent capable of specifically forming a complex with a domain present on an HCV envelope glycoprotein; and (b) (I) contacting the sample with a suitable amount of an aqueous-soluble agent which (1) is capable of specifically forming a complex with a domain present on an HIV-1 envelope glycoprotein, and (2) comprises a moiety capable of specifically forming a complex with a known ligand, thereby forming a complex between the agent and HCV if present in the sample, and (II) removing any complex so formed from the resulting sample by contacting the resulting sample with an immobilized form of the known ligand, with the proviso that step (a) may either precede or follow step (b).

35 The methods of the subject invention may further comprise the step of removing target cells from the bodily fluid sample. In the one embodiment, the target cells are

leukocytes. Methods of removing leukocytes from a bodily fluid sample are well known to those skilled in the art and include, for example, leukofiltration.

5 As used herein, a bodily fluid is any fluid which is present in a subject's body and is capable of containing HCV in an HCV-infected subject. Bodily fluids include, but are not limited to, whole blood or derivatives thereof (e.g., red blood cell and platelet preparations), saliva, cerebrospinal
10 fluid, tears, vaginal secretions, urine, alveolar fluid, synovial fluid, semen, pleural fluid and bone marrow. In the preferred embodiment, the bodily fluid is a fluid which is to be administered to a subject. Also in the preferred embodiment, the bodily fluid sample is selected from the
15 group consisting of whole blood, a red blood cell preparation, a platelet preparation and semen.

The bodily fluid samples such as whole blood may further comprise exogenous substances added thereto for clinical or
20 storage purposes. Such exogenous substances include, by way of example, anticoagulants (e.g., citrate) and preservatives (e.g., dextrose).

In one embodiment, the contacting steps of the methods of
25 the subject invention are performed at about 4°C. In another embodiment, the contacting steps of the methods of the subject invention are performed at about 20°C. In still another embodiment, the contacting steps of the methods of the subject invention are performed at about 37°C.

30

The invention also provides a kit for treating a bodily fluid sample so as to substantially reduce the likelihood of a subject's becoming infected with HCV as a result of contact with the sample which comprises the above-described
35 article of manufacture.

This invention further provides a kit for treating a bodily

fluid sample so as to substantially reduce the likelihood of a subject's becoming infected with HCV as a result of contact with the sample which comprises, in separate compartments: (a) an article of manufacture comprising a solid support having operably affixed thereto an agent capable of specifically forming a complex with a domain present on an HCV envelope glycoprotein; and (b) an aqueous-soluble agent capable of specifically forming a complex with a domain present on an HCV envelope glycoprotein.

10

This invention further provides a kit for treating a bodily fluid sample so as to substantially reduce the likelihood of a subject's becoming infected with HCV as a result of contact with the sample which comprises, in separate compartments: (a) an article of manufacture comprising a solid support having operably affixed thereto an agent capable of specifically forming a complex with a domain present on an HCV envelope glycoprotein; (b) an aqueous soluble agent which (1) is capable of specifically forming a complex with a domain present on an HCV envelope glycoprotein, and (2) comprises a moiety capable of specifically forming a complex with a known ligand, which moiety permits the removal of the agent from a sample via contact with an immobilized form of the known ligand; and (c) an article of manufacture comprising a solid support having operably affixed thereto the known ligand capable of specifically forming a complex with the moiety of the aqueous-soluble agent of step (b).

30 This invention provides a kit for treating a bodily fluid sample so as to substantially reduce the likelihood of a subject's becoming infected with HCV as a result of contact with the sample which comprises, in separate compartments: (a) an aqueous-soluble agent which (i) is capable of specifically forming a complex with a domain present on an HCV envelope glycoprotein, and (ii) comprises a moiety capable of specifically forming a complex with a known

ligand, which moiety permits the removal of the agent from a sample via contact with an immobilized form of the known ligand; and (b) an article of manufacture comprising a solid support having operably affixed thereto the known ligand
5 capable of specifically forming a complex with the moiety of said aqueous-soluble agent.

This invention also provides a kit for reducing the amount of HCV or HCV envelope glycoprotein present in a bodily
10 fluid sample which comprises the above-described article of manufacture. In an embodiment, the bodily fluid is blood.

The kits of the subject invention may further comprise suitable buffers.

15

In order to facilitate an understanding of the following examples, certain frequently occurring methods and/or terms are best described in Sambrook et al. (1989).

20 The methods described herein to capture the HCV virions may be used for any purpose known to one skilled in the art. In one embodiment, the method is employed so as to reduce the infectivity of a subject's sample. In one embodiment, the method is employed for concentrating the HCV virions so as
25 to enable a greater chance of HCV detection, such as in a PCR assay for HCV nucleic acid, such as HCV RNA.

Obtaining a sample of HCV envelope glycoprotein⁺ cells may be performed according to methods well known to those
30 skilled in the art. HCV envelope glycoprotein⁺ cells may be obtained from blood or any other bodily fluid known to contain HCV envelope glycoprotein⁺ cells in HCV-infected subjects.

35 This invention provides a compound or agent capable of inhibiting binding of a DC-SIGN protein to an HCV envelope glycoprotein, thereby inhibiting HCV infection of a cell.

This invention provides a compound or agent capable of inhibiting binding of a DC-SIGNR protein to an HCV envelope glycoprotein, thereby inhibiting HCV infection of a cell.

5 This invention provides an antibody or portion thereof capable of inhibiting binding of a DC-SIGN protein to an HCV envelope glycoprotein, which antibody binds to an epitope located within a region of the DC-SIGN protein, which region of the DC-SIGN protein binds to an HCV envelope glycoprotein. This invention provides an antibody or
10 portion thereof capable of inhibiting binding of a DC-SIGNR protein to an HCV envelope glycoprotein, which antibody binds to an epitope located within a region of the DC-SIGNR protein, which region of the DC-SIGNR protein binds to an
15 HCV envelope glycoprotein.

This invention provides an antibody or portion thereof capable of inhibiting binding of a DC-SIGN protein to an HCV envelope glycoprotein, which antibody binds to an epitope
20 located within a region of the HCV envelope glycoprotein, which region of the HCV envelope glycoprotein binds to a DC-SIGN protein. This invention provides an antibody or portion thereof capable of inhibiting binding of a DC-SIGN protein to an HCV envelope glycoprotein, which antibody
25 binds to an epitope located within a region of the HCV envelope glycoprotein, which region of the HCV envelope glycoprotein binds to a DC-SIGNR protein.

In one embodiment of the antibodies or portions thereof
30 described herein, the antibody is a monoclonal antibody. In one embodiment of the antibodies or portions thereof described herein, the antibody is a polyclonal antibody. In one embodiment of the antibodies or portions thereof described herein, the antibody is a humanized antibody. In
35 one embodiment of the antibodies or portions thereof described herein, the antibody is a chimeric antibody. In one embodiment of the antibodies or portions thereof

described herein, the portion of the antibody comprises a light chain of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a heavy chain of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a Fab portion of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises an $F(ab')_2$ portion of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises an Fd portion of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises an Fv portion of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a variable domain of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises one or more CDR domains of the antibody.

In one embodiment of the antibodies or portions thereof described herein, the antibody binds to an epitope located within a region of an E1 HCV envelope glycoprotein. In one embodiment of the antibodies or portions thereof described herein, the antibody binds to an epitope located within a region of an E2 HCV envelope glycoprotein.

The invention embraces antibodies or fragments of antibodies having the ability to block the interaction between HCV and DC-SIGN and/or the interaction between HCV and DC-SIGNR. The antibodies may have specificity to HCV, DC-SIGN or DC-SIGNR. According to a further aspect of the invention, there is provided an antibody with the above specificity for use in the treatment of all HCV infection and in the manufacture of a medicament for the treatment of an HCV infection. The antibody is preferably a monoclonal

antibody. Such an antibody can be used to temporarily block the DC-SIGNR receptor preventing infection from HCV, for example, immediately after an accidental infection with HCV-infected blood.

5

As used herein, "antibody" includes both naturally occurring and non-naturally occurring antibodies. Specifically, "antibody" includes polyclonal and monoclonal antibodies, and monovalent and divalent fragments thereof. Furthermore, 10 "antibody" includes chimeric antibodies, wholly synthetic antibodies, single chain antibodies, and fragments thereof. The antibody may be a human or nonhuman antibody. A nonhuman antibody may be humanized by recombinant methods to reduce its immunogenicity in man. Antibodies are prepared 15 according to conventional methodology. Monoclonal antibodies may be generated using the method of Kohler and Milstein (Nature, 256:495, 1975). To prepare monoclonal antibodies useful in the invention, a mouse or other appropriate host animal is immunized at suitable intervals 20 (e.g., twice-weekly, weekly, twice-monthly or monthly) with antigenic forms of HCV, HCV envelope glycoproteins, DC-SIGN, or DC-SIGNR. The animal may be administered a final "boost" of antigen within one week of sacrifice. It is often desirable to use an immunologic adjuvant during 25 immunization. Suitable immunologic adjuvants include Freund's complete adjuvant, Freund's incomplete adjuvant, alum, Ribi adjuvant, Hunter's Titermax, saponin adjuvants such as QS21 or Quil A, or CpG-containing immunostimulatory oligonucleotides. Other suitable adjuvants are well-known 30 in the field. The animals may be immunized by subcutaneous, intraperitoneal, intramuscular, intravenous, intranasal or other routes. A given animal may be immunized with multiple forms of the antigen by multiple routes.

35 In one embodiment, HCV is purified from the plasma of HCV-infected individuals using the method of sucrose gradient centrifugation. Alternatively, recombinant HCV E1 and/or E2

envelope glycoproteins, which are available commercially from a variety of sources, such as Austral Biologicals (San Ramon, CA, Cat # HCA-090-2), Immunodiagnostics (Woburn, MA, Cat #4001) and Accurate Chemical (Westbury, MA, Cat #YVS8921). The recombinant HCV envelope glycoproteins may be provided by surface expression on recombinant cell lines. DC-SIGN may be provided in the form of human dendritic cells, whereas DC-SIGNR may be provided as liver sinusoidal cells. Recombinant forms of DC-SIGN and DC-SIGNR may be provided using previously described methods {Pohlmann, Soilleux, et al. 2001 ID: 1081}. Alternatively, the antigen may be provided as synthetic peptides corresponding to antigenic regions of interest.

Following the immunization regimen, lymphocytes are isolated from the spleen, lymph node or other organ of the animal and fused with a suitable myeloma cell line using an agent such as polyethylene glycol to form a hydridoma. Following fusion, cells are placed in media permissive for growth of hybridomas but not the fusion partners using standard methods, as described (Goding, Monoclonal Antibodies: Principles and Practice: Production and Application of Monoclonal Antibodies in Cell Biology, Biochemistry and Immunology, 3rd edition, Academic Press, New York, 1996).

Following culture of the hybridomas, cell supernatants are analyzed for the presence of antibodies of the desired specificity, i.e., that selectively bind the antigen. Suitable analytical techniques include ELISA, flow cytometry, immunoprecipitation, and western blotting. Other screening techniques are well-known in the field. Preferred techniques are those that confirm binding of antibodies to conformationally intact, natively folded antigen, such as non-denaturing ELISA, flow cytometry, and immunoprecipitation.

Significantly, as is well-known in the art, only a small

portion of an antibody molecule, the paratope, is involved in the binding of the antibody to its epitope (see, in general, Clark, W.R. (1986) The Experimental Foundations of Modern Immunology Wiley & Sons, Inc., New York; Roitt, I. (1991) Essential Immunology, 7th Ed., Blackwell Scientific Publications, Oxford). The pFc' and Fc regions, for example, are effectors of the complement cascade but are not involved in antigen binding. An antibody from which the pFc' region has been enzymatically cleaved, or which has been produced without the pFc' region, designated an F(ab')₂ fragment, retains both of the antigen binding sites of an intact antibody. Similarly, an antibody from which the Fc region has been enzymatically cleaved, or which has been produced without the Fc region, designated an Fab fragment, retains one of the antigen binding sites of an intact antibody molecule. Proceeding further, Fab fragments consist of a covalently bound antibody light chain and a portion of the antibody heavy chain denoted Fd. The Fd fragments are the major determinant of antibody specificity (a single Fd fragment may be associated with up to ten different light chains without altering antibody specificity) and Fd fragments retain epitope-binding ability in isolation.

Within the antigen-binding portion of an antibody, as is well-known in the art, there are complementarity determining regions (CDRs), which directly interact with the epitope of the antigen, and framework regions (FRs), which maintain the tertiary structure of the paratope (see, in general, Clark, 1986; Roitt, 1991). In both the heavy chain Fd fragment and the light chain of IgG immunoglobulins, there are four framework regions (FR1 through FR4) separated respectively by three complementarity determining regions (CDR1 through CDR3). The CDRs, and in particular the CDR3 regions, and more particularly the heavy chain CDR3, are largely responsible for antibody specificity.

It is now well-established in the art that the non-CDR regions of a mammalian antibody may be replaced with similar regions of conspecific or heterospecific antibodies while retaining the epitopic specificity of the original antibody.

5 This is most clearly manifested in the development and use of "humanized" antibodies in which non-human CDRs are covalently joined to human FR and/or Fc/pFc' regions to produce a functional antibody.

10 This invention provides in certain embodiments compositions and methods that include humanized forms of antibodies. As used herein, "humanized" describes antibodies wherein some, most or all of the amino acids outside the CDR regions are replaced with corresponding amino acids derived from human
15 immunoglobulin molecules. Methods of humanization include, but are not limited to, those described in U.S. patents 4,816,567, 5,225,539, 5,585,089, 5,693,761, 5,693,762 and 5,859,205, which are hereby incorporated by reference. One of ordinary skill in the art will be familiar with other
20 methods for antibody humanization.

In one embodiment of the humanized forms of the antibodies, some, most or all of the amino acids outside the CDR regions have been replaced with amino acids from human
25 immunoglobulin molecules but where some, most or all amino acids within one or more CDR regions are unchanged. Small additions, deletions, insertions, substitutions or modifications of amino acids are permissible as long as they would not abrogate the ability of the antibody to bind a
30 given antigen. Suitable human immunoglobulin molecules would include IgG1, IgG2, IgG3, IgG4, IgA and IgM molecules. A "humanized" antibody retains a similar antigenic specificity as the original antibody. However, using certain methods of humanization, the affinity and/or
35 specificity of binding of the antibody may be increased using methods of "directed evolution", as described by Wu et al., *J. Mol. Biol.* 294:151, 1999, the contents of which are

incorporated herein by reference.

Fully human monoclonal antibodies also can be prepared by immunizing mice transgenic for large portions of human immunoglobulin heavy and light chain loci. See, e.g., U.S. patents 5,591,669, 5,598,369, 5,545,806, 5,545,807, 6,150,584, and references cited therein, the contents of which are incorporated herein by reference. These animals have been genetically modified such that there is a functional deletion in the production of endogenous (e.g., murine) antibodies. The animals are further modified to contain all or a portion of the human germ-line immunoglobulin gene locus such that immunization of these animals will result in the production of fully human antibodies to the antigen of interest. Following immunization of these mice (e.g., XenoMouse (Abgenix), HuMAb mice (Medarex/GenPharm)), monoclonal antibodies can be prepared according to standard hybridoma technology. These monoclonal antibodies will have human immunoglobulin amino acid sequences and therefore will not provoke human anti-mouse antibody (HAMA) responses when administered to humans.

In vitro methods also exist for producing human antibodies. These include phage display technology (U.S. patents 5,565,332 and 5,573,905) and *in vitro* stimulation of human B cells (U.S. patents 5,229,275 and 5,567,610). The contents of these patents are incorporated herein by reference.

Thus, as will be apparent to one of ordinary skill in the art, the present invention also provides for $F(ab')_2$, Fab, Fv and Fd fragments; chimeric antibodies in which the Fc and/or FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric $F(ab')_2$ fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric Fab fragment antibodies in which the FR

and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; and chimeric Fd fragment antibodies in which the FR and/or CDR1 and/or CDR2 regions have been replaced by homologous
5 human or non-human sequences. The present invention also includes so-called single chain antibodies.

The various antibody molecules and fragments may derive from any of the commonly known immunoglobulin classes, including
10 but not limited to IgA, secretory IgA, IgE, IgG and IgM. IgG subclasses are also well known to those in the art and include but are not limited to human IgG1, IgG2, IgG3 and IgG4.

15 Monoclonal antibodies may be produced by mammalian cell culture in hybridoma or recombinant cell lines such as Chinese hamster ovary cells or murine myeloma cell lines. Such methods are well-known to those skilled in the art. Bacterial, yeast, and insect cell lines can also be used to
20 produce monoclonal antibodies or fragments thereof. In addition, methods exist to produce monoclonal antibodies in transgenic animals or plants (Pollock et al., J. Immunol. Methods, 231: 147, 1999; Russell, Curr.Top. Microbiol. Immunol. 240: 119, 1999).

25 In one embodiment of the agents described herein, the agent is an antibody or portion of an antibody. As used herein, "antibody" means an immunoglobulin molecule comprising two heavy chains and two light chains and which recognizes an
30 antigen. The immunoglobulin molecule may derive from any of the commonly known classes, including but not limited to IgA, secretory IgA, IgG and IgM. IgG subclasses are also well known to those in the art and include but are not limited to human IgG1, IgG2, IgG3 and IgG4. It includes, by
35 way of example, both naturally occurring and non-naturally occurring antibodies. Specifically, "antibody" includes polyclonal and monoclonal antibodies, and monovalent and

divalent fragments thereof. Furthermore, "antibody" includes chimeric antibodies, wholly synthetic antibodies, single chain antibodies, and fragments thereof. Optionally, an antibody can be labeled with a detectable marker.

