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(57) Abstract: The present invention provides identification and characterization of conformational epitopes of the envelope protein E2 of the Hepatitis C virus (HCV). The present invention provides a panel of human monoclonal antibodies that recognize conformational epitopes of E2. The antibodies are derived from patients infected with HCV. The present invention provides methods for utilizing HCV antibodies as therapeutic, diagnostic, and/or prophylactic agents. The present invention provides mimotopes with conformational epitopes intact and methods of using mimotopes. The present invention provides methods of stratifying patients based on their response to HCV. The present invention provides pharmaceutical compositions for prevention and treatment of HCV comprising one or more HCV antibodies.



WO 2010/039154 A1

HEPATITIS C ANTIBODIES AND USES THEREOF

Government Support

[0001] This invention was made with government support under grant number HL079381 awarded by the United States National Institutes of Health. The government has certain rights in the invention.

Background

[0002] Over 170 million people worldwide are infected with hepatitis C virus (HCV). Acute infection is usually silent, but the majority of infected individuals develop persistent infections (Major *et al.*, 2001, "Hepatitis C viruses," *Fields Virology*, 1127-1162; incorporated herein by reference). A small percentage of acute infections however resolve viremia with disease resolution. Cellular immunity is necessary, as a robust and sustained CD4⁺ T cell response is temporally associated with virus clearance leading to disease resolution (reviewed in Shoukry *et al.*, 2004, *Annu. Rev. Microbiol.*, 58:391; incorporated herein by reference).

[0003] Antiviral drugs (*e.g.*, interferon or PEGylated interferon) taken alone or in combination with ribavirin (*i.e.*, nucleoside analog which interferes with viral genome replication) can be used for the treatment of persons with chronic hepatitis C, but the cost of treatment is very high. Treatment with interferon alone is effective in about 10% to 20% of patients, while interferon combined with ribavirin is effective in about 30% to 50% of patients.

[0004] The present invention encompasses the recognition that antibodies may be developed that can be useful for prophylaxis, treatment, and/or diagnosis of HCV. A significant challenge for antibody-based vaccine development is defining conserved protective epitopes and generating antibodies that specifically recognize these epitopes.

Summary of the Invention

[0005] The present invention provides monoclonal antibodies, including human monoclonal antibodies, which bind to multiple hepatitis C virus (HCV) genotypes and/or subtypes. Such antibodies are useful in the prophylaxis, treatment, diagnosis, and/or study of HCV. In particular, the present invention provides monoclonal antibodies binding to conserved conformational epitopes of HCV envelope glycoprotein E2. In some embodiments, HCV

antibodies in accordance with the present invention are able to bind to many, most, or all cases of HCV. In some embodiments, monoclonal antibodies find use in a variety of diagnostic assays. HCV antibodies in accordance with the invention find use in passive immunotherapy for reducing viral load of infected individuals. Use of HCV antibodies in accordance with the invention may also interfere with the infection of target cells. Antibodies recognizing conserved epitopes can be used to provide a template for the rational design of peptide and conformationally defined epitope mimetics (*e.g.*, organic compounds, organometallic compounds, inorganic compounds, small molecules, *etc.*). In some embodiments, conserved regions of HCV E2 protein and fragments thereof are provided for use in therapeutics, prophylaxis, diagnostics, and/or other purposes.

[0006] In some embodiments, antibodies in accordance with the invention are directed to conformational epitopes of the E2 protein of HCV. Conformational and linear epitopes of E2 have been identified using a panel of monoclonal antibodies and a series of deletion constructs of E2. Previous studies (see, *e.g.*, U.S. Patent 6,692,908; and U.S. Patent Publications 2006/0104980 and 2006/0188571; all of which are incorporated herein by reference) have reported a class of antibodies that bind to conformational epitopes between E2 amino acids 411 – 644 from HCV 1b. Antibodies of this class have been found to inhibit the interaction of E2 with CD81. Another class of antibodies has been found to bind to conformational epitopes between HCV 1b E2 amino acids 470 – 644. A third class of antibodies binds to conformational epitopes between HCV 1b E2 amino acids 470 – 644 but fails to inhibit the binding of E2 to CD81. A fourth class binds to conformational epitopes between HCV 1b E2 amino acids 644 – 661. A fifth class binds to conformational epitopes between HCV 1b E1 amino acids 230 – 313. A sixth class binds to a linear HCV E1 epitopes derived from multiple genotypes. A seventh class binds to conformational epitopes that include in part HCV 1a E2 amino acids 657 – 698.

[0007] In some embodiments, conformational epitopes to which HCV antibodies are directed are conserved among HCV genotypes (*e.g.*, genotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and/or combinations thereof). In some embodiments, conformational epitopes to which HCV antibodies are directed are conserved among HCV subtypes (*e.g.*, subtypes 1a, 1b, 1c, 2a, 2b, 2c, 3a, 3b, 4a, 4b, 4c, 4d, 4e, 5a, 6a, 7a, 7b, 8a, 8b, 9a, 10a, 11a, and/or combinations thereof). In some embodiments, conformational epitopes to which HCV antibodies are directed are

conserved among HCV strains. It is estimated that over 100 strains of HCV exist, which are given numerical designations (*e.g.*, 1, 2, 3, *etc.*) after the genotype and subtype indication.

[0008] The present invention provides monoclonal antibodies that recognize HCV E2 epitopes that are more precisely defined than any of the epitopes described above. Antibodies in accordance with the invention cross-compete with some of the previously-described antibodies. However, some antibodies in accordance with the invention neutralize HCV in respected model systems with greater potency than previously-described antibodies. Thus, the present invention encompasses the recognition that the newly-identified antibodies, described herein, may provide greater therapeutic and/or prophylactic benefit than previously-described antibodies.

[0009] In some embodiments, anti-HCV E2 antibodies are selected from the group consisting of human monoclonal antibody HC-1 which is secreted by the hybridoma cell line deposited in the American Type Culture Collection (ATCC) under Accession number PTA-9416; human monoclonal antibody HC-3 secreted by the hybridoma cell line deposited in the ATCC under Accession number PTA-9417; human monoclonal antibody HC-11 secreted by the hybridoma cell line deposited in the ATCC under Accession number PTA-9418; and human monoclonal antibody CBH-23 secreted by the hybridoma cell line deposited in the ATCC under Accession number PTA-9419.

[0010] Antibodies in accordance with the present invention may be combined with pharmaceutically acceptable excipients to provide pharmaceutical formulations. The present invention provides pharmaceutical compositions for treatment, prevention, and/or diagnosis of hepatitis C infection. In some embodiments, pharmaceutical compositions in accordance with the invention comprise human antibodies capable of binding to the HCV envelope glycoprotein E2 and capable of neutralizing HCV infection *in vitro* and *in vivo*.

[0011] In some embodiments, pharmaceutical compositions in accordance with the invention may comprise fragments of HCV antibodies (*e.g.*, HC-1, HC-3, HC-11, and/or CBH-23) that substantially retain the antigen binding characteristics of the whole antibody. In some embodiments, pharmaceutical compositions may comprise HCV antibodies produced by recombinant methods that are well known in the art.

[0012] The present invention provides various therapeutic, prophylactic, and/or diagnostic uses of HCV antibodies in accordance with the invention. In some embodiments, pharmaceutical compositions comprising HC-1, HC-3, HC-11, and CBH-23 antibodies may be

used for the treatment of chronic and/or acute hepatitis C infection by administering to patients a therapeutically effective amount of the antibodies, or fragments thereof, capable of binding to HCV E2. A therapeutically effective amount is an amount sufficient to achieve one or more particular biological effects. A therapeutically effective amount may be administered in a single dose at a single time, or may be distributed over time in multiple individual doses (of the same or different sizes), or in continuous delivery (at a constant or variable rate). In certain embodiments, a therapeutically effective amount is an amount effective to achieve one or more desired biological results including, but not limited to: (i) alleviating one or more symptoms of HCV infection; (ii) reducing the number of circulating viral particles in an individual and/or in any one of an individual's organs (*e.g.*, liver); (iii) preventing reemergence, reducing the likelihood of reemergence, and/or delaying the reemergence of one or more symptoms of HCV infection; and/or (iv) preventing, reducing the likelihood of, and/or delaying the onset of an increase in the number of circulating viral particles in an HCV-infected individual and/or in any one of an individual's organs (*e.g.*, liver). Pharmaceutical compositions in accordance with the invention may be used to prevent, reduce the recurrence of, and/or delay the onset of HCV infection. They may be used, for example, for passive immunization of individuals recently exposed to HCV or at risk of being exposed to HCV, newborn babies born to HCV-positive mothers, and/or liver transplantation patients (*e.g.*, to prevent possible recurrent HCV infections in such patients).

[0013] The invention also provides pharmaceutical compositions comprising therapeutically effective amounts of antibodies in accordance with the invention combined with at least one other anti-viral agent as an additional active ingredient. Such agents may include, but are not limited to, interferons (*e.g.*, interferon α -2b, interferon- γ , *etc.*), anti-HCV monoclonal antibodies, anti-HCV polyclonal antibodies, RNA polymerase inhibitors, protease inhibitors, ribavirin, IRES inhibitors, helicase inhibitors, immunomodulators, antisense compounds, short interfering RNAs, short hairpin RNAs, micro RNAs, RNA aptamers, ribozymes, and combinations thereof.

[0014] Antibodies in accordance with the present invention define conformational epitopes in the HCV E2 protein, and compositions and compounds containing such epitopes are provided. For example, the present invention provides proteins, peptides, and small molecules comprising conformational epitopes of HCV E2 protein. Peptides may contain one or more epitopes recognized by antibodies in accordance with the present invention. In certain embodiments,

proteins are strings of concatenated peptides with optional linking sequences, at least one of which contains at least one conformational epitope of HCV. Peptides of the string may contain different conformational epitopes of HCV E2 protein. Alternatively, peptides of the string may all contain the same epitope. In general, it is desirable that the peptides of the string fold properly in order to display the conformational epitope(s) substantially as it appears in nature. Such proteins and peptides may be used in formulating vaccines or used in diagnostic tests.

[0015] The present invention also provides methods for stratifying patients based on their immunological response to HCV and for identifying those patients likely to respond well to HCV immunotherapy. For example, a patient's serum may be used to test for the presence of antibodies directed against a particular epitope of HCV. If the patient does not have adequate levels of antibodies directed to such an epitope, specifically a conformational epitope of HCV E2 protein, human monoclonal antibodies directed against the epitope may be administered to the patient. The patient's own immune response may be supplemented with antibodies in accordance with the invention or compositions thereof. In certain embodiments, immunotherapy aids in clearance of HCV virus and/or resolution of HCV infection. In certain embodiments, immunotherapy in accordance with the present invention treats and/or prevents chronic HCV infection.

Definitions

[0016] *Amino acid:* As used herein, term "amino acid," in its broadest sense, refers to any compound and/or substance that can be incorporated into a polypeptide chain. In some embodiments, an amino acid has the general structure $\text{H}_2\text{N}-\text{C}(\text{H})(\text{R})-\text{COOH}$. In some embodiments, an amino acid is a naturally occurring amino acid. In some embodiments, an amino acid is a synthetic amino acid; in some embodiments, an amino acid is a D-amino acid; in some embodiments, an amino acid is an L-amino acid. "Standard amino acid" refers to any of the twenty standard L-amino acids commonly found in naturally occurring peptides. "Nonstandard amino acid" refers to any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or obtained from a natural source. As used herein, "synthetic amino acid" encompasses chemically modified amino acids, including but not limited to salts, amino acid derivatives (such as amides), and/or substitutions. Amino acids, including carboxy- and/or amino-terminal amino acids in peptides, can be modified by

methylation, amidation, acetylation, protecting groups, and/or substitution with other chemical groups that can change the peptide's circulating half-life without adversely affecting their activity. Amino acids may participate in a disulfide bond. Amino acids may comprise one or posttranslational modifications, such as association with one or more chemical entities (*e.g.*, methyl groups, acetate groups, acetyl groups, phosphate groups, formyl moieties, isoprenoid groups, sulfate groups, polyethylene glycol moieties, lipid moieties, carbohydrate moieties, biotin moieties, *etc.*). The term "amino acid" is used interchangeably with "amino acid residue," and may refer to a free amino acid and/or to an amino acid residue of a peptide. It will be apparent from the context in which the term is used whether it refers to a free amino acid or a residue of a peptide.

[0017] *Animal:* As used herein, the term "animal" refers to any member of the animal kingdom. In some embodiments, "animal" refers to humans, of either sex and at any stage of development. In some embodiments, "animal" refers to non-human animals, at any stage of development. In certain embodiments, the non-human animal is a mammal (*e.g.*, a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, and/or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, insects, and/or worms. In certain embodiments, the animal is susceptible to infection by HCV. In some embodiments, an animal may be a transgenic animal, genetically engineered animal, and/or a clone.

[0018] *Antibody:* As used herein, the term "antibody" refers to any immunoglobulin, whether natural or wholly or partially synthetically produced. All derivatives thereof which maintain specific binding ability are also included in the term. The term also covers any protein having a binding domain which is homologous or largely homologous to an immunoglobulin-binding domain. Such proteins may be derived from natural sources, or partly or wholly synthetically produced. An antibody may be monoclonal or polyclonal. An antibody may be a member of any immunoglobulin class, including any of the human classes: IgG, IgM, IgA, IgD, and IgE. In certain embodiments, an antibody may be a member of the IgG immunoglobulin class. As used herein, the terms "antibody fragment" or "characteristic portion of an antibody" are used interchangeably and refer to any derivative of an antibody which is less than full-length. In general, an antibody fragment retains at least a significant portion of the full-length antibody's specific binding ability. Examples of antibody fragments include, but are not limited to, Fab,

Fab', F(ab')₂, scFv, Fv, dsFv diabody, and Fd fragments. An antibody fragment may be produced by any means. For example, an antibody fragment may be enzymatically or chemically produced by fragmentation of an intact antibody and/or it may be recombinantly produced from a gene encoding the partial antibody sequence. Alternatively or additionally, an antibody fragment may be wholly or partially synthetically produced. An antibody fragment may optionally comprise a single chain antibody fragment. Alternatively or additionally, an antibody fragment may comprise multiple chains which are linked together, for example, by disulfide linkages. An antibody fragment may optionally comprise a multimolecular complex. A functional antibody fragment typically comprises at least about 50 amino acids and more typically comprises at least about 200 amino acids. In some embodiments, an antibody may be a human antibody. In some embodiments, an antibody may be a humanized antibody.

[0019] *Approximately:* As used herein, the term “approximately” or “about,” as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term “approximately” or “about” refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0020] *Biologically active:* As used herein, the phrase “biologically active” refers to a characteristic of any substance that has activity in a biological system (*e.g.*, cell culture, organism, *etc.*). For instance, a substance that, when administered to an organism, has a biological effect on that organism, is considered to be biologically active. In particular embodiments, where a protein or polypeptide is biologically active, a portion of that protein or polypeptide that shares at least one biological activity of the protein or polypeptide is typically referred to as a “biologically active” portion.