5 Detectable markers include, for example, radioactive or fluorescent markers. The antibody may be a human or nonhuman antibody. The nonhuman antibody may be humanized by recombinant methods to reduce its immunogenicity in man. Methods for humanizing antibodies are known to those skilled

10 in the art. As used herein, "monoclonal antibody," also designated as mAb, is used to describe antibody molecules whose primary sequences are essentially identical and which exhibit the same antigenic specificity. Monoclonal antibodies may be produced by hybridoma, recombinant,

15 transgenic or other techniques known to one skilled in the art. The term "antibody" includes, but is not limited to, both naturally occurring and non-naturally occurring antibodies. Specifically, the term "antibody" includes polyclonal and monoclonal antibodies, and antigen-binding

20 fragments thereof. Furthermore, the term "antibody" includes chimeric antibodies, wholly synthetic antibodies, and antigen-binding fragments thereof. Accordingly, in one embodiment, the antibody is a monoclonal antibody. In one embodiment, the antibody is a polyclonal antibody. In one

25 embodiment, the antibody is a humanized antibody. In one embodiment, the antibody is a chimeric antibody. Such chimeric antibodies may comprise a portion of an antibody from one source and a portion of an antibody from another source.

30

In one embodiment, the portion of the antibody comprises a light chain of the antibody. As used herein, "light chain" means the smaller polypeptide of an antibody molecule composed of one variable domain (VL) and one constant domain

35 (CL), or fragments thereof. In one embodiment, the portion of the antibody comprises a heavy chain of the antibody. As used herein, "heavy chain" means the larger polypeptide of

an antibody molecule composed of one variable domain (VH) and three or four constant domains (CH1, CH2, CH3, and CH4), or fragments thereof. In one embodiment, the portion of the antibody comprises a Fab portion of the antibody. As used
5 herein, "Fab" means a monovalent antigen binding fragment of an immunoglobulin that consists of one light chain and part of a heavy chain. It can be obtained by brief papain digestion or by recombinant methods. In one embodiment, the portion of the antibody comprises an F(ab')₂ portion of the
10 antibody. As used herein, "F(ab')₂ fragment" means a bivalent antigen binding fragment of an immunoglobulin that consists of both light chains and part of both heavy chains. It can be obtained by brief pepsin digestion or recombinant methods. In one embodiment, the portion of the antibody
15 comprises an Fd portion of the antibody. In one embodiment, the portion of the antibody comprises an Fv portion of the antibody. In one embodiment, the portion of the antibody comprises a variable domain of the antibody. In one embodiment, the portion of the antibody comprises a constant
20 domain of the antibody. In one embodiment, the portion of the antibody comprises one or more CDR domains of the antibody. As used herein, "CDR" or "complementarity determining region" means a highly variable sequence of amino acids in the variable domain of an antibody.

25

This invention provides humanized forms of the antibodies described herein. As used herein, "humanized" describes antibodies wherein some, most or all of the amino acids outside the CDR regions are replaced with corresponding
30 amino acids derived from human immunoglobulin molecules. In one embodiment of the humanized forms of the antibodies, some, most or all of the amino acids outside the CDR regions have been replaced with amino acids from human immunoglobulin molecules but where some, most or all amino
35 acids within one or more CDR regions are unchanged. Small additions, deletions, insertions, substitutions or modifications of amino acids are permissible as long as they

would not abrogate the ability of the antibody to bind a given antigen. Suitable human immunoglobulin molecules would include IgG1, IgG2, IgG3, IgG4, IgA and IgM molecules. A "humanized" antibody would retain a similar antigenic
5 specificity as the original antibody.

One skilled in the art would know how to make the humanized antibodies of the subject invention. Various publications, several of which are hereby incorporated by reference into
10 this application, also describe how to make humanized antibodies. For example, the methods described in United States Patent No. 4,816,567 comprise the production of chimeric antibodies having a variable region of one antibody and a constant region of another antibody.

15 United States Patent No. 5,225,539 describes another approach for the production of a humanized antibody. This patent describes the use of recombinant DNA technology to produce a humanized antibody wherein the CDRs of a variable
20 region of one immunoglobulin are replaced with the CDRs from an immunoglobulin with a different specificity such that the humanized antibody would recognize the desired target but would not be recognized in a significant way by the human subject's immune system. Specifically, site directed
25 mutagenesis is used to graft the CDRs onto the framework.

Other approaches for humanizing an antibody are described in United States Patent Nos. 5,585,089 and 5,693,761 and WO 90/07861 which describe methods for producing humanized
30 immunoglobulins. These have one or more CDRs and possible additional amino acids from a donor immunoglobulin and a framework region from an accepting human immunoglobulin. These patents describe a method to increase the affinity of an antibody for the desired antigen. Some amino acids in
35 the framework are chosen to be the same as the amino acids at those positions in the donor rather than in the acceptor. Specifically, these patents describe the preparation of a

humanized antibody that binds to a receptor by combining the CDRs of a mouse monoclonal antibody with human immunoglobulin framework and constant regions. Human framework regions can be chosen to maximize homology with the mouse sequence. A computer model can be used to identify amino acids in the framework region which are likely to interact with the CDRs or the specific antigen and then mouse amino acids can be used at these positions to create the humanized antibody.

10

The above patents 5,585,089 and 5,693,761, and WO 90/07861 also propose four possible criteria which may be used in designing the humanized antibodies. The first proposal was that for an acceptor, use a framework from a particular human immunoglobulin that is unusually homologous to the donor immunoglobulin to be humanized, or use a consensus framework from many human antibodies. The second proposal was that if an amino acid in the framework of the human immunoglobulin is unusual and the donor amino acid at that position is typical for human sequences, then the donor amino acid rather than the acceptor may be selected. The third proposal was that in the positions immediately adjacent to the 3 CDRs in the humanized immunoglobulin chain, the donor amino acid rather than the acceptor amino acid may be selected. The fourth proposal was to use the donor amino acid residue at the framework positions at which the amino acid is predicted to have a side chain atom within 3D of the CDRs in a three dimensional model of the antibody and is predicted to be capable of interacting with the CDRs. The above methods are merely illustrative of some of the methods that one skilled in the art could employ to make humanized antibodies.

This invention provides isolated nucleic acids encoding the agents and/or compounds described herein. In one embodiment, the nucleic acid encodes the antibodies described herein or their humanized versions. The nucleic

acid can be RNA, DNA or cDNA. In one embodiment, the nucleic acid encodes the light chain. In one embodiment, the nucleic acid encodes the heavy chain. In one embodiment, the nucleic acid encodes both the heavy and light chains. In one embodiment, one or more nucleic acids encode the Fab portion. In one embodiment, one or more nucleic acids encode CDR portions. In one embodiment, the nucleic acid encodes the variable domain.

10 This invention provides the nucleic acids described herein, wherein the nucleic acids may be altered by the insertion, deletion and/or substitution of one or more nucleotides, which could result in an alteration of the nucleic acid sequence. In one embodiment, the nucleotide changes do not
15 result in a mutation at the amino acid level. One embodiment, the nucleotide change may result in an amino acid change. Such amino acid change could be one which does not affect the protein's function.

20 This invention provides a vector which comprises a nucleic acid described herein. On embodiment, the vector is a plasmid. This invention provides a host vector system which comprises the vector described herein and suitable host cell. This invention provides a method of producing a
25 polypeptide which comprises growing the host vector system described herein under suitable conditions for producing the polypeptide and recovering the polypeptide so produced.

In one embodiment of the agents described herein, the agent
30 is a polypeptide. In one embodiment of the agents described herein, the agent is an oligopeptide. As used herein, "polypeptide" means two or more amino acids linked by a peptide bond.

35 This invention provides a polypeptide capable of inhibiting binding of a DC-SIGN protein to an HCV envelope glycoprotein, which polypeptide comprises consecutive amino

acids having a sequence which corresponds to the sequence of at least a portion of an extracellular domain of a DC-SIGN protein, which portion binds to an HCV envelope glycoprotein.

5

In one embodiment, the polypeptide corresponds to an extracellular domain of DC-SIGN. In one embodiment of the polypeptide, the extracellular domain comprises consecutive amino acids having a sequence which begins with the lysine at position 62 and ends with the carboxy terminal amino acid as set forth in SEQ ID NO: 1.

In one embodiment of the polypeptide, the extracellular domain is a C-type lectin binding domain or portion thereof.

15

In one embodiment of the polypeptide, the C-type lectin domain comprises consecutive amino acids having a sequence which begins with the leucine at position 229 and ends with the carboxy terminal amino acid as set forth in SEQ ID NO 1.

20

This invention provides a polypeptide capable of inhibiting binding of a DC-SIGNR protein to an HCV envelope glycoprotein, which polypeptide comprises consecutive amino acids having a sequence which corresponds to the sequence of at least a portion of an extracellular domain of a DC-SIGNR protein, which portion binds to an HCV envelope glycoprotein. In one embodiment of the polypeptide, the extracellular domain comprises consecutive amino acids having a sequence which begins with the lysine at position 74 and ends with the carboxy terminal amino acid as set forth in SEQ ID NO: 2.

In one embodiment of the polypeptide, the C-type lectin domain comprises consecutive amino acids having a sequence which begins with the leucine at position 241 and ends with the carboxy terminal amino acid as set forth in SEQ ID NO 2.

35

This invention provides a polypeptide capable of inhibiting binding of a DC-SIGN protein to an HCV envelope glycoprotein, which polypeptide comprises consecutive amino acids having a sequence which corresponds to the sequence of
5 at least a portion of an extracellular domain of an HCV envelope glycoprotein, which portion binds to a DC-SIGN protein.

In one embodiment, the polypeptide comprises consecutive
10 amino acids having a sequence which corresponds to the sequence of at least a portion of an extracellular domain of an E1 HCV envelope glycoprotein, which portion binds to a DC-SIGN protein. In one embodiment, the polypeptide comprises consecutive amino acids having the sequence as set
15 forth in SEQ ID NO: 3 from position 192 to position 346, or a portion thereof.

In one embodiment, the polypeptide comprises consecutive amino acids having a sequence which corresponds to the
20 sequence of at least a portion of an extracellular domain of an E2 HCV envelope glycoprotein, which portion binds to a DC-SIGN protein. In one embodiment, the polypeptide comprises consecutive amino acids having the sequence as set forth in SEQ ID NO: 3 from position 383 to position 717, or
25 a portion thereof.

This invention provides a polypeptide capable of inhibiting binding of a DC-SIGNR protein to an HCV envelope glycoprotein, which polypeptide comprises consecutive amino
30 acids having a sequence which corresponds to the sequence of at least a portion of an extracellular domain of an HCV envelope glycoprotein, which portion binds to a DC-SIGNR protein.

35 In one embodiment, the polypeptide comprises consecutive amino acids having a sequence which corresponds to the sequence of at least a portion of an extracellular domain of

an E1 HCV envelope glycoprotein, which portion binds to a DC-SIGNR protein. In one embodiment, the polypeptide comprises consecutive amino acids having the sequence as set forth in SEQ ID NO: 3 from position 192 to position 346, or
5 a portion thereof.

In one embodiment, the polypeptide comprises consecutive amino acids having a sequence which corresponds to the sequence of at least a portion of an extracellular domain of
10 an E2 HCV envelope glycoprotein, which portion binds to a DC-SIGNR protein. In one embodiment, the polypeptide comprises consecutive amino acids having the sequence as set forth in SEQ ID NO: 3 from position 383 to position 717, or a portion thereof.

15

The compounds and/or agents described herein may be made by any means known to one skilled in the art. For example, a protein may be made by recombinant expression from a nucleic acid, such as a plasmid or vector comprising the encoding
20 nucleic acid, wherein the plasmid or vector is in a suitable host cell, i.e., a host-vector system for the production of the polypeptide of interest. A suitable vector may be made which comprises suitable regulatory sequences, such as enhancers and promoters. The host cell may be of any type,
25 including but not limited to mammalian, bacteria and yeast cells. Suitable bacterial cells include *Escherichia coli* cells. Suitable mammalian cells include but are not limited to human embryonic kidney (HEK) 293T cells, HeLa cells, NIH 3T3 cells, Chinese hamster ovary (CHO) cells and COS cells.

30

If the protein is produced recombinantly, it may be expressed from a plasmid containing a synthetic nucleic acid insert. Such insertion site in the plasmid may allow linking the protein to a tag, such as a poly-histidine tag.
35 Such a tag facilitates later protein purification.

A nucleic acid encoding the polypeptide, protein or

functional equivalent thereof may be cloned under the control of an inducible promoter, thereby allowing regulation of protein expression. Suitable inducible systems are known to those of skill in the art.

5

Vectors for expressing the protein or functional equivalents described herein may be selected from commercial sources or constructed for a particular expression system. Such vectors may contain appropriate regulatory sequences, such as promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences and marker genes. Vectors may be plasmids, or viral-based. One skilled may consult Molecular Cloning: a laboratory manual (Sambrook et al., 1989). Many known techniques and protocols for the manipulation of nucleic acids and analysis of proteins are described in detail in "Short protocols in molecular biology", second addition, Ausubel et al. (John Wiley & Sons 1992).

20 Methods for the isolation and purification of recombinant proteins are known to those of skill in the art and described in various sources such as in Sambrook et al. (1989). In bacteria such as *E. coli*, the recombinant protein may form inclusion bodies within the bacterial cell, thus facilitating its preparation. If produced in inclusion bodies, the carrier protein may require refolding to a natural conformation.

30 Additionally, in order to tailor the properties of the protein or functional equivalent thereof, one skilled appreciates that alterations may be made at the nucleic acid level from known protein sequences, such as by adding, substituting, deleting or inserting one or more nucleotides. Site-directed mutagenesis is the method of preference that may be employed to make mutated proteins. There are many site-directed mutagenesis techniques known to those skilled in the art, including but not limited to oligonucleotide-

directed mutagenesis using PCR, such as is described in Sambrook et al. (1989), or using commercially available kits.

5 Suitable vectors may be selected or constructed, containing appropriate regulatory sequences, including promoter sequences, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. The vectors include but are not limited to plasmids, such as
10 viral e.g., phage, or phagemid, and as described in Sambrook et al. (1989). Techniques and protocols for manipulating nucleic acids, such as in preparing nucleic acid constructs, mutagenesis, sequencing, introducing nucleic acids into cells and gene expression, and analysis of proteins, are
15 described in detail in *Short Protocols in Molecular Biology*, Second Edition, Ausubel et al. Eds, John Wiley & Sons, 1992, which is incorporated by reference.

This invention also provides soluble forms of the
20 polypeptides described herein. Accordingly, for example, a transmembrane domain for a polypeptide expressed on a cell surface may be removed such that the polypeptide would become soluble.

25 This invention provides a nonpeptidyl agent capable of inhibiting binding of a DC-SIGN protein to an HCV envelope glycoprotein, which nonpeptidyl binds to an epitope located within a region of the DC-SIGN protein, which region of the DC-SIGN protein binds to an HCV envelope glycoprotein. This
30 invention provides a nonpeptidyl agent capable of inhibiting binding of a DC-SIGNR protein to an HCV envelope glycoprotein, which nonpeptidyl binds to an epitope located within a region of the DC-SIGNR protein, which region of the DC-SIGNR protein binds to an HCV envelope glycoprotein.

35

This invention provides a nonpeptidyl agent capable of inhibiting binding of a DC-SIGN protein to an HCV envelope

glycoprotein, which nonpeptidyl agent binds to at least a portion of an extracellular domain of an HCV envelope glycoprotein, which portion binds to a DC-SIGN protein. This invention provides a nonpeptidyl agent capable of inhibiting binding of a DC-SIGNR protein to an HCV envelope glycoprotein, which nonpeptidyl agent binds to at least a portion of an extracellular domain of an HCV envelope glycoprotein, which portion binds to a DC-SIGNR protein.

10 In one embodiment of the nonpeptidyl agents described herein, the nonpeptidyl agent binds to at least a portion of an extracellular domain of an E1 HCV envelope glycoprotein. In one embodiment of the nonpeptidyl agents described herein, the nonpeptidyl agent binds to at least a portion of
15 an extracellular domain of an E2 HCV envelope glycoprotein.

In one embodiment of the nonpeptidyl agents described herein, the nonpeptidyl agent is a carbohydrate. The carbohydrate may one known to those of skill in the art, including but not limited to mannose, mannan and methyl- α -D-mannopyranoside.

As used herein, "nonpeptidyl agent" means an agent that does not consist in its entirety of a linear sequence of amino acids linked by peptide bonds. A nonpeptidyl molecule may, however, contain one or more peptide bonds. In one embodiment, the nonpeptidyl agent is a compound having a molecular weight less than 500 daltons. As used herein, a "small molecule" or small molecular weight molecule is one
25 having a molecular weight less than 500 daltons.
30

This invention provides a composition which comprises a carrier and a compound which inhibits binding of HCV to DC-SIGN and/or DC-SIGNR on the surface of a cell. In one embodiment, the composition comprises an amount of the compound effective to inhibit binding of HCV to DC-SIGN and/or DC-SIGNR on the surface of a cell.