[0021] *Characteristic portion:* As used herein, the term a “characteristic portion” of a substance, in the broadest sense, is one that shares some degree of sequence or structural identity with respect to the whole substance. In certain embodiments, a characteristic portion shares at least one functional characteristic with the intact substance. For example, a “characteristic portion” of a protein or polypeptide is one that contains a continuous stretch of amino acids, or a collection of continuous stretches of amino acids, that together are characteristic of a protein or

polypeptide. In some embodiments, each such continuous stretch generally contains at least 2, 5, 10, 15, 20, 50, or more amino acids. In general, a characteristic portion of a substance (*e.g.*, of a protein, antibody, *etc.*) is one that, in addition to the sequence and/or structural identity specified above, shares at least one functional characteristic with the relevant intact substance. In some embodiments, a characteristic portion may be biologically active.

[0022] *Expression*: As used herein, “expression” of a nucleic acid sequence refers to one or more of the following events: (1) production of an RNA template from a DNA sequence (*e.g.*, by transcription); (2) processing of an RNA transcript (*e.g.*, by splicing, editing, 5’ cap formation, and/or 3’ end formation); (3) translation of an RNA into a polypeptide or protein; and/or (4) post-translational modification of a polypeptide or protein.

[0001] *Functional*: As used herein, a “functional” biological molecule is a biological molecule in a form in which it exhibits a property and/or activity by which it is characterized.

[0023] *Gene*: As used herein, the term “gene” has its meaning as understood in the art. It will be appreciated by those of ordinary skill in the art that the term “gene” may include gene regulatory sequences (*e.g.*, promoters, enhancers, *etc.*) and/or intron sequences. It will further be appreciated that definitions of gene include references to nucleic acids that do not encode proteins but rather encode functional RNA molecules such as tRNAs, RNAi-inducing agents, *etc.* For the purpose of clarity we note that, as used in the present application, the term “gene” generally refers to a portion of a nucleic acid that encodes a protein; the term may optionally encompass regulatory sequences, as will be clear from context to those of ordinary skill in the art. This definition is not intended to exclude application of the term “gene” to non-protein-coding expression units but rather to clarify that, in most cases, the term as used in this document refers to a protein-coding nucleic acid.

[0024] *Gene product or expression product*: As used herein, the term “gene product” or “expression product” generally refers to an RNA transcribed from the gene (pre-and/or post-processing) or a polypeptide (pre- and/or post-modification) encoded by an RNA transcribed from the gene.

[0025] *Homology*: As used herein, the term “homology” refers to the overall relatedness between polymeric molecules, *e.g.*, between nucleic acid molecules (*e.g.*, DNA molecules and/or RNA molecules) and/or between polypeptide molecules. In some embodiments, polymeric molecules are considered to be “homologous” to one another if their sequences are at least 25%,

30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%

identical. In some embodiments, polymeric molecules are considered to be “homologous” to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% similar.

[0026] *Identity:* As used herein, the term “identity” refers to the overall relatedness between polymeric molecules, *e.g.*, between nucleic acid molecules (*e.g.*, DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of the percent identity of two nucleic acid sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second nucleic acid sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or substantially 100% of the length of the reference sequence. The nucleotides at corresponding nucleotide positions are then compared. When a position in the first sequence is occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, the percent identity between two nucleotide sequences can be determined using the algorithm of Meyers and Miller (CABIOS, 1989, 4: 11-17), which has been incorporated into the ALIGN program (version 2.0) using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity between two nucleotide sequences can, alternatively, be determined using the GAP program in the GCG software package using an NWSgapdna.CMP matrix.

[0027] *Isolated:* As used herein, the term “isolated” refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) produced, prepared, and/or manufactured by the hand of man. Isolated substances and/or entities may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about

70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% of the other components with which they were initially associated. In some embodiments, isolated agents are about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is “pure” if it is substantially free of other components. As used herein, calculation of percent purity of isolated substances and/or entities should not include excipients (*e.g.*, buffer, solvent, water, *etc.*)

[0028] *Mimotope*: As used herein, the term “mimotope” refers to a macromolecule which mimics the structure of an epitope. In some embodiments, a mimotope elicits an antibody response identical or similar to that elicited by its corresponding epitope. In some embodiments, an antibody that recognizes an epitope also recognizes a mimotope which mimics that epitope. In some embodiments, a mimotope is a peptide. In some embodiments, a mimotope is a small molecule, carbohydrate, lipid, or nucleic acid. In some embodiments, mimotopes are peptide or non-peptide mimotopes of conformationally-conserved HCV epitopes. In some embodiments, by mimicking the structure of the conformationally defined viral epitope, a mimotope interferes with the ability of HCV virus particles to bind to its natural binding partners (*e.g.*, HCV target receptor, E1 protein, *etc.*), *e.g.*, by binding to the natural binding partner itself.

[0029] *Nucleic acid*: As used herein, the term “nucleic acid,” in its broadest sense, refers to any compound and/or substance that is or can be incorporated into an oligonucleotide chain. In some embodiments, a nucleic acid is a compound and/or substance that is or can be incorporated into an oligonucleotide chain via a phosphodiester linkage. In some embodiments, “nucleic acid” refers to individual nucleic acid residues (*e.g.*, nucleotides and/or nucleosides). In some embodiments, “nucleic acid” refers to an oligonucleotide chain comprising individual nucleic acid residues. As used herein, the terms “oligonucleotide” and “polynucleotide” can be used interchangeably. In some embodiments, “nucleic acid” encompasses RNA as well as single and/or double-stranded DNA and/or cDNA. Furthermore, the terms “nucleic acid,” “DNA,” “RNA,” and/or similar terms include nucleic acid analogs, *i.e.*, analogs having other than a phosphodiester backbone. For example, the so-called “peptide nucleic acids,” which are known in the art and have peptide bonds instead of phosphodiester bonds in the backbone, are considered within the scope of the present invention. The term “nucleotide sequence encoding

an amino acid sequence” includes all nucleotide sequences that are degenerate versions of each other and/or encode the same amino acid sequence. Nucleotide sequences that encode proteins and/or RNA may include introns. Nucleic acids can be purified from natural sources, produced using recombinant expression systems and optionally purified, chemically synthesized, *etc.* Where appropriate, *e.g.*, in the case of chemically synthesized molecules, nucleic acids can comprise nucleoside analogs such as analogs having chemically modified bases or sugars, backbone modifications, *etc.* A nucleic acid sequence is presented in the 5' to 3' direction unless otherwise indicated. The term “nucleic acid segment” is used herein to refer to a nucleic acid sequence that is a portion of a longer nucleic acid sequence. In many embodiments, a nucleic acid segment comprises at least 3, 4, 5, 6, 7, 8, 9, 10, or more residues. In some embodiments, a nucleic acid is or comprises natural nucleosides (*e.g.*, adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine); nucleoside analogs (*e.g.*, 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyladenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O(6)-methylguanine, and 2-thiocytidine); chemically modified bases; biologically modified bases (*e.g.*, methylated bases); intercalated bases; modified sugars (*e.g.*, 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose); and/or modified phosphate groups (*e.g.*, phosphorothioates and 5'-*N*-phosphoramidite linkages). In some embodiments, the present invention is specifically directed to “unmodified nucleic acids,” meaning nucleic acids (*e.g.*, polynucleotides and residues, including nucleotides and/or nucleosides) that have not been chemically modified in order to facilitate or achieve delivery.

[0030] *Protein:* As used herein, the term “protein” refers to a polypeptide (*i.e.*, a string of at least two amino acids linked to one another by peptide bonds). Proteins may include moieties other than amino acids (*e.g.*, may be glycoproteins, proteoglycans, *etc.*) and/or may be otherwise processed or modified. Those of ordinary skill in the art will appreciate that a “protein” can be a complete polypeptide chain as produced by a cell (with or without a signal sequence), or can be a characteristic portion thereof. Those of ordinary skill will appreciate that a protein can sometimes include more than one polypeptide chain, for example linked by one or more disulfide bonds or associated by other means. Polypeptides may contain L-amino acids, D-amino acids, or

both and may contain any of a variety of amino acid modifications or analogs known in the art. Useful modifications include, *e.g.*, terminal acetylation, amidation, methylation, *etc.* In some embodiments, proteins may comprise natural amino acids, non-natural amino acids, synthetic amino acids, and combinations thereof. The term “peptide” is generally used to refer to a polypeptide having a length of less than about 100 amino acids, less than about 50 amino acids, less than 20 amino acids, or less than 10 amino acids. In some embodiments, proteins are antibodies, antibody fragments, biologically active portions thereof, and/or characteristic portions thereof.

[0031] *Recurrent HCV infection:* As used herein, a “recurrent HCV infection” refers to reemergence of clinical and/or laboratory evidence of infection, *e.g.*, one or more symptoms of infection or the presence of circulating HCV particles and/or HCV particles in the subject’s liver. In some embodiments, a recurrent HCV infection refers to such a condition in a subject who has been previously infected with HCV but who has received a liver transplant.

[0032] *Small Molecule:* In general, a “small molecule” is understood in the art to be an organic molecule that is less than about 2000 g/mol, less than about 1500 g/mol, less than about 1000 g/mol, less than about 800 g/mol, or less than about 500 g/mol. In some embodiments, small molecules are non-polymeric. In some embodiments, small molecules are not proteins, peptides, or amino acids. In some embodiments, small molecules are not nucleic acids or nucleotides. In some embodiments, small molecules are not saccharides or polysaccharides.

[0033] *Subject:* As used herein, the term “subject” or “patient” refers to any organism to which a composition of this invention may be administered, *e.g.*, for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include animals (*e.g.*, mammals such as mice, rats, rabbits, non-human primates, and humans; insects; worms; *etc.*). In some embodiments, a subject may be infected with, suffering from, and/or susceptible to HCV.

[0034] *Substantially:* As used herein, the term “substantially” refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term “substantially” is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[0035] *Suffering from*: An individual who is “suffering from” a disease, disorder, and/or condition (e.g., HCV infection) has been diagnosed with and/or displays one or more symptoms of a disease, disorder, and/or condition.

[0002] *Susceptible to*: An individual who is “susceptible to” a disease, disorder, and/or condition (e.g., HCV infection) has not been diagnosed with a disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition may exhibit symptoms of the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition may not exhibit symptoms of the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will develop the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will not develop the disease, disorder, and/or condition.

[0036] *Therapeutically effective amount*: As used herein, the term “therapeutically effective amount” means an amount of inventive composition that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition (e.g., HCV infection), to treat, diagnose, prevent, and/or delay the onset of the disease, disorder, and/or condition.

[0037] *Therapeutic agent*: As used herein, the phrase “therapeutic agent” refers to any agent that, when administered to a subject, has a therapeutic effect and/or elicits a desired biological and/or pharmacological effect. In some embodiments, a therapeutic agent is any substance that can be used to alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of and/or reduce incidence of one or more symptoms or features of HCV infection.

[0038] *Treating*: As used herein, the term “treat,” “treatment,” or “treating” refers to any method used to partially or completely alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of and/or reduce incidence of one or more symptoms or features of a disease, disorder, and/or condition (e.g., HCV infection). Treatment may be administered to a subject who does not exhibit signs of a disease, disorder, and/or condition. In some embodiments, treatment may be administered to a subject who exhibits only early signs of the disease, disorder, and/or condition for the purpose of decreasing the risk of developing pathology associated with the disease, disorder, and/or condition.

[0039] *Vector*: As used herein, “vector” refers to a nucleic acid molecule capable of transporting another nucleic acid to which it is associated. In some embodiment, vectors are capable of extra-chromosomal replication and/or expression of nucleic acids to which they are linked in a host cell such as a eukaryotic and/or prokaryotic cell. Vectors capable of directing the expression of operatively linked genes are referred to herein as “expression vectors.”

HCV Nomenclature

[0040] It is well known by those skilled in the art that HCV nomenclature typically utilizes an Arabic numeral (*e.g.*, “1,” “2,” “3,” “4,” *etc.*) that represents HCV genotype and a lowercase letter (*e.g.*, “a,” “b,” *etc.*) that represents HCV subtype. Although the rules of nomenclature are generally accepted in the art, those of ordinary skill in the art recognize that the rules of nomenclature are not always strictly followed by those of skill in the art in publications, presentations, conversation, *etc.* Thus, those skilled in the art would recognize that, for example, it is implicit that “HCV 1a,” “HCV genotype 1a,” and “HCV subtype 1a” could be used interchangeably by one of skill in the art, and that all three terms are intended to refer to HCV genotype 1, subtype a.

[0041] As used herein, Arabic numerals (*e.g.*, “1,” “2,” “3,” “4,” *etc.*) are used to refer to HCV genotype, and lowercase letters (*e.g.*, “a,” “b,” *etc.*) are used to refer to HCV subtypes. It will also be understood that, when HCV of a particular genotype is referred to herein, it is meant to encompass all subtypes of the named genotype. To give but one example, “genotype 1” is used herein to refer to all subtypes of genotype 1 (*e.g.*, genotype 1, subtype a; genotype 1, subtype b; *etc.*).

[0042] As used herein, any Arabic numerals (*e.g.*, “1,” “2,” “3,” “4,” *etc.*) that are present *after* the genotype and subtype designations will be understood to refer to the HCV strain.

Brief Description of the Drawing

[0043] *Figure 1: Binding to different HCV genotypes by indirect immunofluorescent assay.* 293T cells were transfected with the constructs bearing E1E2 sequences of genotypes 1 to 6. Twenty-four hours post-transfection, cells were fixed onto slides, and antibody binding to fixed

cells was detected by fluorescence microscopy. HC-1, HC-3, HC-11, and CBH-23 bound to genotypes 1a, 1b, 2a, 2b, 3a, 4, 5, and 6.

[0044] *Figure 2: Human monoclonal antibodies V_L and V_H domain CDR sequences translated from cDNA sequences.* Analysis revealed that each antibody contains distinct V_L and V_H CDR sequences.

[0045] *Figure 3: IgG subclass typing.* HC-1 is IgG2, whereas HC-3, HC-11, and CBH-23 are IgG1.

[0046] *Figure 4: Neutralization with different HCV genotypes.* Each antibody was tested at 20 μ g/ml for its ability to neutralize different HCV genotypes. The numbers are percent neutralization compared to no antibody control. RO4 is an isotype-matched human monoclonal antibody to HCMV and serves as a negative control.

[0047] *Figure 5: Antibody competition assay.* Competition analysis with representative biotin-labeled domains A (CBH-4D), B (CBH-5), and C (CBH-7) HCV antibodies was performed. HC-1 and HC-11 showed minimum competition with domains A and C antibodies, and 60% – 80% competition with CBH-5, suggesting that epitopes recognized by these new antibodies are located within domain B. CBH-23 showed minimum or no competition with domains A and B antibodies, and >90% competition with CBH-7, suggesting that this epitope is located within domain C. HC-3 showed minimum or no competition with domains A, B, and C antibodies, suggesting that this antibody recognizes a new distinct domain. Each control antibody (*i.e.*, CBH-4D, CBH-5 and CBH-7) inhibits itself and minimally inhibits the other two antibodies.

[0048] *Figure 6: Inhibition of E2 binding to CD81 by human monoclonal antibodies.*

Genotype 1b E1E2 expressed in 293T containing 1 μ g/ml E2 was incubated with each test antibody at 10 μ g/ml and the antibody-antigen complex was then added onto CD81 pre-coated wells. Detection of bound E2 to CD81 was measured with biotinylated CBH-4D. CBH-5 was used as a positive control and RO4 as a negative control. Preincubation of E2 glycoproteins with HC-1, HC-11, CBH-23, or CBH-5 reduced by over 90% E2 binding to CD81 compared to the RO4 negative control. Similar to other domain B or C HCV antibodies, these HCV antibodies neutralize HCV by blocking E2 binding to CD81. In contrast, preincubation of E2 glycoproteins in the presence of HC-3 did not reduce E2 binding to CD81. Experiments were performed twice in triplicate. Error bars indicate one standard deviation from the mean.