This invention provides a composition which comprises an antibody or portion thereof described herein and a carrier. This invention provides a composition which comprises a polypeptide described herein and a carrier. This invention provides a composition which comprises a nonpeptidyl agent described herein and a carrier. The carriers include but are not limited to an aerosol, intravenous, oral and topical carriers. Accordingly, the invention provides the above composition adapted for aerosol, intravenous, oral or topical applications or other applications known to one skilled in the art.

This invention provides the agents, compounds and/or compositions described herein and carrier. Such carrier may be a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are well known to those skilled in the art. Such pharmaceutically acceptable carriers may include but are not limited to aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like.

As used herein, "composition" means a mixture. The compositions include but are not limited to those suitable for oral, rectal, intravaginal, topical, nasal, ophthalmic,

or parenteral administration to a subject. As used herein, "parenteral" includes but is not limited to subcutaneous, intravenous, intramuscular, or intrasternal injections or infusion techniques. As used herein, "administering" may be
5 effected or performed using any of the methods known to one skilled in the art. The methods for administration to the subject include but are not limited to oral, rectal, intravaginal, topical, nasal, ophthalmic, parenteral, subcutaneous, intravenous, intramuscular, or intrasternal
10 injections or infusion techniques.

This invention provides DC-SIGN and DC-SIGNR proteins, or functional equivalents thereof, for use in the therapy or diagnosis of HCV. The invention provides a compound that
15 binds specifically to DC-SIGN and/or DC-SIGNR proteins for use in the therapy or diagnosis of HCV.

As used herein, a functional equivalent of DC-SIGN or DC-SIGNR is a compound which is capable of binding to HCV, thereby preventing its interaction with DC-SIGN and/or DC-SIGNR. Preferably, the functional equivalent is a peptide or protein. The term "functional equivalent" includes fragments, mutants, and muteins of DC-SIGN and DC-SIGNR. Functional equivalents include molecules that bind HCV,
20 preferably the HCV envelope glycoproteins, and comprise all or a portion of the extracellular domains of DC-SIGN or DC-SIGNR.
25

The functional equivalents include soluble forms of the DC-SIGN or DC-SIGNR proteins. A suitable soluble form of these
30 proteins, or functional equivalents thereof, might comprise, for example, a truncated form of the protein from which the transmembrane domain has been removed by chemical, proteolytic or recombinant methods. The transmembrane
35 domain of DC-SIGN starts at about glycine 49 and ends at about serine 61, whereas the transmembrane domain of DC-SIGNR starts at about glycine 49 and ends at about serine

73.

In one embodiment, the functional equivalent comprises all or a portion of the extracellular domain of DC-SIGN or DC-SIGNR. The extracellular region of DC-SIGN begins at about lysine 62 and includes the carboxy-terminal amino acids, whereas the extracellular region of DC-SIGNR begins at about lysine 74 and includes the carboxy-terminal amino acids. Preferably, the functional equivalent is at least 80% homologous to the corresponding protein. In a preferred embodiment, the functional equivalent is at least 90% homologous as assessed by any conventional analysis algorithm such as for example, the Pileup sequence analysis software (Program Manual for the Wisconsin Package, 1996). Amino acid numbering is as provided in GenBank Protein Accession Number AAK20997 for DC-SIGN and AAG13848 for DC-SIGNR.

The term "a functionally equivalent fragment" as used herein also may mean any fragment or assembly of fragments of DC-SIGN and/or DC-SIGNR that binds to HCV, preferably that binds to the HCV envelope glycoproteins. The C-type lectin binding domain of DC-SIGN begins at about leucine 229 and includes the carboxy-terminal amino acids, whereas the C-type lectin binding domain of DC-SIGNR begins at about leucine 241 and includes the carboxy-terminal amino acids. The complete protein, extracellular domain, or C-type lectin domain may be truncated at one or both ends or portions may be removed internally provided that the protein retains the defined function.

Proteinaceous, functionally equivalent fragments or analogues may belong to the same protein family as the human DC-SIGN and DC-SIGNR proteins identified herein. By "protein family" is meant a group of proteins that share a common function and exhibit common sequence homology. Homologous proteins may be derived from non-human species.

Preferably, the homology between functionally equivalent protein sequences is at least 25% across the whole of amino acid sequence of the complete protein or of the complete EC2 fragment (amino acids 113-201). More preferably, the
5 homology is at least 50%, even more preferably 75% across the whole of amino acid sequence of the protein or protein fragment. More preferably, homology is greater than 80% across the whole of the sequence. More preferably, homology is greater than 90% across the whole of the sequence. More
10 preferably, homology is greater than 95% across the whole of the sequence.

The term "functionally equivalent analogue" is used to describe a compound that possesses an analogous function to
15 an activity of the DC-SIGN and DC-SIGNR proteins and may, for example comprise a peptide, cyclic peptide, polypeptide, antibody or antibody fragment. Such a compound may be a protein, or may be a synthetic agent designed so as to mimic certain structures or epitopes on the inhibitor protein.
20 Preferably, the compound is an antibody or antibody fragment.

The term "functionally equivalent analogue" also includes any analogue of DC-SIGN or DC-SIGNR obtained by altering
25 the amino acid sequence, for example, by one or more amino acid deletions, substitutions or additions such that the protein analogue retains the ability to bind to HCV, preferably the envelope glycoproteins of HCV. Amino acid substitutions may be made, for example, by point mutation of
30 the DNA encoding the amino acid sequence. In one embodiment, the analogue retains the ability to bind HCV but does not bind ICAM-3.

The functional equivalent of DC-SIGN or DC-SIGNR may be an
35 analogue of a fragment of the DC-SIGN or DC-SIGNR. The DC-SIGN or DC-SIGNR or functional equivalent may be chemically modified, provided it retains its ability to bind to HCV,

preferably the envelope glycoproteins of HCV.

This invention also provides functional equivalents of such polypeptides and fragments thereof. Such functional
5 equivalents may be at least 75% homologous to the native sequence. Such functional equivalents may also be at least 80%, at least 85%, at least 90%, at least 95% or at least 100% homologous to the native sequence. A functionally equivalent fragment may be a fragment of the polypeptide
10 that still binds to its target ligand. For example, a functionally equivalent fragment of the E1 ectodomain would be a fragment that has a deletion of at least one amino acid at its amino terminal end, at its carboxy terminal end, internally, or a combination thereof, yet still binds to its
15 ligand on the cell susceptible to HCV infection.

This invention also provides functionally equivalent analogues of such polypeptides and polypeptide fragments. Such analogues would have an activity which is analogous to
20 the polypeptide or fragment. Such analogues may be obtained by changing the amino acid sequence, such as by an insertion, deletion or substitution of at least one amino acid. Such an analogue would still bind to its ligand. For example, an E1 analogue would still bind to its ligand on
25 the cell susceptible to HCV infection. Amino acid substitutions may be conservative substitutions. Such conservative substitutions may be ones within the following groups: (1) glycine and alanine; (2) valine, isoleucine, and leucine; (3) aspartic acid and glutamic acid; (4) asparagine
30 and glutamine; (5) serine and threonine; (6) lysine and arginine; (7) phenylalanine and tyrosine. Such substitutions may also be homologous substitutions such as within the following groups: (a) glycine, alanine, valine, leucine, and isoleucine; (b) phenylalanine, tyrosine, and
35 tryptophan; (c) lysine, arginine, and histidine; (d) aspartic acid, and glutamic acid; (e) asparagine and glutamine; (f) serine and threonine; (g) cysteine and

methionine.

The functional equivalent may also be modified such as by a chemical modification, yet wherein it still binds to its
5 respective ligand.

It is envisaged that such molecules will be useful in preventative therapy of HCV infection, because these molecules will bind specifically to the virus and will thus
10 prevent entry of the virus into cells. As used herein, "binding specifically" means that the functionally equivalent analogue has high affinity for HCV or the HCV envelope glycoproteins but not for control proteins. Specific binding may be measured by a number of techniques
15 such as ELISA, flow cytometry, western blotting, or immunoprecipitation. Preferably, the functionally equivalent analogue specifically binds to HCV or the HCV envelope glycoproteins at nanomolar or picomolar concentrations.

20

This invention also provides a compound that binds to DC-SIGN and/or DC-SIGNR for use in the diagnosis or therapy of HCV. Preferably the compound binds specifically to DC-SIGN and/or DC-SIGNR at nanomolar or picomolar concentrations.
25 Such compounds may be used to prevent the virus binding and infecting target cells. The compound includes but is not limited to an antibody, a carbohydrate, a small molecule, a peptide, a polypeptide, and an oligopeptide.

30 The DC-SIGN protein, DC-SIGNR protein, or functional equivalent thereof may be produced by any suitable means, as will be apparent to those of skill in the art. In order to produce sufficient amounts of the DC-SIGN protein, DC-SIGNR protein, or functional equivalents thereof for use in
35 accordance with the present invention, expression may conveniently be achieved by culturing under appropriate conditions recombinant host cells containing the DC-SIGNR

protein, or functional equivalent thereof. Preferably, the DC-SIGN or DC-SIGNR protein is produced by recombinant means, by expression from an encoding nucleic acid molecule. Systems for cloning and expression of a polypeptide in a
5 variety of different host cells are well known.

When expressed in recombinant form, the DC-SIGN protein, DC-SIGNR protein or functional equivalent thereof is preferably generated by expression from an encoding nucleic acid in a
10 host cell. Any host cell may be used, depending upon the individual requirements of a particular system. Suitable host cells include bacteria mammalian cells, plant cells, yeast and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous
15 polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells and many others. Bacteria are also preferred hosts for the production of recombinant protein, due to the ease with which bacteria may be manipulated and grown. A common, preferred bacterial host
20 is *E. coli*.

The nucleic acids, polypeptides and antibodies or any other agent or compound described herein may be isolated and/or purified. One skilled in the art would know how to isolate
25 and/or purify them. Methods are provided in any laboratory manual such as "Molecular Cloning" by Sambrook et al. (1989).

As used herein, the following standard abbreviations are
30 used throughout the specification to indicate specific amino acids: A = ala = alanine; R = arg = arginine; N = asn = asparagine D = asp = aspartic acid; C = cys = cysteine; Q = gln = glutamine; E = glu = glutamic acid; G = gly = glycine; H = his = histidine; I = ile = isoleucine; L = leu =
35 leucine; K = lys = lysine; M = met = methionine; F = phe = phenylalanine; P = pro = proline; S = ser = serine; T = thr = threonine; W = trp = tryptophan; Y = tyr = tyrosine; and V

= val = valine.

This invention provides a transgenic nonhuman animal which comprises a transgene encoding the polypeptide of interest
5 or a functional equivalent thereof. The following U.S. patents are hereby incorporated by reference: U.S. Patent No. 6,025,539, IL-5 transgenic mouse; U.S. Patent No. 6,023,010, Transgenic non-human animals depleted in a mature lymphocytic cell-type; U.S. Patent No. 6,018,098, In vivo
10 and in vitro model of cutaneous photoaging; U.S. Patent No. 6,018,097, Transgenic mice expressing human insulin; U.S. Patent No. 6,008,434, Growth differentiation factor-11 transgenic mice; U.S. Patent No. 6,002,066; H2-M modified transgenic mice; U.S. Patent No. 5,994,618, Growth
15 differentiation factor-8 transgenic mice; U.S. Patent No. 5,986,171, Method for examining neurovirulence of polio virus, U.S. Patent No. 5,981,830, Knockout mice and their progeny with a disrupted hepsin gene; U.S. Patent No. 5,981,829, DELTA.Nur77 transgenic mouse; U.S. Patent No. 5,936,138;
20 Gene encoding mutant L3T4 protein which facilitates HIV infection and transgenic mouse expressing such protein; U.S. Patent No. 5,912,411, Mice transgenic for a tetracycline-inducible transcriptional activator; U.S. Patent No. 5,894,078, Transgenic mouse expressing C-100 app.

25

The methods used for generating transgenic mice are well known to one of skill in the art. For example, one may use the manual entitled "Manipulating the Mouse Embryo" by Brigid Hogan et al. (Ed. Cold Spring Harbor Laboratory)
30 1986. See for example, Leder and Stewart, U.S. Patent No. 4,736,866 for methods for the production of a transgenic mouse.

For sometime it has been known that it is possible to carry
35 out the genetic transformation of a zygote (and the embryo and mature organism which result therefrom) by the placing or insertion of exogenous genetic material into the nucleus

of the zygote or to any nucleic genetic material which ultimately forms a part of the nucleus of the zygote. The genotype of the zygote and the organism which results from a zygote will include the genotype of the exogenous genetic material. Additionally, the inclusion of exogenous genetic material in the zygote will result in a phenotype expression of the exogenous genetic material.

The genotype of the exogenous genetic material is expressed upon the cellular division of the zygote. However, the phenotype expression, e.g., the production of a protein product or products of the exogenous genetic material, or alterations of the zygote's or organism's natural phenotype, will occur at that point of the zygote's or organism's development during which the particular exogenous genetic material is active. Alterations of the expression of the phenotype include an enhancement or diminution in the expression of a phenotype or an alteration in the promotion and/or control of a phenotype, including the addition of a new promoter and/or controller or supplementation of an existing promoter and/or controller of the phenotype.

The genetic transformation of various types of organisms is disclosed and described in detail in U.S. Pat. No. 4,873,191, issued October 10, 1989, which is incorporated herein by reference to disclose methods of producing transgenic organisms. The genetic transformation of organisms can be used as an in vivo analysis of gene expression during differentiation and in the elimination or diminution of genetic diseases by either gene therapy or by using a transgenic non-human mammal as a model system of a human disease. This model system can be used to test putative drugs for their potential therapeutic value in humans.

35

The exogenous genetic material can be placed in the nucleus of a mature egg. It is preferred that the egg be in a

fertilized or activated (by parthenogenesis) state. After the addition of the exogenous genetic material, a complementary haploid set of chromosomes (e.g., a sperm cell or polar body) is added to enable the formation of a zygote. 5 The zygote is allowed to develop into an organism such as by implanting it in a pseudopregnant female. The resulting organism is analyzed for the integration of the exogenous genetic material. If positive integration is determined, the organism can be used for the *in vivo* analysis of the 10 gene expression, which expression is believed to be related to a particular genetic disease.

The "transgenic non-human animals" of the invention are produced by introducing "transgenes" into the germline of 15 the non-human animal. Embryonal target cells at various developmental stages can be used to introduce transgenes. Different methods are used depending on the stage of development of the embryonal target cell. The zygote is the best target for micro-injection. In the mouse, the male 20 pronucleus reaches the size of approximately 20 micrometers in diameter which allows reproducible injection of 1-2 pl of DNA solution. The use of zygotes as a target for gene transfer has a major advantage in that in most cases the injected DNA will be incorporated into the host gene before 25 the first cleavage (Brinster, et al. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 4438-4442). As a consequence, all cells of the transgenic non-human animal will carry the incorporated transgene. This will in general also be reflected in the efficient transmission of the transgene to 30 offspring of the founder since 50% of the germ cells will harbor the transgene. Microinjection of zygotes is the preferred method for incorporating transgenes in practicing the invention.

35 Retroviral infection can also be used to introduce transgene into a non-human animal. The developing non-human embryo can be cultured *in vitro* to the blastocyst stage. During

this time, the blastomeres can be targets for retroviral infection (Jaenich, R. (1976) Proc. Natl. Acad. Sci U.S.A. 73: 1260-1264). Efficient infection of the blastomeres is obtained by enzymatic treatment to remove the zona pellucida (Hogan, et al. (1986) in Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). The viral vector system used to introduce the transgene is typically a replication-defective retrovirus carrying the transgene (Jahner, et al. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 6927-6931; Van der Putten, et al. (1985) Proc. Natl. Acad. Sci U.S.A. 82: 6148-6152). Transfection is easily and efficiently obtained by culturing the blastomeres on a monolayer of virus-producing cells (Van der Putten, supra; Stewart, et al. (1987) EMBO J. 6: 383-388). Alternatively, infection can be performed at a later stage. Virus or virus-producing cells can be injected into the blastocoele (Jahner, D., et al. (1982) Nature 298, 623-628). Most of the founders will be mosaic for the transgene since incorporation occurs only in a subset of the cells which formed the transgenic non-human animal. Further, the founder may contain various retroviral insertions of the transgene at different positions in the genome which generally will segregate in the offspring. In addition, it is also possible to introduce transgenes into the germ line, albeit with low efficiency, by intrauterine retroviral infection of the midgestation embryo (Jahner, D. et al. (1982) supra).

A third type of target cell for transgene introduction is the embryonal stem cell (ES). ES cells are obtained from pre-implantation embryos cultured in vitro and fused with embryos (Evans, M. J., et al. (1981) Nature 292, 154-156; Bradley, M. O., et al. (1984) Nature 309, 255-258; Gossler, et al. (1986) Proc. Natl. Acad. Sci U.S.A. 83, 9065-9069; and Robertson, et al. (1986) Nature 322, 445-448). Transgenes can be efficiently introduced into the ES cells by DNA transfection or by retrovirus-mediated transduction.

Such transformed ES cells can thereafter be combined with blastocysts from a non-human animal. The ES cells thereafter colonize the embryo and contribute to the germ line of the resulting chimeric animal. For review, see
5 Jaenisch, R. (1988) Science 240, 1468-1474.

As used herein, a "transgene" is a DNA sequence introduced into the germline of a non-human animal by way of human intervention such as by way of the above described methods.

10

The invention is illustrated in the Experimental Details section which follows. These experimental details are set forth to aid in an understanding of the invention, but they are not intended to, and should not be construed, to limit
15 in any way the invention as set forth in the claims which follow thereafter.

EXPERIMENTAL DETAILS

First Set of Experiments

5 Binding of Hepatitis C Virus structural glycoprotein E2 to Human C-Type Lectins, DC-SIGN and DC-SIGNR

Summary

10 DC-SIGN, a human C-type lectin, is expressed on the surface of dendritic cells (DC), and a highly-homologous protein, DC-SIGNR, is found at high levels on sinusoidal endothelial cells of the liver and lymph node. These molecules bind HIV envelope glycoprotein, gp120, and facilitate virus
15 transmission in trans by attachment to DC. HCV E2 is the functional equivalent of HIV gp120 and contains abundant high-mannose type oligosaccharides which may bind to lectin molecules, DC-SIGN and DC-SIGNR. To test this hypothesis, HeLa cell lines expressing DC-SIGN or DC-SIGNR were
20 constructed and binding to E2 protein and HCV virions was evaluated. Using a fluorometric bead assay, it was demonstrated for the first time that purified E2 binds to DC-SIGN and DC-SIGNR and these interactions are inhibited by a monoclonal antibody to DC-SIGN/DC-SIGNR in addition to
25 mannan and calcium chelators. From these experiments it appears that DC-SIGN and DC-SIGN-R function as attachment co-receptors for HCV and that their expression on DC, and on endothelium in the liver and placenta have important implications for HCV disease.

30

Materials and Methods

Plasmids and cells

Plasmids pCDNA3-DC-SIGN and pCDNA3-DC-SIGNR (Item # 5444 and
35 6749 respectively, AIDS Research and Reference Reagent Program, Rockville, MD) were transfected into HeLa cells using a lipid formulation (Effectene, Qiagen, Valencia, CA)

according to the manufacturer's suggested protocol. Two days post-transfection, cells were treated with standard growth media (Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS; Hyclone, Logan, UT), penicillin/streptomycin (Life Technologies, Carlsbad, CA), and L-glutamine (Life Technologies) supplemented with 600 ug/ml Geneticin (Life Technologies). After 2 weeks, surviving colonies of cells were selected, expanded, and screened by flow cytometry using monoclonal antibodies that recognize DC-SIGN (507D, DC-SIGN-R (612X), or both DC- and DC-SIGNR (604L). The transfected HeLa cell lines were routinely cultured in DMEM supplemented with 10% FBS, penicillin/streptomycin, L-Glutamine with Geneticin (600 ug/ml). Growing cells were divided for maintenance culture using cell dissociation solution (Sigma, St. Louis, MO).

Antibodies and recombinant proteins

The following mAbs were used:

Anti-E2 mAb HCM-091-a-5 (Clone 4F6/2) from Austral Biologicals (San Ramon, CA) is a mouse IgG1 mAb which reacts with linear epitope in E2 and with serum from HCV seropositive donors. H31, H33, H44, H48, H50, H52, H53, H54, H60, H61 are all conformation-specific anti-HCV-E2 mouse mAbs (from Dr. Jean Dubuisson, Institut Pasteur de Lille, France) that cross-react with conformation epitopes (Deleersnyder et al, J.Virol, 71, 697-704 (1997), Flint et al, J. Virol, 73, 6782-6790 (1999)).

120507 (507D) from BD Pharmingen (San Diego, CA) is a DC-SIGN-specific, lectin binding domain targeted, conformation dependent, mouse IgG2b. 507D blocks SIV and HIV infection and ICAM-3 adhesion (Jameson et al, J. Virol, 76, 1866-1875 (2002), Wu et al, J. Virol, 76, 5905-5914 (2002)).

120604 (604L) from BD Pharmingen (San Diego, CA) is a DC-SIGNR-specific, lectin binding domain targeted, conformation dependent, mouse IgG2b. 604L does not block binding to SIV

or HIV, and exhibits only weak or no blocking of ICAM-3 adhesion (Jameson et al, J. Virol, 76, 1866-1875 (2002), Wu et al, J. Virol, 76, 5905-5914 (2002)).

- 5 120612 (612X) from BD Pharmingen (San Diego, CA) is a mouse IgG2a that recognizes the lectin binding domain of both DC-SIGN and DC-SIGNR). 612X blocks ICAM-3 adhesion and HIV infection (Jameson et al, J. Virol, 76, 1866-1875 (2002), Wu et al, J. Virol, 76, 5905-5914 (2002)).

10

- DC6 (item # 5442, AIDS Research and Reference Reagent Program, Rockville, MD) is a mouse IgG1 that recognizes both DC-SIGN and DC-SIGNR, via the neck or repeat region, and not the lectin-binding domain. DC4 does not block ICAM-3
15 binding or SIV transmission (Baribaud et al, J. Virol, 10281-10289 (2001)).

- DC28 (item # 5443, AIDS Research and Reference Reagent Program, Rockville, MD) is a mouse IgG2a that recognizes
20 both DC-SIGN and DC-SIGNR, via the neck or repeat region, and not the lectin-binding domain. DC28 does not block ICAM-3 binding or SIV transmission (Baribaud et al, J. Virol, 10281-10289 (2001)).

- 25 Control isotype-matched murine IgG (mIgG; Caltag) was used to establish background levels of binding. Recombinant E2 protein (Accurate Chemical, Westbury, NY) was expressed in secreted form in CHO cells, and encompasses amino acids 384-665 of the HCV polyprotein. Recombinant ICAM-2 or ICAM-3 was
30 used as soluble Fc fusion proteins (R&D Systems).

Immunofluorescence

- Cells were stained in PBS/0.5%BSA at 4°C for 30 minutes with primary mAbs and washed before addition of isotype-specific
35 FITC-conjugated secondary mAbs (anti-mouse-FITC, BD Pharmingen (San Diego, CA) for a further 30 minutes at 4°C. After washing, cells were analyzed by flow cytometry using a

FACScan (Becton Dickinson, Mountain View, CA). Isotype-specific controls were included to establish quadrant positions.

5 *Preparation of HCV-E2 fluorescent beads*

Carboxylate-modified FluoSpheres® NeutrAvidin™ labeled microspheres (505/515 nm, 1.0 µm; Molecular Probes, Eugene, OR) were coated with HCV E2 glycoprotein as described for ICAM-1 beads (Geijtenbeek et al, Blood, 94, 754-764 (1999)).
10 Briefly, NeutrAvidin™-coated beads were sonicated, and washed in PBS/BSA (0.5%). Beads were incubated with biotinylated-Sp-conjugated AffiniPure F(ab')₂ goat anti-mouse IgG F(ab')₂ fragment specific (6 µg/ml in PBS/BSA (0.5%); Jackson ImmunoResearch Laboratories, Inc., West
15 Grove, PA) for 2 hours at 37°C. After washing, beads were incubated with mouse anti-E2 antibodies (6 µg/ml in PBS/BSA (0.5%)) at 4°C overnight. The beads were washed and incubated with 250 ng/ml purified HCV E2 produced in CHO cells (Accurate Chemicals, NY) overnight at 4°C. Identity
20 of E2 protein was confirmed by Western blot analysis with anti-E2 mAbs (data not shown).

Fluorescent bead adhesion assay

This was performed as described by Geijtenbeek et al,
25 (Blood, 94, 754-764 (1999)), with modifications. Cells were removed from culture by cell dissociation solution (Sigma) for 5 minutes at 37°C and washed three times in adhesion buffer (20 mM Tris-HCl [pH 8.0], 150 mM NaCl, 1 mM CaCl₂, 2 mM MgCl₂, and 0.5% BSA). Cells were resuspended at a final
30 concentration of 5 x 10⁶ cells/ml in adhesion buffer for 30 minutes at 4°C to recharge Ca²⁺ levels. Cells (5 x 10⁵) were preincubated with mannan (20 µg/ml; Sigma), antibodies (0.1-20 µg/ml), EDTA (5 mM) or EGTA (5 mM) for 10 minutes at room temperature. HCV-E2-coated fluorescent beads (20
35 beads/cell) were prepared with the H53 capture mAb and added, and the suspension incubated for 30 minutes at 37°C. Adhesion was determined by measuring the percentage of cells

that bound fluorescent beads by flow cytometry using a FACScan (Becton Dickinson, Oxnard, CA).

Results

5

To investigate whether DC-SIGN and DC-SIGNR are receptors for HCV E2, stable HeLa cell lines were produced by transfection of cDNAs encoding either DC-SIGN or DC-SIGNR. Flow cytometric analysis was performed on selected clones of these cells (HeLa-DC-SIGN and HeLa-DC-SIGNR) using a panel of anti-DC-SIGN or anti-DC-SIGNR specific antibodies that have been reported to react with human tissues (Figure 4 and Table 1 below). High levels of DC-SIGN and DC-SIGNR molecules were expressed at the cell surface of the respective cell lines. HeLa parent cells did not stain with any of the anti-DC-SIGN or anti-DC-SIGNR antibodies.

15

Table 1. Cell surface expression of DC-SIGN and DC-SIGNR in HeLa-DC-SIGN and HeLa-DC-SIGNR stable cell lines.

20

Antibody Clone	Specificity	% Positive Cells (Mean fluorescence intensity)		
		HeLa-DC-SIGN	HeLa-DC-SIGNR	HeLa parent
507 (D)	DC-SIGN	90.6 (81.1)	3.5 (17.1)	0.8 (7.8)
612 (X)	DC-SIGN, DC-SIGNR	67.3 (46.6)	94.8 (102.1)	0.3 (8.1)
604 (L)	DC-SIGNR	1.1 (14.2)	96.0 (128.9)	0.1 (8.6)
Isotype control	none	1.0 (16.9)	2.8 (13.7)	0.1 (7.9)

To determine whether DC-SIGN and DC-SIGNR bind to HCV-E2 glycoprotein, a flow cytometric adhesion assay (Geijtenbeek et al, Blood, 94, 754-764 (1999)) was adapted. HCV-E2

protein produced in CHO cells was captured on fluorescent beads using a panel of anti-E2 mAbs, which were incubated with DC-SIGN- and DC-SIGNR-HeLa cells at a ratio of 20 beads per cell. The HCV-E2 coated beads bound efficiently to both cell types, and binding was efficiently inhibited by mannan (Figure 5) and EDTA or EGTA (data not shown), which are chelators for the calcium ions required for the structural integrity and carbohydrate-binding properties of the C-type lectins. Low levels of background adhesion were observed in the non-transfected HeLa parent cell line, which does not express DC-SIGN or DC-SIGNR. Binding levels were dependent on the anti-E2 mAb used for coating, however the trend was similar for DC-SIGN and DC-SIGNR cells. Beads conjugated with antibody only, and without E2 protein, did not bind to cells (data not shown).

We also tested a panel of anti-DC-SIGNR and anti-DC-SIGN mAbs for their effect on E2 binding (Fig. 6). MAb to the lectin domain of the SIGN molecules mediated similar levels of inhibition compared to mannan, whereas mAbs to the membrane-proximal heptad-repeat region were less effective. The patterns of inhibition of E2 binding by these mAbs largely parallel those observed for inhibition of HIV gp120 binding (Baribaud, F. et al., 2002, J. Virol. 76, 9135-9142).

Soluble ICAM-2 and ICAM-3 Fc fusion proteins had little effect on E2 binding to either SIGN molecule (Fig. 6). Previous studies have suggested differences in the recognition of gp120 and ICAM-3 by DC-SIGN (Wu, et al., 2002 J. Virol. 76, 5905-5914; Geijtenbeek, T.B. et al., 2002, J. Biol. Chem.: 11314-11320.), and a similar situation may apply to HCV. Similarly, anti-E2 mAbs did not inhibit binding of E2 beads to either SIGN molecule (data not shown). This finding is consistent with lectin recognition of glycans distributed over the surface of E2, and the attendant difficulty of blocking such interactions with

monospecific agents. Similarly, gp120 binding to DC-SIGN is not blocked either by anti-gp120 mAbs or by mutation of individual N-linked glycosylation sites on gp120 (Hong, P.W., et al., J. Virol. 76: 12855-12865).

5

Discussion

It has therefore been demonstrated that HCV-E2 glycoprotein interacts with DC-SIGN and DC-SIGNR, and that mannan, calcium chelators and an anti-DC-SIGN/DC-SIGNR mAb inhibit this interaction. These findings are novel, and the expression of these potential HCV receptors on DC and endothelium has important implications for viral life cycle. DC-SIGN expressed on DC may transmit HCV in trans to susceptible cells in a similar fashion to HIV, and expression of DC-SIGNR in the liver and placenta may dictate viral tropism and subsequent pathogenesis.

Liver sinusoidal endothelial cells are in continuous contact with passing leukocytes, and may capture viruses, apoptotic cells and antigens from the blood and promote trans-infection of target cells. It is thus possible that DC-SIGNR promotes infection of these cells, thereby establishing a reservoir for production of new virus to pass on to hepatocytes. A similar mechanism may operate for vertical transmission of HCV via term placenta, a tissue that contains high levels of DC-SIGNR. Inhibition of these interactions represents therapeutic and prophylactic strategies for HCV disease. The role of DC-SIGN-R and DC-SIGN as tethering molecules that orchestrates HCV trafficking and localization to the liver remain to be elucidated; however, their interactions with HCV-E2 represent novel targets for therapeutic intervention.

Second Set of Experiments

Summary

- 5 An inhibitor of HCV attachment to DC-SIGN and/or DC-SIGNR is described that abrogates binding of HCV positive sera, or purified virions in the assay described below. This inhibitor may interact with the virus, or the receptor, or both.

10

Serum binding to cells

- HeLa cell lines (HeLa-DC-SIGN, HeLa-DC-SIGNR) or parental HeLa cells are cultured overnight in DMEM containing 10% FBS in a 24-well plate at 1×10^5 cells/well. The following day, the cells are washed once with adherence buffer and then blocked with adherence buffer containing 10% heat-inactivated goat serum for 20 minutes at 37°C. The cells are washed once with adherence buffer, and inhibitor(s) are added for 1 hour in adhesion buffer to half of the wells.
- 15
- 20 Ten μ l of either HCV RNA+ (virus positive) or HCV RNA- serum (virus negative) are diluted in advance for a final volume of 200 μ l, and are added to the wells. Inhibitor(s) may also be added to aliquots of the sera for 1 hour to enable interaction with virus. The virus is allowed to bind to
- 25 cells for 1 hour at 37°C with gentle agitation every 15 minutes. Finally, the serum is removed and the cells are washed five times with adherence buffer.

Viral RNA extraction

- 30 Viral RNA is extracted from cells using a QIAmp Viral RNA Mini Spin kit (Qiagen) with modifications. Briefly, two extractions with 280 μ l of lysis buffer are added per well and transferred to a 1.7-ml tube. The empty plate is washed with 140 μ l of Dulbecco's phosphate buffered saline with
- 35 calcium and magnesium, and pooled into the same tube. RNA extraction and binding to spin columns is done using the manufacturer's guidelines. Following a wash with wash

buffer, DNA on the column is removed by treatment with RNase-free DNase (Qiagen) using the manufacturer's guidelines. RNA is washed and eluted in two steps using 30 μ l and 40 μ l elution buffer, and the eluate is combined.

5

HCV-specific RT-PCR

One half nmol of primer RJD-5 is combined with 0.5 μ l of extracted RNA in a final volume of 6 μ l. Samples are heated for 10 minutes at 70°C and then cooled to 4°C using a GeneAmp PCR system (Perkin Elmer). In a 10 μ l reaction mixture, the pre-heated template is combined with 1x First Strand Buffer, 10 mM DTT, 5 mM deoxyribonucleoside triphosphates (dNTPs), and 7.5 U of ThermoScript (Invitrogen), incubated at 58°C for 50 minutes, then 85°C for 5 minutes before cooling to 4°C. From this RT reaction, 5 μ l are used as the template for PCR in a 50 μ l reaction containing 1x High Fidelity PCR Buffer, 2 mM MgSO₄, 2 mM dNTPs, 50 pmol primers RJD-1 and RJD-5, and 1.25 U Platinum Taq high fidelity DNA polymerase (Invitrogen). PCR amplification is accomplished using the method of Young et al. (Young KK, Resnik RM, Myers TW. Detection of hepatitis C virus RNA by a combined reverse transcription-polymerase chain reaction assay. J. Clin. Microbiol. 1993 31:882-886.)

25 *Blotting of RT-PCR products*

Ten μ l of each RT-PCR reaction is resolved on a 1% agarose gel containing a biotinylated DNA ladder (NEB). The gel is capillary blotted onto a Protran nitrocellulose membrane (type BA-85, Schleicher and Schull) following the Southern blot method described in Sambrook, Fritsch, and Maniatis (Sambrook, J., Fritsch, E.F., and Maniatis, T., in Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, NY, Vol. 1, 2, 3 (1989). The following day the DNA is crosslinked to the membrane at 2400 J/m² using a StrataLinker (Stratagene) and dried at room temperature for at least 1 hour.