[0049] *Figure 7: Epitope mapping of HC-1 and HC-11 by alanine replacement.* Mutations were introduced into E2 from genotype 1a H77c strain (GenBank accession no. AF009606) by site-directed mutagenesis (Stratagene, CA). Mutated E2 proteins were expressed in 293T cells and analyzed by ELISA. Mutated amino acids are depicted on the y-axis. The number at the beginning of each peptide corresponds to the position in the polyprotein of reference strain H. (A) HC-1 and (B) HC-11 HCV antibodies binding to each mutant is expressed as the percent of binding value normalized by the binding of CBH-17 and divided by HC antibody binding to the wild-type on the x-axis.

[0050] *Figure 8: Epitope mapping of HC-3 by alanine replacement.* Alanine replacement was performed as shown in Figure 7.

[0051] *Figure 9: Time course of antibody-mediated neutralization of HCVpp infection.* To approximate the step that HC-3 inhibits during the entry pathway, a time course study was performed compared to domain B and an antibody to CD81. There are similar patterns of progressive loss in blocking virus entry with both anti-CD81 and the domain B HCV antibody, HC-11. This is expected as domain B HCV antibodies inhibit virus entry by blocking E2 binding to CD81. The similarity in patterns with HC-3 with these two antibodies suggests that HC-3 inhibits virus entry at a temporal step near virus interaction with CD81. This includes the possible inhibition of E2 interaction with a different HCV co-receptor, such as SR-B1 or a step immediately following CD81 engagement.

[0052] *Figure 10: HC-3 epitope is involved in E1-E2 dimerization.* Compared to H77c, HCVpp mutants R657A and D658A (and to a lesser extent, F679A) showed significant reduction in E1 in the cushion pellet HCVpp. This suggests that these residues are involved in heterodimer formation between E1 and E2. These residues have not been previously identified as being involved in E1E2 dimerization. Similar observations were observed with E1E2 from cell lysates. The E431A mutant serves as a control of a mutation not affecting heterodimer formation.

[0053] *Figure 11: Effect of HC-3 epitope mutagenesis on the infectivity of HCVpp.* Infectivity of virus having R657A, D658A and F679A, L692A, I696A, or D698A mutations was determined. Each mutation is lethal to the virus. A control mutation at E431A showed no change.

Description of Certain Embodiments of the Invention

[0054] The present invention provides antibodies which recognize hepatitis C virus (HCV) envelope glycoprotein 2 (E2). In some embodiments, HCV E2 antibodies are monoclonal antibodies, such as human monoclonal antibodies. In some embodiments, HCV E2 antibodies bind to E2 associated with one or more hepatitis C virus (HCV) genotypes and/or subtypes. In some embodiments, HCV E2 antibodies are useful for therapeutic, diagnostic, and/or prophylactic applications. The present invention provides pharmaceutical compositions comprising HCV E2 antibodies and methods for treating patients using HCV E2 antibodies.

[0055] In some embodiments, HCV E2 antibodies are identified and isolated from serum from infected patients. As described in the examples, a group of human monoclonal antibodies from peripheral B-cells of three HCV-infected individuals having high serum neutralization of binding titers were produced and characterized. Four human monoclonal antibodies to HCV E2 were produced and isolated, HC-1, HC-3, HC-11, and CBH-23. All four antibodies bind to genotypes 1a, 1b, 2a, 2b, 3a, 4, 5, and 6 of HCV (Figure 1). These antibodies bind to conformational epitopes, which are conserved across virus types and genotypes. HC-1, HC-11 and CBH-23 inhibit the interaction of E2 protein with human CD81 (Figure 6). HC-3 does not inhibit E2 binding to CD81 (Figure 6).

[0056] In some embodiments, HCV E2 antibodies in accordance with the invention provide for neutralization of a broad spectrum of HCV genotypes and/or subtypes. Both breadth of reactivity to multiple HCV genotypes and/or subtypes and the ability to interfere with the binding of HCV virions to susceptible cells are desirable for a therapeutically useful neutralizing antibody. The present invention also provides for design of peptide and non-peptide structural mimetics of HCV envelope proteins.

Hepatitis C Virus (HCV)

[0057] Hepatitis C virus (HCV) is an enveloped virus the genetic information for which is encoded in a 9.5 kb positive strand RNA genome. A highly conserved noncoding region of 341 bp is localized at the 5'-end of this viral genome, which is followed by a long open-reading frame coding for a polyprotein of approximately 3,010 amino acids. Two putative envelope glycoproteins E1 (gp35) and E2 (gp72) have been identified with 5 or 6 and 11 *N*-linked glycosylation sites, respectively. A high level of genetic variability is associated with the envelope genes. This variability is highly accentuated at the 5'-end of the E2 gene, where two

hypervariable regions termed HVR1 and HVR2, have been described. Antibodies to HVR1 appear to mediate virus neutralization in cell culture and chimpanzee protection studies (Farci *et al.*, 1996, *Proc. Natl. Acad. Sci., USA*, 93:15394-15399; and Shimizu *et al.*, 1994, *J. Virol.*, 68:1494-1500; both of which are incorporated herein by reference). Although progress has been made at inducing a broader immune response to HVR1 related sequences (Puntoriero *et al.*, 1998, *EMBO J.*, 17:3521-3533; incorporated herein by reference), unfortunately, antibodies to HVR1 tend to be isolate-specific and over time drive the replication of new viral variants that the existing immune response does not recognize (Farci *et al.*, 1994, *Proc. Natl. Acad. Sci., USA*, 91:7792-7796; Weiner *et al.*, 1992, *Proc. Natl. Acad. Sci., USA*, 89:3468-3472; and Kato *et al.*, 1993, *J. Virol.*, 67:3923-3930; all of which are incorporated herein by reference). HCV envelope antigens appear to be highly immunogenic when expressed in glycosylated forms (da Silva Cardoso *et al.*, 1997, *Ann. Hematol.*, 74:135-7; incorporated herein by reference). Preliminary data suggest the existence of conserved epitopes within the E2 protein (Lesniewski *et al.*, 1995, *J. Med. Virol.*, 45:415-22; incorporated herein by reference). The existence of neutralizing antibodies in serum from infected patients has been proposed.

[0058] Studies using HCV E1-E2 proteins expressed in mammalian cells have shown that infected individuals have an antibody response to HCV E2 composed in part to epitopes that are both conformational and linear in nature (Harada *et al.*, 1994, *J. Gen. Virol.*, 76:1223-1231; incorporated herein by reference). Studies involving the isolation of human monoclonal or recombinant antibodies to HCV E2 protein showed that a substantial fraction of these antibodies recognize conformational epitopes (da Silva Cardoso *et al.*, 1998, *J. Med. Virol.*, 55:28-34; incorporated herein by reference). As to biological function of these domains, investigators have employed surrogate assays to provide insights into virus neutralization since the virus cannot be grown *in vitro* (Houghton, "Hepatitis C viruses," In Fields, Knipe, Howley (eds.) *Virology*, Lippincott-Raven, Philadelphia, 1035-1058; incorporated herein by reference). One surrogate assay, the neutralization of binding (NOB) assay, evaluates the ability of a given antibody or serum to prevent the association of HCV E2 protein with a human T-cell line (Rosa *et al.*, 1996 *Proc. Natl. Acad. Sci., USA*, 93:1759-1763; incorporated herein by reference). The finding that serum antibodies obtained from chimpanzees protected by vaccination were strongly positive in the NOB assay provides support for the relevance of the assay as a measure of virus

neutralization activity (Rosa *et al.*, *supra*; and Ishii *et al.*, 1998, *Hepatology*, 28:1117-1120; both of which are incorporated herein by reference).