Hybridization to detect HCV

The blot is incubated for 4 hours at 63°C in prehybridization solution. Prehybridization solution contains 5x Denhardt's [0.2% (w/v) fatty acid-free BSA (JRH Biosciences), 0.2% (w/v) polyvinylpyrrolidone (PVP, Sigma), 0.2% (w/v) Ficoll-400 (Sigma)], 6x SSC (0.9 M NaCl, 90 mM sodium citrate pH 7.4), 0.5% (w/v) sodium dodecylsulfate (SDS, Promega), and 0.1 mg/ml herring sperm DNA (Invitrogen). After the incubation, 1 pmol/ml of primer RJD-6 or RJD-7 is added to the prehybridization solution to make the hybridization solution, and is incubated overnight at 63°C. The following morning, the blot is washed twice for 5 minutes in wash buffer [2x SSC, 0.1% (w/v) SDS] at room temperature, twice for 15 minutes in wash buffer at 63°C, and once more in wash buffer at room temperature. The blot is then washed once for 5 minutes in PBST [Dulbecco's phosphate buffered saline without calcium and magnesium, 0.05% (v/v) Tween-20]. The blot is then incubated with strepavidin-HRP (Amersham) at 1/1500 in PBST for 45 minutes at room temperature. The blot is washed twice quickly then three times for 15 minutes in PBST. The blot is developed using Western Lightening (NEN/Perkin Elmer) and Kodak film. An HCV RNA positive signal is exemplified by a specific band of 243 base pairs. The intensity of the 243 base pair band is compared in the presence and the absence of inhibitor, and a reduction in intensity indicates inhibition of HCV binding.

Oligo name	Sequence
RJD-1 (KY80 ¹)	5'-GCA GAA AGC GTC TAG CCA TGG CGT-3'
RJD-5 (KY78 ¹)	5'-CTC GCA AGC ACC CTA TCA GGC AGT-3'
RJD-6	5' biotin-GGA GAG CCA TAG TGG TCT GCG GAA C-3'
RJD-7 (KY88 ¹)	5' biotin-GTT GGG TCG CGA AAG GCC TTG TGG T-3'

Third Set of Experiments

Summary

5 A novel, enhanced method for detecting HCV in samples from humans (blood, serum, plasma, tissue, amniotic fluid, et al) is disclosed that utilizes the assay described in the example vide infra. In this method, the samples are tested in the cell binding assay (SIGN assay) for attachment to
10 HeLa cells expressing DC-SIGN and/or DC-SIGNR. The standard cell-free assay is used as a control at varying dilutions of sample to determine the limit of detection and linearity of the SIGN assay. This test provides additional quantitative information on HCV viral load, (e.g., an increased
15 sensitivity in detecting the presence of HCV in a biological sample), in addition to qualitative properties (DC-SIGN or DC-SIGNR binding) on the virus present in the sample. This assay provides novel information relevant for receptor usage, the distribution of viral quasi-species (e.g.,
20 pathogenic phenotypes) and thus has utility in monitoring clinical disease progression.

Sample binding to cells

HeLa cell lines (HeLa-DC-SIGN, HeLa-DC-SIGNR) or parental
25 HeLa cells are cultured overnight in DMEM containing 10% FBS in a 24-well plate at 1×10^5 cells/well. The following day, the cells are washed once with adherence buffer and blocked with adherence buffer containing 10% heat-inactivated goat serum for 20 minutes at 37°C. The cells
30 are washed once with adherence buffer. A fixed volume (e.g., 10-1000 μ l) of either HCV RNA+ (virus positive) or HCV RNA- (virus negative) serum, or other samples (plasma, tissue extracts, etc.) is diluted in adherence buffer for a final volume of 200 μ l, and a range of 10-fold serial
35 dilutions is prepared. These suspensions are added to wells for 1 hour to enable interaction with virus and are incubated at 37°C with gentle agitation every 15 minutes.

Finally, the sample is removed and the cells are washed five times with adherence buffer. To determine the limit of detection and linearity of the assay, aliquots of the same samples are used without cell binding (cell-free samples) in the subsequent steps as discussed below.

Viral RNA extraction

Viral RNA is extracted from cells, or cell-free samples, using a QIAmp Viral RNA Mini Spin kit (Qiagen) with modifications. Briefly, two extractions with 280 µl of lysis buffer are added per well and transferred to a 1.7-ml tube. The empty plate is washed with 140 µl of Dulbecco's phosphate buffered saline with calcium and magnesium, and pooled into the same tube. RNA extraction and binding to spin columns is carried out using the manufacturer's guidelines. Following a wash with wash buffer, DNA on the column is removed by treatment with RNase-free DNase (Qiagen) using the manufacturer's guidelines. RNA is washed and eluted in two steps using 30 µl and 40 µl elution buffer, and the eluates are combined.

HCV-specific RT-PCR

One half nmol of primer RJD-5 is combined with 0.5 µl of extracted RNA in a final volume of 6 µl. Samples are heated for 10 minutes at 70°C and then cooled to 4°C using a GeneAmp PCR system (Perkin Elmer). In a 10 µl reaction mixture, the pre-heated template is combined with 1x First Strand Buffer, 10 mM DTT, 5 mM deoxyribonucleoside triphosphates (dNTPs), and 7.5 U of ThermoScript (Invitrogen), incubated at 58°C for 50 minutes, then at 85°C for 5 minutes before cooling to 4°C. From this RT reaction, 5 µl is used as the template for PCR in a 50 µl reaction containing 1x High Fidelity PCR Buffer, 2 mM MgSO₄, 2 mM dNTPs, 50 pmol primers RJD-1 and RJD-5, and 1.25 U Platinum Taq high fidelity DNA polymerase (Invitrogen). PCR amplification is accomplished using the method of Young et al. (Young KK, Resnick RM, Myers TW. Detection of hepatitis

C virus RNA by a combined reverse transcription-polymerase chain reaction assay. J Clin Microbiol. 1993, 31(4):882-6).

Blotting of RT-PCR products

5 Ten μ l of each RT-PCR reaction is resolved on a 1% agarose gel containing a biotinylated DNA ladder (NEB). The gel is capillary blotted onto a Protran nitrocellulose membrane (type BA-85, Schleicher and Schull) following the Southern blot method described in Sambrook, Fritsch, and Maniatis (Sambrook, J. Fritsch, E.F., and Maniatis, T., in Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, NY, Vol 1, 2, 3 (1989). The following day the DNA is crosslinked to the membrane at 2400 J/m² using a StrataLinker (Stratagene) and then dried at room temperature
10
15 for at least 1 hour.

Hybridization to detect HCV

The blot is incubated for 4 hours at 63°C in prehybridization solution. The prehybridization solution
20 contains 5x Denhardt's [0.2% (w/v) fatty acid-free BSA (JRH Biosciences), 0.2% (w/v) polyvinylpyrrolidone (PVP, Sigma), 0.2% (w/v) Ficoll-400 (Sigma)], 6x SSC (0.9M NaCl, 90 mM sodium citrate pH 7.4), 0.5% (w/v) sodium dodecylsulfate (SDS, Promega), and 0.1 mg/ml herring sperm DNA
25 (Invitrogen). After the incubation, 1 pmol/ml of primer RJD-6 or RJD-7 is added to the prehybridization solution to make the hybridization solution, which is incubated overnight at 63°C. The following morning, the blot is washed twice for 5 minutes in wash buffer [2x SSC, 0.1%
30 (w/v) SDS] at room temperature, twice for 15 minutes in wash buffer at 63°C, and once more in wash buffer at room temperature. The blot is then washed once for 5 minutes in PBST [Dulbecco's phosphate buffered saline without calcium and magnesium, 0.05% (v/v) Tween-20]. The blot is then
35 incubated with streptavidin-HRP (Amersham) at 1/1500 in PBST for 45 minutes at room temperature. The blot is washed twice quickly then three times for 15 minutes in PBST. The

blot is developed using Western Lightning (NEN/Perkin Elmer) and Kodak film. An HCV RNA positive signal is exemplified by a specific band of 243 base pairs. The intensity of the 243 base pair band is compared between
 5 identical samples in the cell binding (SIGN) assay, and the cell-free assay for quantitative and qualitative differences. The difference in signal intensities observed in the DC-SIGN and DC-SIGN-R assays provides information relevant to HCV receptor usage and tropism, and ultimately
 10 to clinical progression.

Oligo name	Sequence
RJD-1 (KY801)	5'-GCA GAA AGC GTC TAG CCA TGG CGT-3'
RJD-5 (KY781)	5'-CTC GCA AGC ACC CTA TCA GGC AGT-3'
RJD-6	5' biotin-GGA GAG CCA TAG TGG TCT GCG GAA C-3'
RJD-7 (KY881)	5' biotin-GTT GGG TCG CGA AAG GCC TTG TGG T-3'

Numerous other embodiments of the above assay can be envisaged. For example, HCV captured onto HeLa-DC-SIGN
 15 and/or HeLa-DC-SIGN-R cells can be quantitated using other conventional readouts, such as by Western blot analysis of HCV proteins using antibodies to viral proteins. In another embodiment, purified DC-SIGN and/or DC-SIGN-R proteins can be immobilized onto a surface, such as a plate or bead using
 20 conventional technologies, and used to capture and concentrate HCV from patient specimens. The amount of HCV can be quantified using by measuring the number of viral genomes by RT-PCR methods as described, by Western blot analysis of viral proteins, by ELISA, or by other standard
 25 methodologies that are well known to those skilled in the art.

Fourth Set of Experiments

Virus binding assay

5 *Virus-cell binding*

HeLa cell lines were cultured overnight in DMEM containing 10% FBS in a 96-well plate at 1×10^4 cells/well. Cells were blocked with AB containing 10% heat-inactivated goat serum for 20 minutes at 37°C. Cells were washed once with
10 AB, and mannan (20 µg/ml) added for 15 minutes in adherence buffer at room temperature. After washing, sera (10-20 µl) from HCV RNA+ (virus positive) or HCV RNA- (virus negative) serum donors (all HIV seronegative) were diluted in AB, and allowed to bind to cells for 1 hour at 37°C with gentle
15 agitation every 15 minutes, after which cells were washed five times with adherence buffer.

RNA extraction

Viral RNA was extracted from cells using a QIAmp Viral RNA
20 Mini Spin kit (Qiagen) with modifications. Briefly, RNA was extracted with lysis buffer followed by binding to spin columns, and DNA was removed by treatment with RNase-free DNase (Qiagen). RNA was washed and eluted in elution buffer.

25

Southern blot

HCV RNA was amplified by RT-PCR as described previously (Young, K.K. et al. 1993, J. Clin. Microbiol. 31, 882-886) with modifications. Primer KY78 (5'-
30 CTCGCAAGCACCTATCAGGCAGT-3', 0.5 nmol) (nt 276-299) was combined with 0.5 µl of extracted RNA in a final volume of 6 µl and preheated followed by addition of cDNA synthesis buffer, 10 mM DTT, 5 mM deoxyribonucleoside triphosphates (dNTPs), and 7.5 U of ThermoScript (Invitrogen), incubated
35 at 58°C for 50 min then 85°C for 5 min before cooling to 4°C. From this RT reaction, 5 µl was used as the template for PCR in a 50 µl reaction containing 1X High Fidelity PCR

Buffer, 2 mM MgSO₄, 2 mM dNTPs, 50 pmol primers KY80 (5'-GCAGAAAGCGTCTAGCCATGGCGT-3') (nt 56-79) and KY78, and 1.25 U Platinum Taq high fidelity DNA polymerase (Invitrogen). The amplification product was resolved on a 1% agarose gel and
5 blotted onto a nitrocellulose membrane (BioRad).

For detection, the blot was incubated for 4 hours at 63°C in prehybridization solution [5x Denhardt's [0.2% (w/v) fatty acid free-BSA (JRH Biosciences), 0.2% (w/v)
10 polyvinylpyrrolidone (Sigma), 0.2% (w/v) Ficoll-400 (Sigma)], 6x SSC (0.9 M NaCl, 90 mM sodium citrate pH 7.4), 0.5% (w/v) sodium dodecylsulfate (SDS, Promega), and 0.1 mg/ml herring sperm DNA (Invitrogen)]. Following incubation, 1 pmol/ml of primer RJD-6 (5' biotin-
15 GGAGAGCCATAGTGGTCTGCGGAA C-3') (nt 120-144) or KY88 (5' biotin-GTTGGGTCGCGAAAGGCCTTGTGGT-3') (nt 251-275) was added to the prehybridization solution and incubated overnight at 63°C. After extensive high stringency washing, the blot was incubated with streptavidin-HRP (Pierce), washed and
20 developed using Western Lightening Plus (NEN/Perkin Elmer). An HCV RNA positive signal is exemplified by a specific band of 243 base pairs.

Real-time PCR

25 HCV QuantasureTM Plus assay was utilized at Laboratory Corporation of America (Research Triangle Park, NC), and has been demonstrated to be sensitive, specific to HCV and has a linear dynamic range of 10 to 100,000,000 IU/ml in comparative studies to 228 Roche COBAS Amplicor assay
30 (Turnmire, C., 2002 Clearwater Virology Symposium). Briefly, a 4 µl-aliquot of extracted RNA was added to a one-step RT-PCR reaction mixture containing sense and anti-sense primers specific for HCV and a Taqman probe (proprietary sequences; LabCorp, Inc.). The cycle at which the
35 amplification plot crosses the threshold was defined as the threshold cycle (C_T), and was predictive of the number of HCV RNA copies in the sample. A standard curve was

calculated for quantification using serial 10-fold dilutions of a reference HCV-positive sample.

Results

5

HCV virus binding to DC-SIGN-R and DC-SIGN

The ability of the SIGN molecules to bind HCV virions was compared. In the absence of a method for culturing HCV *in vitro*, a novel assay to measure the interaction of the SIGN molecules with HCV virions present in the sera of infected individuals was developed. There are no prior reports on the binding of naturally occurring viruses to either DC-SIGN-R or DC-SIGN.

15 In the virus-binding assay, HCV-positive or HCV-negative sera were combined for one hour with HeLa cells expressing DC-SIGNR, DC-SIGN or neither receptor. Following removal of unbound virus and RNA extraction, HCV genomes were detected by real-time PCR (Taqman) or by RT-PCR followed by
20 qualitative Southern blot.

DC-SIGNR transfectants specifically bound 3 of 3 HCV-positive sera as determined by Taqman analysis, whereas DC-SIGN mediated specific binding for 1 of 3 sera. The levels
25 of virus binding to DC-SIGNR ranged from 4- to 7- fold greater than the background levels observed for parental HeLa (Figs. 7a, 7b). HCV binding to DC-SIGN-R was abrogated by more than 90% following mannan treatment (Fig. 7c, 7d). Overall, there was a good concordance in results obtained in
30 the hybridization and Taqman assays, although the latter was more quantitative and sensitive to detect the low levels of binding (Fig. 7).

During natural infection, HCV serum titers show significant
35 inter- and intra- patient variation over time (Lauer, G.H., et. al. 2001, N. Engl. J. Med. 345, 41-52). To further investigate the breadth and robustness of HCV binding to DC-

SIGNR and DC-SIGN, the assay was repeated using sera from patients with higher viral loads. For these, both DC-SIGN-R and DC-SIGN mediated specific, mannan-inhibitable binding for 3 of 3 sera (Fig. 8), with the highest-titered sera exhibiting 129- and 58-fold enhanced binding to DC-SIGN-R and DC-SIGN cells, respectively. Only background signals were observed for HCV-negative sera. *In toto*, DC-SIGNR specifically bound HCV for 6 of 6 donor sera independent of viral load, whereas DC-SIGN mediated binding in 4 of 6 cases with a bias towards high-titered sera.

In addition, mAbs to the lectin domains of the SIGN molecules inhibited virus binding to DC-SIGNR and DC-SIGN by 78% and 91%, respectively. In contrast, mAbs to E2 had no effect on virus binding (data not shown). These findings mirror those observed for purified E2 protein, and thus support the notion that E2 plays a major role in mediating HCV binding to the SIGN molecules.

These findings demonstrate for the first time that HCV interacts specifically with DC-SIGN-R and DC-SIGN, and this interaction is mediated at least in part by E2. Binding was blocked by relevant inhibitors, including mannan, calcium chelators, and mAbs to DC-SIGNR and DC-SIGN. Intriguingly, DC-SIGNR was somewhat more efficient than DC-SIGN at capturing virions at low viral loads.

The patterns of HCV binding and inhibition suggest that the interaction is mediated by high-mannose glycans on HCV and E2. That is, binding was competitively inhibited by mannan and by mAbs to the lectin domain of the SIGN molecules. Binding also was abrogated by chelators of the calcium ions that are required by these C-type lectins. However, binding was not inhibited by mAbs to other regions of the SIGN molecules or by anti-E2 mAbs, at least when used individually.