[0059] The human tetraspannin cell surface protein CD81 (also known as TAPA-1; for review see Levy *et al.*, 1998, *Ann. Rev. Immunol.*, 16:89-109; incorporated herein by reference) is the target protein bound by HCV E2 in the NOB assay (Pileri *et al.*, 1998, *Science*, 282:938-941; incorporated herein by reference). Furthermore, human CD81 binds to free virions, and subsequently is a possible receptor for HCV (Pileri *et al.*, *supra*). However, little is known about the conservation of the epitopes recognized by the previously identified NOB positive antibodies in HCV E2 proteins of different genotypes and/or subtypes.

[0060] Other approaches to detection of and protection against HCV include the development of peptide mimetics. As an example, peptide mimetics of Hepatitis type A and C viral proteins have been created through production of randomly generated synthetic and phage-display peptide libraries for use in detection assays and vaccination therapies (Mattioli *et al.*, 1995, *J. Virol.*, 69:5294-5299; and Prezzi *et al.*, 1996, *J. Immunol.*, 156:4504-4513; both of which are incorporated herein by reference). However, effective antibody binding of these mimotopes has only been compared to linearly defined viral epitopes. The sequential recombinant fusing of several linearly defined immunodominant HCV epitopes has been described for use in diagnostic assays (Chein *et al.*, 1999, *J. Clin. Microbiol.*, 37:1393-1397; incorporated herein by reference). However, this multiple-epitope fusion antigen designed from linear epitopes was not created to function in the same capacity as a conformational mimetic. It was not designed to interfere with binding to a target receptor.

[0061] References providing background information concerning HCV include Abrignani, 1997, *Springer Semin. Immunopathology*, 19:47-55; Simmonds, 1995, *Hepatology*, 21:570:583; and Mahaney *et al.*, 1994, *Hepatology*, 20:1405-1411; all of which are incorporated herein by reference. Vaccinia virus or baculovirus constructs having a portion of the HCV genome are described by Ralston *et al.* (1993, *J. Virology*, 67:6733-6761; incorporated herein by reference) and Lanford *et al.* (1993, *Virology*, 197:225-235; incorporated herein by reference).

HCV Antibodies

[0062] HCV envelope glycoprotein genes display some of the highest levels of genetic heterogeneity across genotypes and subtypes, with E2 displaying greater variability than E1. A

hypervariable region (HVR1) found at the N-terminus of E2 is highly immunogenic and is the major determinant for isolate-specific neutralizing antibody responses (Farci *et al.*, 1996, *Proc. Natl. Acad. Sci., USA*, 93:15394; and Shimizu *et al.*, 1994, *J. Virol.*, 68:1494; both of which are incorporated herein by reference). A study of sequential HCV isolates obtained from serum samples collected over a 26-year period from a specific patient showed that the serum antibodies fail to neutralize the concurrent dominant E1E2 species present at the same time point (von Hahn *et al.*, 2007, *Gastroenterology*, 132:667; incorporated herein by reference). Escape is associated with mutations in HVR1 leading to decreased binding and neutralization by monoclonal antibodies to HVR1 that were produced to the earliest isolate obtained from this patient. More broadly neutralizing antibodies are usually directed against conformational epitopes within E2 (Allander *et al.*, 2000, *J. Gen. Virol.*, 81:2451; Bugli *et al.*, 2001, *J. Virol.*, 75:9986; Habersetzer *et al.*, 1998, *Virology*, 249:32; Hadlock *et al.*, 2000, *J. Virol.*, 74:10407; and Ishii *et al.*, 1998, *Hepatology*, 28:1117; all of which are incorporated herein by reference).

[0063] The present inventors previously described a panel of neutralizing and nonneutralizing human monoclonal antibodies to conformational epitopes on HCV E2 derived from peripheral B cells of an individual infected with genotype 1b HCV. By cross-competition analysis three immunogenic clusters of overlapping epitopes with distinct functions and properties were identified (Keck *et al.*, 2005, *J. Virol.*, 79:13199; and Keck *et al.*, 2004, *J. Virol.*, 78:9224; both of which are incorporated herein by reference). All nonneutralizing antibodies fell within one cluster designated as domain A (Keck *et al.*, 2005, *J. Virol.*, 79:13199; incorporated herein by reference). Neutralizing antibodies segregated into two clusters, designated as domains B and C, with domain B antibodies having greater potency than domain C antibodies in blocking genotype 2a infectious cell culture virus (HCVcc) infection (Keck *et al.*, 2007, *J. Virol.*, 81:1043; incorporated herein by reference). All domain B antibodies as well as domain C antibodies were shown to inhibit E2 binding to CD81, a receptor for HCV shown to be essential for HCVpp and HCVcc entry into host cells (Hsu *et al.*, 2003, *Proc. Natl. Acad. Sci., USA*, 100:7271; and Tscherne *et al.*, 2006, *J. Virol.*, 80:1734; both of which are incorporated herein by reference).

[0064] Although four antibodies to overlapping epitopes within domain B were previously isolated from one HCV infected individual, it remains unclear to the extent that domain B epitopes on E2 are dominant targets of the immune response. Example 1 describes the isolation of two new human monoclonal antibodies, HC-1 and HC-11, from a genotype 1a HCV infected

individual that cross-compete with domain B antibodies from the earlier panel, thus, expanding the number of overlapping epitopes within this domain. These antibodies are directed at conformational epitopes conserved among all genotypes and/or subtypes; neutralize HCVpp and HCVcc, with some having greater potency than previously noted with other antibodies to this domain. The mechanism of neutralization with these antibodies is by inhibiting E2 binding to CD81. In addition, alanine substitution studies on E2 in a region that has been defined to engage CD81 (Owsianka *et al.*, 2006, *J. Virol.*, 80:8695; incorporated herein by reference) show that some contact residues within these HCV antibody epitopes are the same contact residues needed for E2 binding to CD81. A third human monoclonal antibody, CBH-23, was isolated from a genotype 1b HCV infected individual. CBH-23 cross-competes with a representative antibody to domain C and mediates neutralization by inhibiting E2 binding to CD81. A fourth human monoclonal antibody, HC-3, was isolated from a genotype 1b HCV infected individual. HC-3 does not cross-compete with representative domain A, B, and C antibodies from the earlier panel, thus indicating a new domain, designated D. HC-3 does not inhibit E2 binding to CD81 (Figure 6). The present invention encompasses the recognition that mechanisms by which HC-3 mediates virus neutralization occur at approximately the same time during virus entry as the virus interacts with CD81. While not wishing to be bound by any one particular theory, HC-3 may mediate virus neutralization by blocking virus attachment, which occurs prior to E2 binding to CD81; blocking virus interaction with a different co-receptor just before or after E2 binds to CD81; and/or blocking a conformational change in one or more viral envelope glycoproteins involved in successful virus entry.

[0065] By virtue of the variety of binding profiles of the antibodies, diagnostic assays may be employed which will detect a plurality of types and genotypes and/or subtypes, so as to provide a pan-anti-HCV antibody. In some embodiments, antibodies may be used for passive immunization, as protective therapy for individuals at risk for HCV or as a therapy for people who are seropositive for HCV.

[0066] References relating to the use of antibodies to HCV include Akatsuka *et al.*, 1993, *Hepatology*, 18:503-510; DeLalla *et al.*, 1993, *J. Hepatol.*, 18:163-167; Mondelli *et al.*, 1994, *J. Virol.*, 68:4829-4836; Siemoneit *et al.*, 1994, *Hybridoma*, 13:9-13; and Moradpour *et al.*, 1996, *J. Med. Virol.*, 48:234-241; all of which are incorporated herein by reference.

[0067] HCV antibodies in accordance with the invention offer several advantages over existing antibodies against HCV. Because non-homologous primary amino acid sequences may still define immunologically identical three-dimensional protein structures, antibodies binding to structurally conserved epitopes can recognize multiple, sequentially divergent HCV genotypes and/or subtypes in native conformation, whereas antibodies recognizing only linear or denatured epitopes may not. In particular, antibodies that recognize conformationally dependent epitopes of E2 may effectively interfere with the interaction of HCV virus with its cellular target receptor. Using antibodies that recognize conformationally dependent epitopes to actively interfere with the ability of native HCV virus to bind to target cell receptors (*e.g.*, CD81) has specific therapeutic application for reducing viral load in infected individuals, and/or preventing infection or re-infection of organs in non-infected individuals (for example, by (i) recognizing HCV E2 proteins encoded by different HCV genotypes and/or subtypes; (ii) binding HCV particles; and/or (iii) preventing attachment and entry of HCV viral particles to their target cells), particularly in recent organ transplant recipients, individuals undergoing renal dialysis, babies born to HCV-infected mothers, and/or individuals undergoing treatment for hemophilia or other blood clotting disorders. In some embodiments, other recipients include individuals recently exposed to HCV (*e.g.*, via exposure to HCV-containing bodily fluids). In some embodiments, HCV antibodies may be useful for treatment of patients with strains of HCV that are identical to the strain of HCV to which the antibodies were raised. In some embodiments, HCV antibodies may be useful for treatment of patients with strains of HCV that are different from the strain of HCV to which the antibodies were raised. Both individual HCV antibodies and a cocktail of several HCV antibodies recognizing several epitopes may be employed.

[0068] In some embodiments, HCV antibodies may interfere with E2-associated viral infection by mechanisms other than preventing direct interaction with CD81. In some embodiments, HCV antibodies may interfere with viral infectivity by a number of possible mechanisms, including preventing E2 binding to co-receptor proteins, disrupting conformational changes in E2 proteins necessary for target cell binding, inhibiting E2-mediated viral fusion to target cells, and/or inhibiting uncoating of HCV virions. In some embodiments, HCV antibodies that directly interfere with E2 binding to CD81 may effectively complement HCV antibodies that interfere with infectivity by other mechanisms.

[0069] HCV antibodies in accordance with the invention which recognize viral epitopes and interfere with virus/target receptor interaction and viral epitopes which bind to such antibodies may also serve as templates for rationally designing peptide and other structural mimics of the viral epitopes. Structural molecular mimics defined by these HCV antibodies find use in their ability to block binding of the native virus to target receptors by binding to the target receptor themselves.

[0070] By producing human or humanized monoclonal antibodies, it is possible to directly analyze the human immune response to HCV. In some cases, it is desirable to use human or humanized monoclonal antibodies. By using human monoclonal antibodies, immune responses against the antibodies themselves as foreign antigens can be minimized, whereas vigorous immune responses are typically generated against monoclonal antibodies produced from non-human sources, because they are recognized as foreign antigens. Selecting for HCV antibodies that recognize conserved viral conformational epitopes affords broader and more effective therapeutic application of these reagents for ameliorating or preventing HCV infection than antibodies able to bind only linear or denatured epitopes. Antibodies described herein recognize conformational epitopes, which are highly conserved HCV E2 proteins of multiple different genotypes and/or subtypes. Thus, antibodies described herein have the advantage that they are more potent against a wide range of HCV isolates than many previously described neutralizing antibodies. An additional advantage is that the high conservation of the epitopes recognized by the antibodies described herein indicates that these antibodies recognize sequences with functional and/or structural significance within the HCV E2 protein. Thus peptides or small molecules isolated with these antibodies have a high probability of being functional regions within HCV E2.

Production and Characterization of HCV Antibodies

[0071] The overall strategy employed for the development of the subject HCV antibodies is described in Example 1. Briefly, (1) individuals with evidence of exposure to HCV were identified; (2) antigen specific B-cells from their peripheral blood were expanded and activated *in vitro*; (3) these cells were immortalized by electrofusion with a suitable mouse-human heteromyeloma; (4) relevant human antibody secreting hybridomas were identified; and (5) the relevant hybridomas were stabilized by cloning. This strategy resulted in the identification of

HCV antibodies that are specific to the HCV E2 protein and which bound to conformational epitopes of E2 of HCV genotypes 1a, 1b, 2a, 2b, 3a, 4, 5, and 6 (Figure 1).

[0072] As described in Example 1, peripheral B cells from individuals with either HCV 1a or 1b infection and high serum antibody neutralization activities were used to produce and characterize a panel of human monoclonal antibodies. The initial screening made use of a genotype 1b E2 protein to screen for antibodies derived from a 1a infected donor, or 1a E2 protein to screen for antibodies derived from a 1b infected donor. This step biased the screening approach used to the selection of antibodies to epitopes conserved between genotypes 1a and 1b since the donor was infected with either a genotype 1a or 1b isolate. Antibodies identified by such screening (*i.e.*, HC-1, HC-3, HC-11, and CBH-23) did not recognize E2 protein by western blot, but did recognize E2 protein by immunoprecipitation. These results suggest that these HCV antibodies recognize conformational epitopes.

[0073] HCV antibodies HC-1, HC-3, HC-11, and CBH-23 were all able to bind to E1E2 of HCV genotypes 1a, 1b, 2a, 2b, 3a, 4, 5, and 6 (Figure 1). HC-1 and HC-11 neutralize the majority of HCV genotypes 1a, 1b, 2a, 2b, 3a, 4, 5, and 6 HCVpp 1a (Figure 4) and 1b infection, as well as HCVcc 1a and 2a infection (Figure 4). HC-3 was able to neutralize HCVpp 1a and HCVcc 2a. HC-3 neutralizes 1a HCVpp and 2a HCVcc (Figure 4), and CBH-23 neutralizes genotype 1a, 1b, 2a HCVpp, and 2a HCVcc (Figure 4). These data indicate that all four HCV antibodies are able to recognize and neutralize multiple HCV genotypes. CD81 capture assays show that three of these four antibodies (*i.e.*, HC-1, HC-11, and CBH-23) are capable of blocking the interaction of E2 and CD81 (Figure 6). The present invention shows that epitopes that partially or fully overlap the CD81 binding site within HCV E2 are both conformational in nature and highly conserved. A high degree of sequence conservation in the CD81 binding site is consistent with the proposition that the interaction between HCV E2 and CD81 is biologically relevant. The present invention encompasses the recognition that, since HC-3 neutralizes HCV 1a and 2a but does not inhibit E2 binding to CD81, HC-3 might utilize alternative mechanisms of virus neutralization.

[0074] Competition analysis has been employed to define antibodies with similar binding sites in HCV E2. Previously identified HCV antibodies that recognize one of HCV E2 domains A, B, or C were biotinylated, and the binding of the biotinylated antibodies to HCV E2 in the presence of increasing amounts of competing HCV antibodies (*i.e.*, HC-1, HC-3, HC-11, and

CBH-23) was determined. HCV antibodies HC-1 and HC-11 effectively competed with antibodies that recognize domain B, but not domains A or C. CBH-23 effectively competed with an antibody recognizing domain C and not domains A or B. HC-3 did not compete with any domain A, B, or C antibodies.

[0075] These results indicate that conformational epitopes within HCV E2 glycoprotein are highly conserved among divergent HCV genotypes and/or subtypes. The antibodies that recognize these epitopes are useful as reagents to better define the structure of HCV E2. Furthermore, the antibodies that inhibited binding of HCV virions to human CD81 were shown to mediate virus neutralization of HCVpp and HCVcc models, which are well-accepted HCV models in the art. Indeed, one of ordinary skill in the art recognizes that successful neutralization of HCVpp and/or HCVcc is predictive of *in vivo* therapeutic activity (see, *e.g.*, Lavie *et al.*, 2007, *Curr. Issues Mol. Biol.*, 9:71-86; Bartosch *et al.*, 2003, *J. Exp. Med.*, 197:633-642; and Law *et al.*, 2008, *Nat. Med.*, 14:25-27; all of which are incorporated herein by reference).