Immune system disorders such as cryoglobulinemia are the chief extrahepatic complications of HCV infection (Dammacco, F., Sansonno, D., Piccolo, C., Racanelli, V., D'Amore, F.P. & Lauletta, G. (2000) *Semin. Liver Dis.* 20, 143-157.), and the interaction of E2 with CD81 on B cells has been posited to be a contributing factor (Flint, M. & McKeating, J.A. (2000) *Rev. Med. Virol.* 10, 101-117.). HCV transcripts have been observed at low levels in DC and other lymphoid cells (Navas, M.C., Fuchs, A., Schvoerer, E., Bohbot, A., Aubertin, A.M. & Stoll-Keller, F. (2002) *J. Med. Virol.* 67, 152-161.; Mellor, J., Haydon, G., Blair, C., Livingstone, W. & Simmonds P. (1998) *J. Gen Virol* 79 (Pt 4) 705-714.), but these do not appear to represent significant reservoirs of HCV. However, DC-SIGNR and DC-SIGN interactions may contribute to immune dysregulation, including the impaired DC function observed in chronic HCV infection (Kanto, T., Hayashi, N., Takehara, T., Tatsum, Y., Kuzushita, N., Ito, A., Sasaki, Y., Kasahara, A. & Hori, M. (1999) *J. Immunol.* 162, 5584-5591; Bain, C., Fatmi, A., Zoulim, F., Zarski, J.P., Trepco, C. & Inchauspe, G. (2001) *Gastroenterology* 120, 512-524; Auffermann-Gretzinger, S., Keefe, E.B. & Levy, S. (2001) *Blood* 97, 3171-3176.). Migratory DC may also mediate trafficking of HCV to liver and other sites and virus binding to DC-SIGN or DC-SIGN-R may modulate HCV immunity to promote maintenance of chronic infection.

One skilled in the art will readily appreciate that the specific methods and results discussed herein are merely illustrative of the invention as described more fully in the claims that follow thereafter.

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10062.

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Gly	Ala	Lys	Asp	Val	Arg	Cys	His	Ala	Arg	Lys	Ala	Val	Ala	His
2525						2530					2535			
Ile	Asn	Ser	Val	Trp	Lys	Asp	Leu	Leu	Glu	Asp	Ser	Val	Thr	Pro
2540						2545					2550			
Ile	Asp	Thr	Thr	Ile	Met	Ala	Lys	Asn	Glu	Val	Phe	Cys	Val	Gln
2555						2560					2565			

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Pro	Glu	Lys	Gly	Gly	Arg	Lys	Pro	Ala	Arg	Leu	Ile	Val	Phe	Pro
2570						2575					2580			
Asp	Leu	Gly	Val	Arg	Val	Cys	Glu	Lys	Met	Ala	Leu	Tyr	Asp	Val
2585						2590					2595			
Val	Ser	Lys	Leu	Pro	Leu	Ala	Val	Met	Gly	Ser	Ser	Tyr	Gly	Phe
2600						2605					2610			
Gln	Tyr	Ser	Pro	Gly	Gln	Arg	Val	Glu	Phe	Leu	Val	Gln	Ala	Trp
2615						2620					2625			
Lys	Ser	Lys	Lys	Thr	Pro	Met	Gly	Phe	Ser	Tyr	Asp	Thr	Arg	Cys
2630						2635					2640			
Phe	Asp	Ser	Thr	Val	Thr	Glu	Ser	Asp	Ile	Arg	Thr	Glu	Glu	Ala
2645						2650					2655			
Ile	Tyr	Gln	Cys	Cys	Asp	Leu	Asp	Pro	Gln	Ala	Arg	Val	Ala	Ile
2660						2665					2670			
Lys	Ser	Leu	Thr	Glu	Arg	Leu	Tyr	Val	Gly	Gly	Pro	Leu	Thr	Asn
2675						2680					2685			
Ser	Arg	Gly	Glu	Asn	Cys	Gly	Tyr	Arg	Arg	Cys	Arg	Ala	Ser	Gly
2690						2695					2700			
Val	Leu	Thr	Thr	Ser	Cys	Gly	Asn	Thr	Leu	Thr	Cys	Tyr	Ile	Lys
2705						2710					2715			
Ala	Arg	Ala	Ala	Cys	Arg	Ala	Ala	Gly	Leu	Gln	Asp	Cys	Thr	Met
2720						2725					2730			
Leu	Val	Cys	Gly	Asp	Asp	Leu	Val	Val	Ile	Cys	Glu	Ser	Ala	Gly
2735						2740					2745			
Val	Gln	Glu	Asp	Ala	Ala	Ser	Leu	Arg	Ala	Phe	Thr	Glu	Ala	Met
2750						2755					2760			
Thr	Arg	Tyr	Ser	Ala	Pro	Pro	Gly	Asp	Pro	Pro	Gln	Pro	Glu	Tyr
2765						2770					2775			
Asp	Leu	Glu	Leu	Ile	Thr	Ser	Cys	Ser	Ser	Asn	Val	Ser	Val	Ala
2780						2785					2790			
His	Asp	Gly	Ala	Gly	Lys	Arg	Val	Tyr	Tyr	Leu	Thr	Arg	Asp	Pro

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2795	2800	2805
Thr Thr Pro Leu Ala Arg Ala Ala Trp Glu Thr Ala Arg His Thr 2810 2815 2820		
Pro Val Asn Ser Trp Leu Gly Asn Ile Ile Met Phe Ala Pro Thr 2825 2830 2835		
Leu Trp Ala Arg Met Ile Leu Met Thr His Phe Phe Ser Val Leu 2840 2845 2850		
Ile Ala Arg Asp Gln Leu Glu Gln Ala Leu Asn Cys Glu Ile Tyr 2855 2860 2865		
Gly Ala Cys Tyr Ser Ile Glu Pro Leu Asp Leu Pro Pro Ile Ile 2870 2875 2880		
Gln Arg Leu His Gly Leu Ser Ala Phe Ser Leu His Ser Tyr Ser 2885 2890 2895		
Pro Gly Glu Ile Asn Arg Val Ala Ala Cys Leu Arg Lys Leu Gly 2900 2905 2910		
Val Pro Pro Leu Arg Ala Trp Arg His Arg Ala Arg Ser Val Arg 2915 2920 2925		
Ala Arg Leu Leu Ser Arg Gly Gly Arg Ala Ala Ile Cys Gly Lys 2930 2935 2940		
Tyr Leu Phe Asn Trp Ala Val Arg Thr Lys Leu Lys Leu Thr Pro 2945 2950 2955		
Ile Ala Ala Ala Gly Arg Leu Asp Leu Ser Gly Trp Phe Thr Ala 2960 2965 2970		
Gly Tyr Ser Gly Gly Asp Ile Tyr His Ser Val Ser His Ala Arg 2975 2980 2985		
Pro Arg Trp Phe Trp Phe Cys Leu Leu Leu Leu Ala Ala Gly Val 2990 2995 3000		
Gly Ile Tyr Leu Leu Pro Asn Arg 3005 3010		

CLAIMS

What is claimed:

- 5 1. A method for determining HCV binding to a cell comprising:
- (a) contacting a cell expressing DC-SIGN or DC-SIGNR with a source of HCV for a time sufficient to allow binding of HCV to the cell; and
- 10 (b) detecting the cell-bound HCV.
2. The method of claim 1, wherein the cell-bound HCV is detected by RT-PCR followed by Southern blot.
- 15 3. The method of claim 1, wherein the cell-bound HCV is detected by real-time PCR.
4. The method of claim 1, wherein the cell-bound HCV is detected using an immunoassay.
- 20 5. The method of claim 1, wherein the cell-bound HCV is detected using an HCV-specific detection reagent.
6. The method of claim 5, wherein the HCV-specific detection reagent is an antibody or an oligonucleotide probe or primer.
- 25 7. A method for detecting the presence of HCV in a biological source comprising:
- 30 (a) contacting the source suspected to contain HCV with a cell expressing DC-SIGN or DC-SIGNR for a time sufficient to allow binding of HCV to the cell; and
- (b) detecting the cell-bound HCV.
- 35 8. The method of claim 7, wherein the cell-bound HCV is detected by RT-PCR followed by Southern blot.

9. The method of claim 7, wherein the cell-bound HCV is detected by real-time PCR.
10. The method of claim 7, wherein the cell-bound HCV is
5 detected using an immunoassay.
11. A method for identifying a compound capable of inhibiting the binding of HCV to a cell expressing DC-SIGN comprising:
- 10 (a) contacting the cell expressing DC-SIGN with a source of HCV in the presence or absence of a test compound for a time sufficient to allow binding of HCV to the cells; and
- 15 (b) detecting the cell-bound HCV, wherein a reduction of cell-bound HCV in the presence of the test compound compared to the amount of cell-bound HCV in the absence of the test compound is indicative of a compound capable of inhibiting the binding of HCV to a cell expressing DC-SIGN.
- 20
12. The method of claim 11, wherein the cell-bound HCV is detected by RT-PCR followed by Southern blot.
13. The method of claim 11, wherein the cell-bound HCV is
25 detected by real-time PCR.
14. The method of claim 11, wherein the cell-bound HCV is detected using an immunoassay.
- 30 15. The method of claim 11, wherein the cell-bound HCV is detected using an HCV-specific detection reagent.
16. The method of claim 15, wherein the HCV-specific detection reagent is an antibody or an oligonucleotide
35 probe or primer.
17. The method of claim 16, wherein the oligonucleotide

probe or primer specifically hybridizes to an HCV genome or portion thereof.

18. The method of claim 11, wherein the source is a
5 biological fluid, a tissue, or a cell.

19. The method of claim 18, wherein the biological fluid is blood, serum, plasma, or amniotic fluid.

10 20. The method of claim 11, wherein the test compound is an antibody, non-antibody polypeptide, or a nonpeptidyl agent.

15 21. A method for identifying a compound capable of inhibiting the binding of HCV to a cell expressing DC-SIGNR comprising:

(a) contacting the cell expressing DC-SIGNR with a source of HCV in the presence or absence of a test compound for a time sufficient to allow binding of
20 HCV to the cells; and

(b) detecting the cell-bound HCV, wherein a reduction of cell-bound HCV in the presence of the test compound compared to the amount of cell-bound HCV in the absence of the test compound is indicative
25 of a compound capable of inhibiting the binding of HCV to a cell expressing DC-SIGNR.

22. The method of claim 21, wherein the cell-bound HCV is detected by RT-PCR followed by Southern blot.
30

23. The method of claim 21, wherein the cell-bound HCV is detected by real-time PCR.

24. The method of claim 21, wherein the cell-bound HCV is
35 detected using an immunoassay.

25. The method of claim 21, wherein the cell-bound HCV is

detected using an HCV-specific detection reagent.

26. The method of claim 25, wherein the HCV-specific detection reagent is an antibody or an oligonucleotide probe or primer.
27. The method of claim 26, wherein the oligonucleotide probe or primer specifically hybridizes to an HCV genome or portion thereof.
28. The method of claim 21, wherein the source is a biological fluid, a tissue, or a cell.
29. The method of claim 28, wherein the biological fluid is blood, serum, plasma, or amniotic fluid.
30. The method of claim 21, wherein the test compound is an antibody, non-antibody polypeptide, or a nonpeptidyl agent.
31. A method for identifying a compound capable of inhibiting an HCV infection of a cell expressing DC-SIGN comprising:
- (a) contacting the cell expressing DC-SIGN with a source of HCV in the presence or absence of a test compound for a time sufficient to allow infection of the cell expressing DC-SIGN by HCV; and
- (b) detecting the HCV in HCV-infected cells, wherein a reduction of HCV in the presence of the test compound compared to the amount of HCV in the absence of the test compound is indicative of a compound capable of inhibiting the infection of the cell expressing DC-SIGN by the HCV.
32. The method of claim 31, wherein the HCV is detected by RT-PCR followed by Southern blot.

33. The method of claim 31, wherein the HCV is detected by real-time PCR.
34. The method of claim 31, wherein the HCV is detected using an immunoassay.
35. The method of claim 31, wherein the HCV is detected using an HCV-specific detection reagent.
36. The method of claim 35, wherein the HCV-specific detection reagent is an antibody or an oligonucleotide probe or primer.
37. The method of claim 36, wherein the oligonucleotide probe or primer specifically hybridizes to an HCV genome or portion thereof.
38. The method of claim 31, wherein the source is a biological fluid, a tissue, or a cell.
39. The method of claim 38, wherein the biological fluid is blood, serum, plasma, or amniotic fluid.
40. The method of claim 31, wherein the test compound is an antibody, non-antibody polypeptide, or a nonpeptidyl agent.
41. A method for identifying a compound capable of inhibiting an HCV infection of a cell expressing DC-SIGNR comprising:
- (a) contacting the cell expressing DC-SIGNR with a source of HCV in the presence or absence of a test compound for a time sufficient to allow infection of the cell expressing DC-SIGNR by HCV; and
 - (b) detecting the HCV in the HCV-infected cell, wherein a reduction of HCV in the presence of the test compound compared to the amount of HCV in

the absence of the test compound is indicative of a compound capable of inhibiting the infection of the cell expressing DC-SIGNR by the HCV.

- 5 42. The method of claim 41, wherein the HCV is detected by RT-PCR followed by Southern blot.
43. The method of claim 41, wherein the HCV is detected by real-time PCR.
- 10 44. The method of claim 41, wherein the HCV is detected using an immunoassay.
45. The method of claim 41, wherein the HCV is detected
15 using an HCV-specific detection reagent.
46. The method of claim 45, wherein the HCV-specific detection reagent is an antibody or an oligonucleotide probe or primer.
- 20 47. The method of claim 46, wherein the oligonucleotide probe or primer specifically hybridizes to an HCV genome or portion thereof.
- 25 48. The method of claim 41, wherein the source is a biological fluid, a tissue, or a cell.
49. The method of claim 48, wherein the biological fluid is blood, serum, plasma, or amniotic fluid.
- 30 50. The method of claim 41, wherein the test compound is an antibody, non-antibody polypeptide, or a nonpeptidyl agent.
- 35 51. A method for identifying a compound capable of inhibiting the infection of a cell by HCV, said cell susceptible to infection by HCV, the method comprising:

- 5 (a) contacting a cell expressing DC-SIGN with a source of HCV for a time sufficient to allow binding of HCV to the cell expressing DC-SIGN;
- (b) contacting the cell-bound HCV with a cell susceptible to infection by HCV in the presence or absence of a test compound for a time sufficient for infection in the absence of the test compound; and
- 10 (c) detecting infection of the cell susceptible to infection by HCV, wherein the absence of infection or the reduction of infection in the presence of the test compound compared to the infection in the absence of the test compound is indicative of a compound capable of inhibiting
- 15 infection.

52. A method for identifying a compound capable of inhibiting the infection of a cell by HCV, said cell susceptible to infection by HCV, the method comprising:
- 20 (a) contacting a cell expressing DC-SIGNR with a source of HCV for a time sufficient to allow binding of HCV to the cell expressing DC-SIGNR;
- (b) contacting the cell-bound HCV with a cell susceptible to infection by HCV in the presence or
- 25 absence of a test compound for a time sufficient for infection in the absence of the test compound; and
- (c) detecting infection of the cell susceptible to infection by HCV, wherein the absence of
- 30 infection or the reduction of infection in the presence of the test compound compared to the infection in the absence of the test compound is indicative of a compound capable of inhibiting infection.

FIGURE 1

msdskeprlq	qlglleeeql	rglgfrqtrg	ykslagclgh	gplvlqllsf	tllagllvqv
skvpssisqe	gsrqdaiyqn	ltqlkaavge	lseksklqei	yqeltqlkaa	vgelpekskl
geiygeltrl	kaavgelpek	sklgeiygel	twlkaavgel	pekskmqei	geltrlkaav
gelpekskqg	eiygeltrlk	aavgelpeks	kqgeiygelt	rlkaavgelp	ekskqgeiyq
eltqlkaave	rlchpcpwew	tffqgnncyfm	snsqrnwhds	itackevgaq	lvviksaeeq
nflqlqssrs	nrftwmglsd	lngegtwqvw	dgspllpfsk	qywnrgepnn	vgeedcaefs
gngwnddkcn	lakfwickks	aascsrdeeq	flspapatpn	pppa	

FIGURE 2

msdskeprvq	qlglleedpt	tsgirlfprd	fqfqqihghk	sstgclghga	lvlqllsfml
lagvlvailv	qvskvpssls	gegseqdaiy	qnltqlkaav	gelseksklq	ei ygeltqlk
aavgelpeks	klgei ygelt	rlkaavgelp	eksklgei yq	eltrlkaavg	elpeksklqe
iygeltrlka	avgelpeksk	lgei ygelte	lkaavgelpe	ksklgei yqe	ltqlkaavge
lpdgskqqqi	ygeltdlhta	ferlcrhchpk	dwtffqgncy	fmsnsqrnwh	dsvtacqevr
aqlvviktae	eqnflqlqts	rsnrfswmgl	sdlnqegtww	wvdgsplsp	fqrywnsgep
nns gnedxae	fsgsgwmdnr	cdvdnywick	kpaacfrde		

FIGURE 3

MSTNPKPQRKTKRNTNRRPQDVKFPGGGQIVGGVYLLPRRGPRL
 GVRATRKTSERSQPRGRROPIPKARRPEGRTWAQPGYPWPLYGNEGCGWAGWLLSPRG
 SRPSWGPTDPRRRSRNLGKVIDTLTCGFADLMGYIPLVGAPLGGAARALAHGVRVLED
 GVNYATGNLPGCSFSIFLLALLSCLTPASAYQVRNSSGLYHVTNDCPNSSIVYEAD
 AILHTPGCVPCVREGNASRCWVAVTPTVATRDGKLPTTQLRRHIDLLVGSATLCSALY
 VGDLCGSVFLVGQLFTFSRRHWTTQDCNCSIYPGHI TGHMAWDMMMNWSPTAALVV
 AQLLRIPQAIMDMIAGAHWGLAGIAYFSMVGNWAKVLVLLLFAGVDAETHVTGGSA
 GRTTAGLVGLLTPGAKQNIQOLINTNGSWHINSTALNCNESLNTGWLGLFYQHKFNSS
 GCPERLASCRRLTDFAGWGPI SYANGSGLDERPYCWHYPPRPGCIVPAKSVCGPVYC
 FTPSPVVVGTTDRSGAPTYSWGANDTDVFLNNTRPPLGNWFGCTWMNSTGFTKVCGA
 PPCVIGGVGNNTLLCPTDCFRKHPEATYSRCGSGPWITPRCMVDYPYRLWHYPCTINY
 TIFKVRMYVGGVEHRLEAACNWTRGERCDLEDNRSELSPLLLSTTQWQVLPSCFTTL
 PALSTGLIHLHQNI VDVQYLYGVGSSIASWAIKWEYVLLFLLLADARVCSCLWMLL
 ISQAEAALENLVILNAASLAGTHGLVSFLVFFCFAWYKGRWVPGAVYAFYGMWPLLL
 LLLALPQRAYALDTEVAASCGGVVLVGLMALTLSPYYKRYISWCMWLOQYFLTRVEAQ
 LHVWVPLNVRGGRDAVILLMCVHPTLVFDITKLLLAIFGPLWILQASLLKVPYFVR
 VOGLLRICALARKIAGGHYVQMAIKLGALTGTIVYNHLTPLRDWAHNGLRDLAVAVE
 PVVFSRMETKLITWGADTAACGDI INGLPVSARRGOEILLGPADGMVSKGWRLAPIT
 AYAQOTRGLLGCIIITSLTGRDKNQVEGEVQIVSTATQOTFLATCINGVCWTVYHGAGR
 TIASPKGPVIQMYTNVDQDLVGWPAPQGSRLTPTCTCGSSDLYLVTRHADVIPVRRRG
 DSRGSLSPRPI SYLKGSSGGPLCPAGHAVGLFRAAVCTRGVAKAVDFIPVENLETT
 MRSFVFTDNSSPPAVPQSFQVAHLHAPTGSKGSTKVPAAAYAAQGYKVLVLNPSVAATL
 GFGAYMSKAHGVDPNI RTGVRTITTGSPITYSTYGKFLADGGCSGGAYDIIICDECHS
 TDATSILGIGTVLDQAEAGARLVVLATATPPGSVTVSHPNIEEVALSTTGEIPFYGK
 AIPLEVIKGRHLIFCHSKKKCDELAACKLVALGINAVAYYRGLDVSVIP TSGDVVVVS
 TDALMTGFTGDFDSVIDCNTCVTQTVDFSLDPTFTIETTTLPQDAVSRTORRGRTGRG
 KPGIYRFVAPGERPSGMFDSSVLCECYDAGCAWYELTPAETTVRLRAYMNTPGLPVCQ
 DHLEFWEGVFTGLTHIDAHFLSOTKQSGENFPYLVAYQATVCARAQAPPSWDQMWKC
 LIRLKPTLHGPTPLLYRLGAVQNEVTLTHPITKYIMTCSADLEVVTSTWVLVGGVLA
 ALAAYCLSTGCVVIVGRIVLSGKPAIIPDREVLQEFDEMEECQHLPIYIEQGMMLAE
 QFKQKALGLLOTASRQAEVITPAVQTNWQKLEVFwakHMWNFISGIQYLAGLSTLPGN
 PAIASLMAFTAAVTSPLTTGOTLLFNI LGGWVAAQLAAPGAATAFVGAGLAGAAIGSV
 GLGKVLVDILAGYGAGVAGALVAFKIMSGEVPSTEDLVNLLPAILSPGALVGVVCAA
 ILRRHVGPGEAGAVQWMNRLIAFASRGNHVSPTHYVPESDAAARVAILSSSLTVTQLLR
 RLHQWISSECTTPCSGSWLRDIWDWICEVLSDFKTWLKAKLMPQLPGIPFVSCQRGYR
 GVWRGDGIMHTRCHCGAEITGHVKNGTMRI VGPRTCRNMWSGTFPINAYTTGPCTPLP
 APNYKFALWRVSAEEYVEIRRVGDFHYVSGMTTDLNLCPCQIPSPFEFFTEL DGVRLHR
 FAPPCKPLREEVSFRVGLHEYVPGSQLPCEPEPDVAVLTSMLTDP SHITAEAGRRL
 ARGSPPSMASSASQLSAPSLKATCTANHDSFDAELIEANLLWRQEMGGNITRVESEN
 KVVILDSFDPLVAEEDEREVSVP AEILRKSRRFARALPVWARPDYNPPLVETWKKPDY
 EPPVHGCPLPPRSPVPPPRKKRTVVLTESTLSTALAEATKSFGSSSTSGITGDN
 TTSSEPA PS GCPPDSDESYSMPPLEGEPGDPDLSGWSWSTVSSGADTEDVVCSSM
 SYSWTGALVTPCAAEEQKL PINALSNSLLRHHNLVYSTTSRSACQROKKVTFDRLOVL
 DSHYQDVLKEVKAAASKVKANLLSVEEACSLTPPHSAKSKFGYGAKDVRCHARKAVAH
 INSVWKDLLEDVTFIDTTIMAKNEVFCVQPEKGGRKPARLIVFPDLGVRVCEKMALY
 DVVSKLPLAVMGSSYGFQYSPGQORVEFLVQAWKSKKTPMGFSYDTRCFDSTVTESDIR
 TEEAIYQCCDLDPQARVAIKSLTERLYVGGPLTNSRGENCYRRCRASGVLTTSCGNT
 LTCYIKARAACRAAGLODCTMLVCGDDLVI CESAGVQEDAASLRAFTEAMTRYSAFP
 GDPPQPEYDLELITSCSSNVSAHDGAGKRVYYLTRDPTTFLARA AWETARHTPVNSW
 LGNIIMFAPTLWARMILMTHFFSVLIARDOLEQALNCEIYGACYSIEPLDLPPIIQRL
 HGLSAFSLHSYSPGEINRVAACLRLKLGVPPLRAWHRARSVRARLLSRGGRAAICGKY
 LFNWAVRTKLKLTPIAAAGRLDLSGWFTAGYSGGDIYHSVSHARPRWFWFCLLLLAAG
 VGIYLLPNR

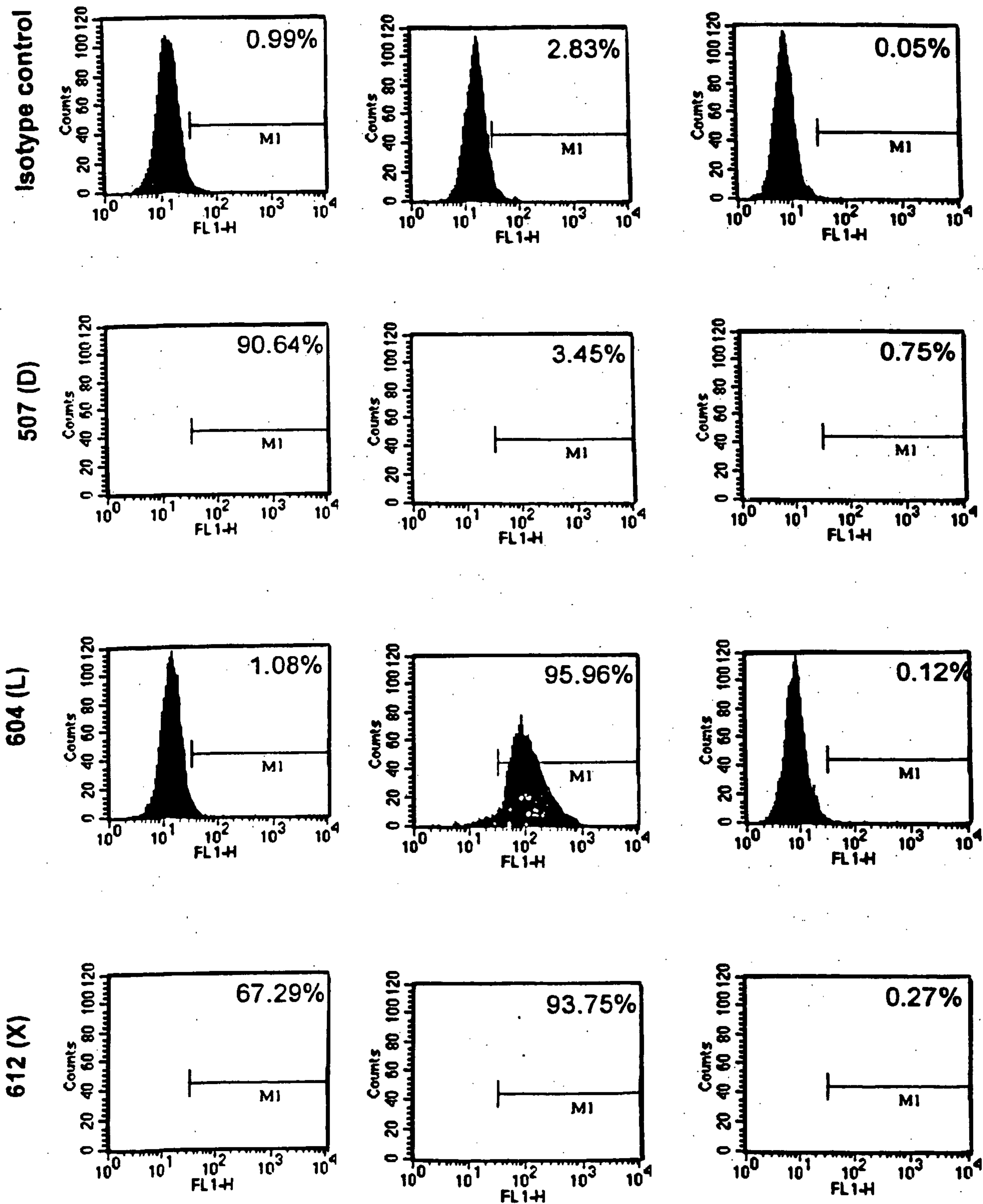
FIGURE 4**HeLa-DC-SIGN****HeLa-DC-SIGN-R****Parent HeLa**

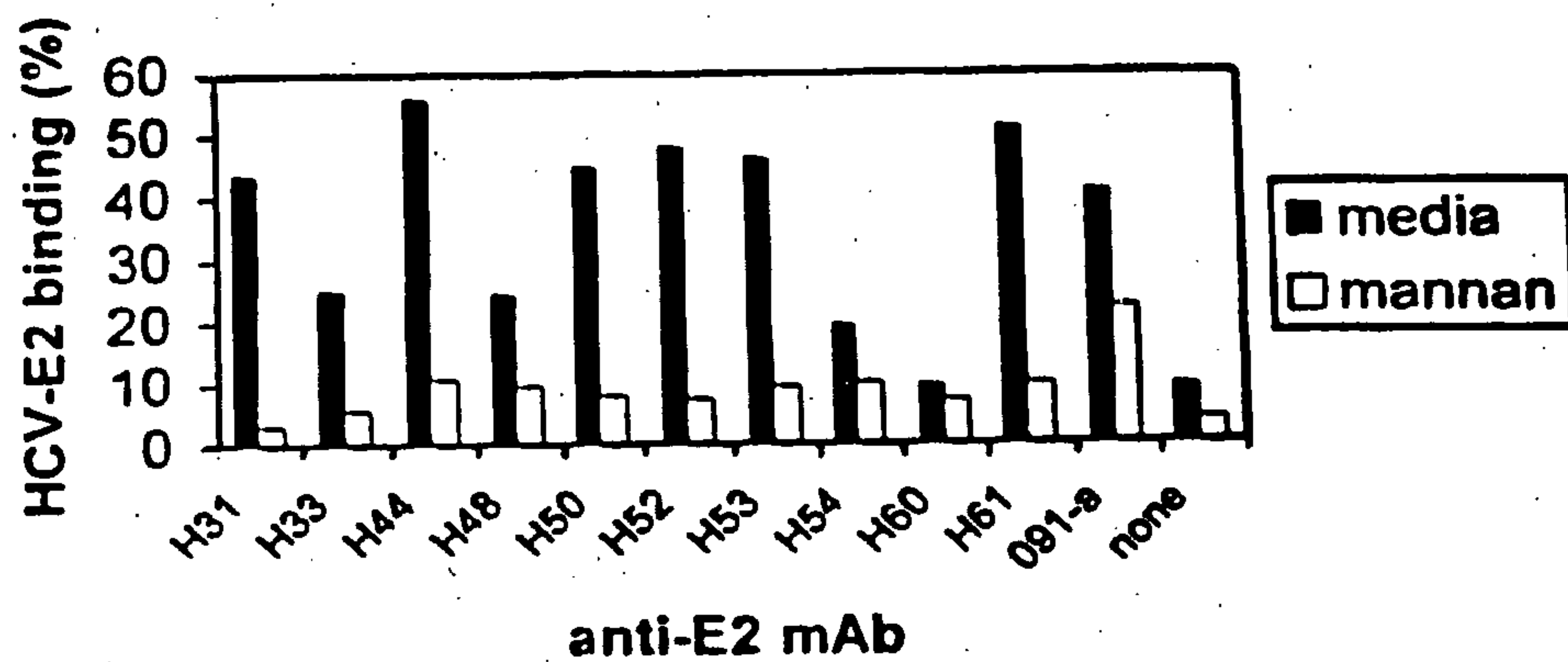
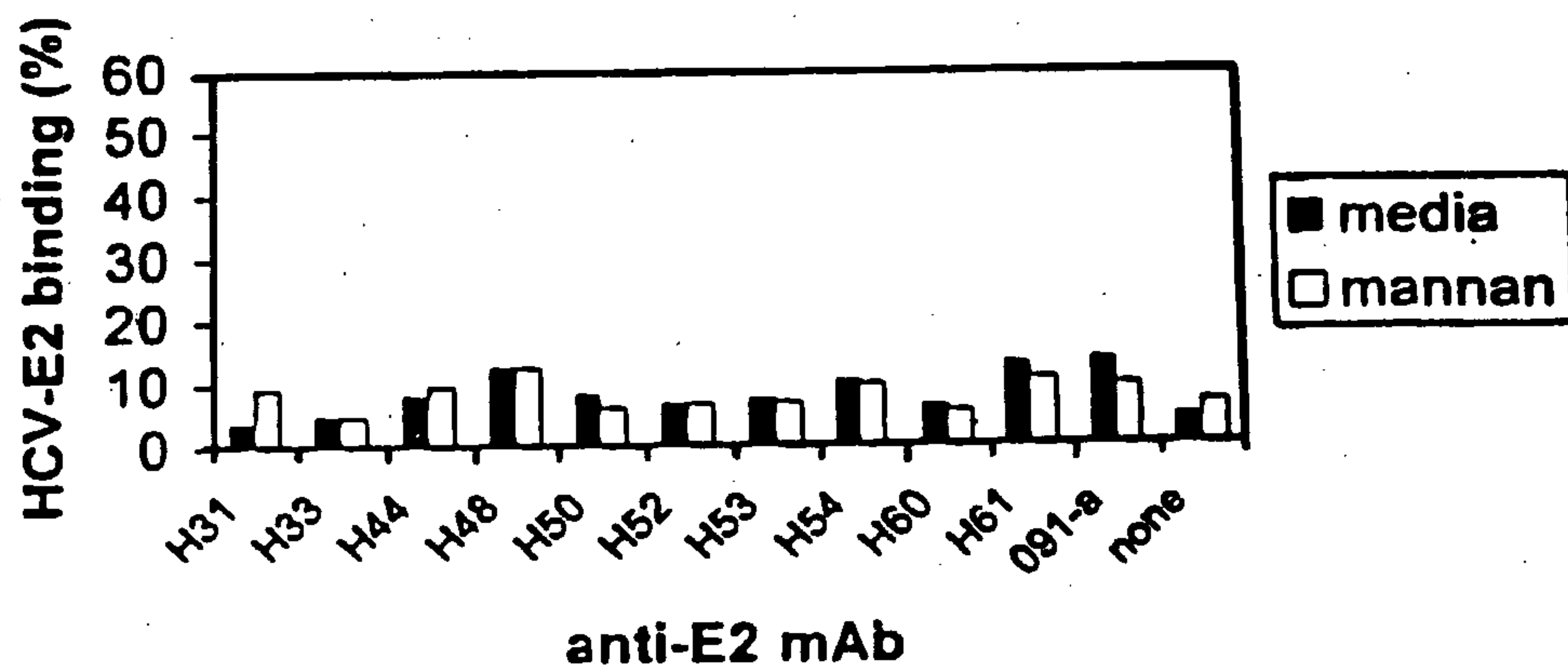
FIGURE 5A**FIGURE 5B****FIGURE 5C**

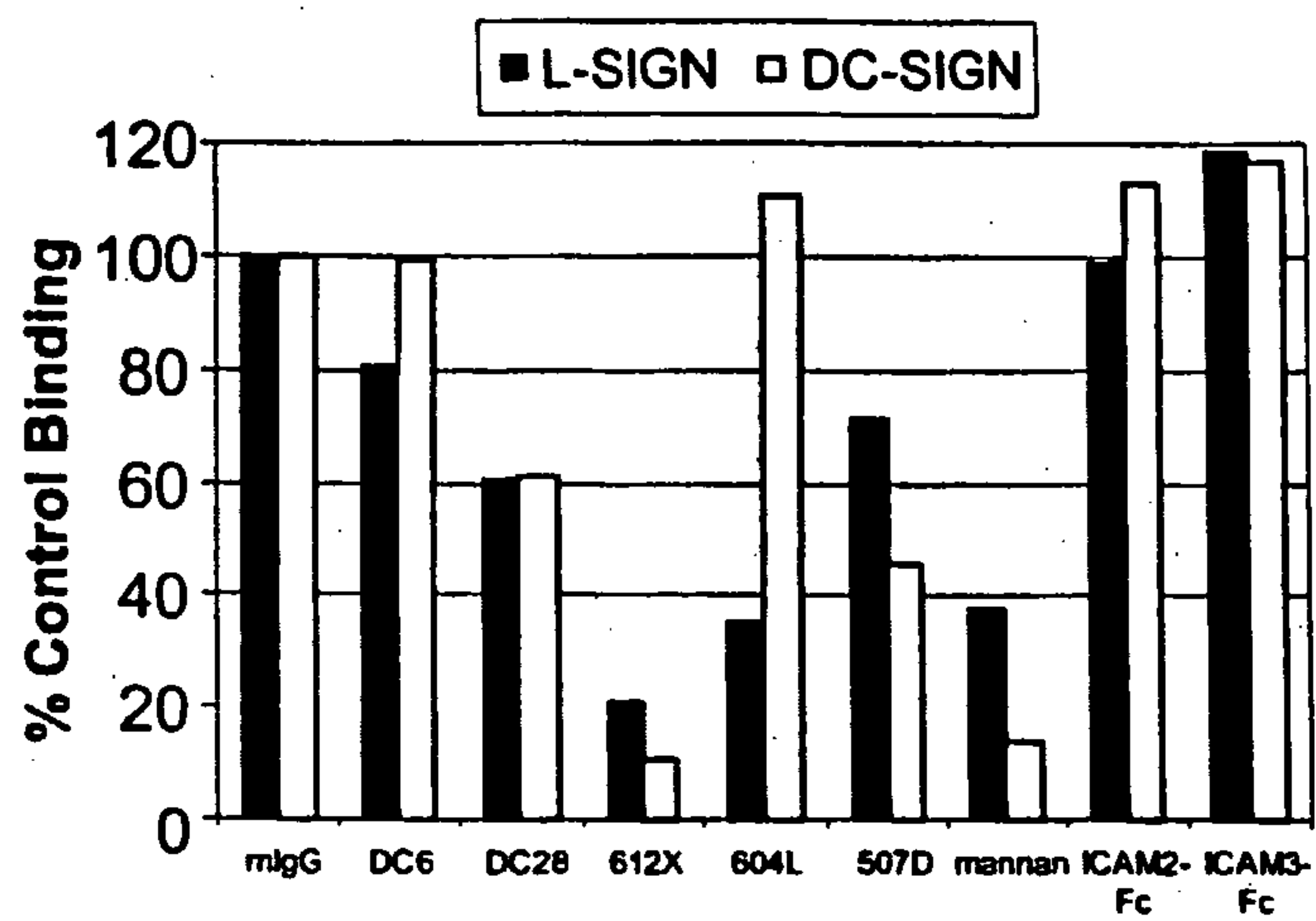
FIGURE 6

FIGURE 7A

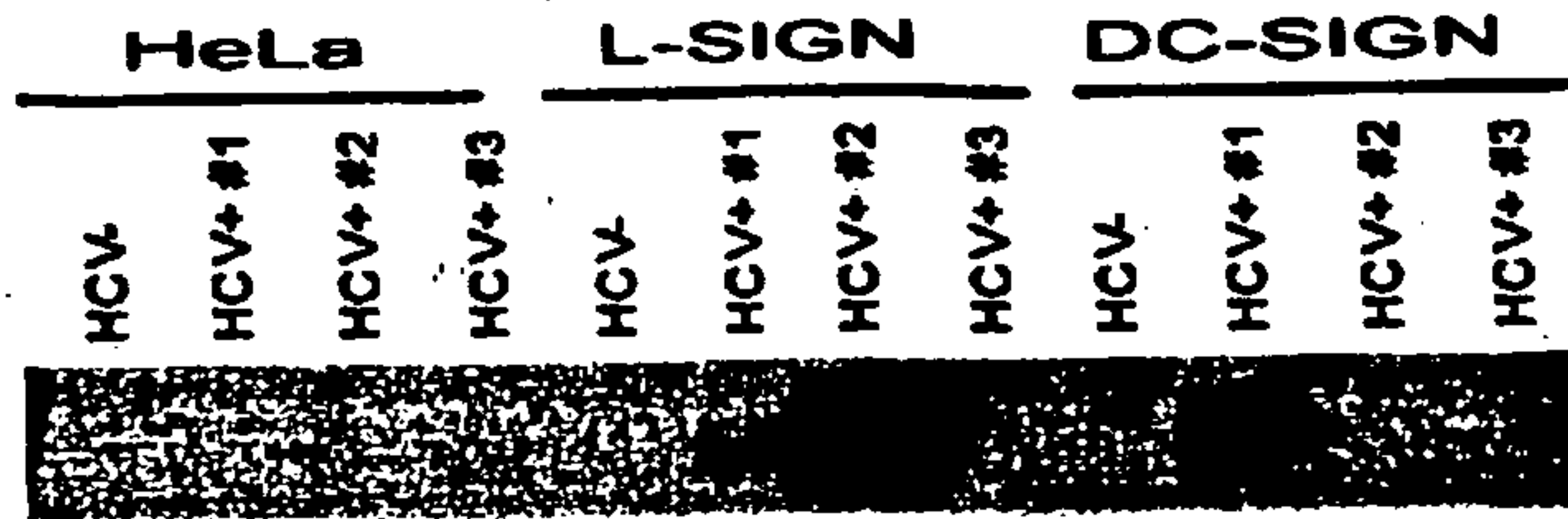


FIGURE 7B

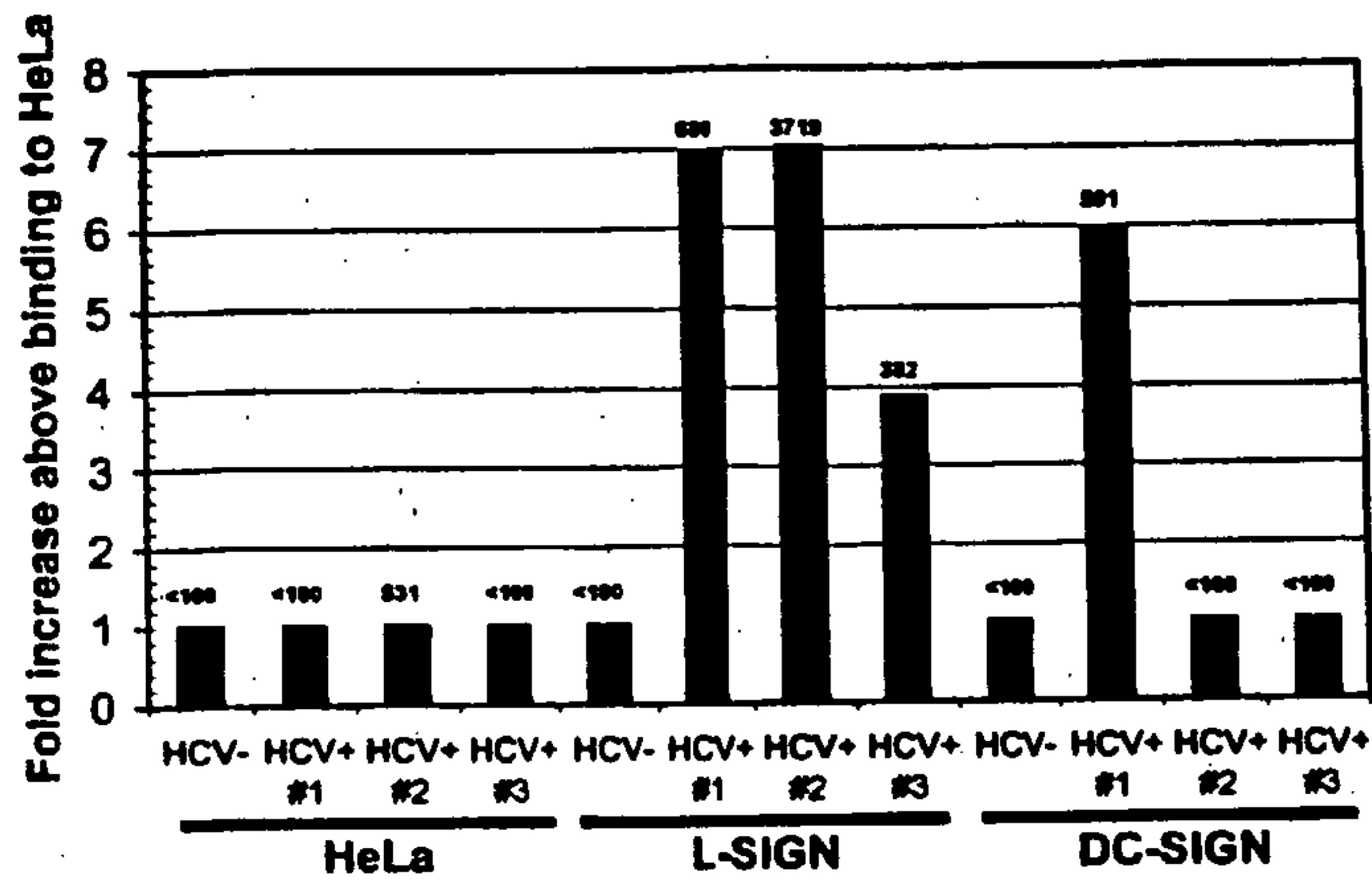


FIGURE 7C

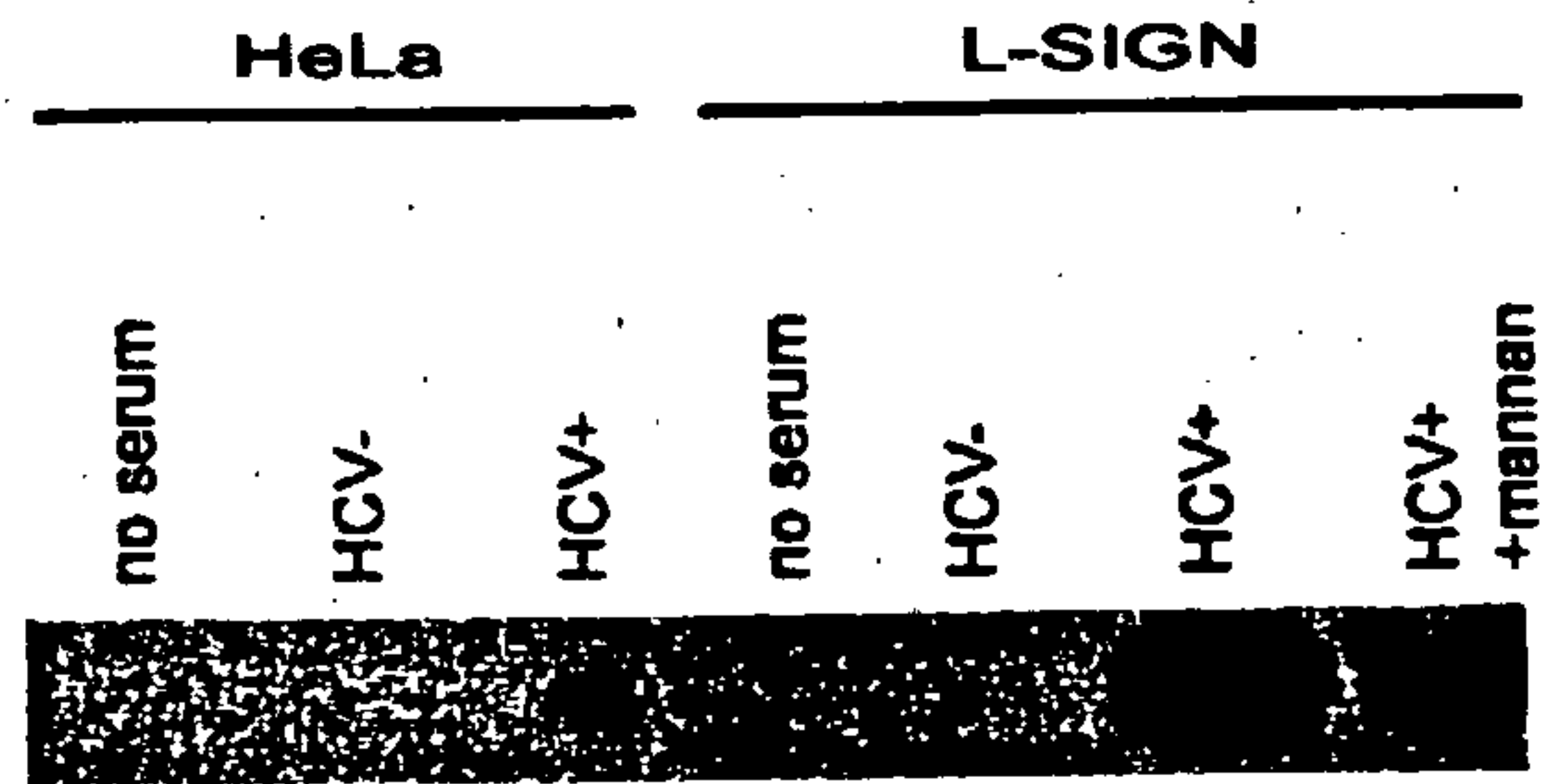


FIGURE 7D

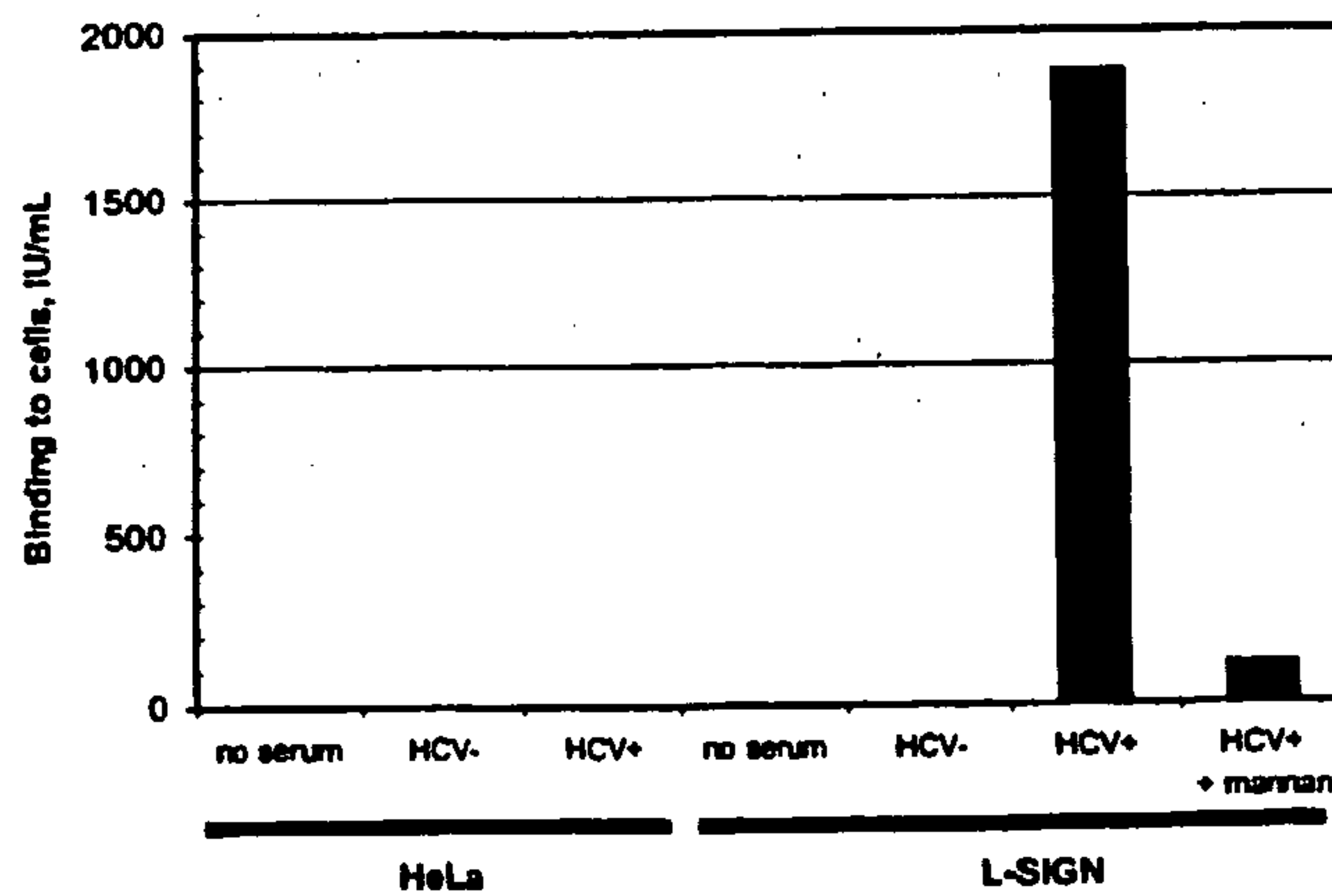


FIGURE 8