[0076] Thus, the present invention provides HCV E2 antibodies HC-1, HC-3, HC-11, and/or CBH-23. The present invention provides antibodies that recognize the same or similar epitopes as HC-1, HC-3, HC-11, and/or CBH-23. In some embodiments, the invention provides antibodies that compete with HC-1, HC-3, HC-11, and/or CBH-23 for binding to E2 protein. In some embodiments, the invention provides antibodies that decrease HC-1, HC-3, HC-11, and/or CBH-23 binding to E2 protein by about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, or more than about 95%.

[0077] In some embodiments, the invention provides agents (*e.g.*, small molecules, peptides, nucleic acids, lipids, nanoparticles, *etc.*) that compete with HC-1, HC-3, HC-11, and/or CBH-23 for binding to E2 protein. In some embodiments, the invention provides (*e.g.*, small molecules, peptides, nucleic acids, lipids, nanoparticles, *etc.*) that decrease HC-1, HC-3, HC-11, and/or CBH-23 binding to E2 protein by about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, or more than about 95%.

[0078] The HCVpp (HCV pseudotyped particle) model comprises infectious retroviral particles pseudotyped with unmodified HCV E1 and E2 proteins (see, *e.g.*, Bartosch *et al.*, 2003, *J. Exp. Med.*, 197:633-642; Drummer *et al.*, 2003; *FEBS Lett.*, 546:385-390; Op De Beeck *et al.*, 2004, *J. Virol.*, 78:2994-3002; and Hsu *et al.*, 2003, *Proc. Natl. Acad. Sci., USA*, 100:7271-7276; all of which are incorporated herein by reference). HCVpp preferentially infect human hepatic

cells, are neutralized by anti-E2 antibodies, are neutralized by HCV-infected patient serum, and mimic the early stages of HCV infection, including viral entry (Bartosch *et al.*, 2003, *supra*; and Lavie *et al.*, 2007, *Curr. Issues Mol. Biol.*, 9:71-86; both of which are incorporated herein by reference). HCVpp-based assays are useful to observe virus entry and correlate with physiological conditions *in vivo*, and are suitable for development of new antiviral therapies (Bartosch *et al.*, 2003, *supra*).

[0079] The HCVcc (cell cultured HCV virion) model provides a cell-culture system that allows production of infectious, fully replicative HCV virions (Lindenbach *et al.*, 2005, *Science*, 309:623-626; Wakita *et al.*, 2005, *Nat. Med.*, 11:791-796; and Zhong *et al.*, 2005, *Proc. Natl. Acad. Sci., USA*, 102:9294-9299; all of which are incorporated herein by reference). The HCVcc system is based on the transfection of the human hepatoma cell line Huh-7 with genomic HCV RNA of the genotype 2a JFH1 strain cloned from an individual with fulminant hepatitis. In order to allow comparative studies between different HCV strains, chimeric genomes encoding structural proteins from different genotypes and non-structural proteins from the JFH1 isolate have also been made (Pietschmann *et al.*, 2006). HCVcc models are able to replicate *in vitro* in cell culture and *in vivo* in chimpanzees (Wakita *et al.*, 2005, *supra*). The HCVcc system allows for the study of the complete viral cycle life and confirms data generated with the HCVpp system.

[0080] It is well-established that the results of virtually all experiments performed using HCVcc virions are consistent with the results of similar experiments performed using HCVpp virions. See Lindenbach *et al.*, 2005, *Science*, 309:623-626; Lavie *et al.*, 2007, *Curr. Issues Mol. Biol.*, 9:71-86; Chapel *et al.*, 2007, *J. Gen. Virol.*, 88:1133-1143; Régeard *et al.*, 2007, *FEBS J.*, 274:4705-4718; Pietschmann *et al.*, 2006, *Proc. Natl. Acad. Sci., USA*, 103:7408-7413; Wakita *et al.*, 2005, *Nature Medicine*, 11:791-796; Yi *et al.*, 2006, *Proc. Natl. Acad. Sci., USA*, 103:2310-2315; Zhong *et al.*, 2005, *Proc. Natl. Acad. Sci., USA*, 102:9294-9299; all of which are incorporated herein by reference.

[0081] Another established model for studying HCV is the "HCV-Trimera" mouse model (Ilan *et al.*, 2002, *J. Infect. Dis.*, 185:153-61; incorporated herein by reference). The HCV-Trimera mouse model was developed by using lethally irradiated mice, reconstituted with Severe Combined Immunodeficiency (SCID) mouse bone marrow cells, in which human liver fragments infected *ex vivo* with HCV had been transplanted. HCV Trimera mice may be useful for

assessing *in vivo* therapeutic efficacy of potential HCV therapeutic and/or diagnostic agents (Ilan *et al.*, 2002, *J. Infect. Dis.*, 185:153-61; Eren *et al.*, 2006, *J. Virol.*, 80:2654-2664; both of which are incorporated herein by reference).

[0082] It is well-established that the results of experiments performed using either HCVcc or HCVpp virions in culture systems are consistent with the results of animal experiments performed using human liver-mouse chimeric models (Eren *et al.*, 2006, *J. Virol.*, 80:2654-2664; and Law *et al.*, 2008, *Nat. Med.*, 14:25-27; both of which are incorporated herein by reference). Furthermore, it has been established that inhibition of HCV replication in both *in vitro* assays and, by extension, *in vivo* small animal human mouse chimera models, *e.g.*, the Trimer mouse model, correlate with inhibition of HCV in non-human primates *in vivo*. For example, in a study that focused in part on the ability of selected polyclonal human IgG preparations in preventing infection in chimpanzees, preparations with high neutralization titers as defined by HCVpp assays were protective while preparations with low neutralization titers were not. See Yu *et al.*, 2004, *Proc. Natl. Acad. Sci., USA*, 101:7705-7710; incorporated herein by reference.

[0083] Given the predictive value of these model systems, one of ordinary skill in the art will readily recognize that HC-1, HC-3, HC-11, and/or CBH-23 (or antibodies that recognize the same epitopes as HC-1, HC-3, HC-11, and/or CBH-23) may be successfully used for neutralization of HCV *in vivo*. Analogous to the success achieved with hepatitis B immunoglobulin in liver transplantation (Dickson, 1998, *Liver Transpl. Surg.*, 4(5 Suppl 1):S73-S78; and Markowitz *et al.*, 1998, *Hepatology* 28:585-589; both of which are incorporated herein by reference), one possible application is to suppress HCV infection in liver transplant recipients with broadly reactive neutralizing human monoclonal antibodies.

[0084] While human monoclonal antibodies are provided, other antibodies from other sources may recognize the same epitopes recognized by the human antibodies described herein, and may also be employed. Generally antibodies from murine sources (*e.g.*, mice, rats, lagomorpha, *etc.*) and domestic animals (*e.g.*, rabbit, guinea pig, goat, sheep, pig, chicken, horse, hamster, *etc.*) find use. One may produce antibodies having the conserved regions of mammalian sources using genetic engineering and replacing the constant regions of the HCV antibodies provided herein or may use the proteins to be described below as immunogens for immunizing the animals and then immortalizing the resulting B cells and screening as described below for immortalized cells which produce monoclonal antibodies having analogous broad range binding specificity.

By screening in competitive assays with the subject HCV antibodies, one can determine whether the non-human antibodies bind to the same epitope.

[0085] Instead of using hybridomas as a source of the antibodies, genes encoding an HCV antibody or portion thereof may be isolated and introduced into an appropriate mammalian host cell, *e.g.*, CHO, CHO-K1, 293T, Huh7, Huh7.5, HeLa, CV1, or the like. Suitable expression plasmids include pcDNA3.1 Zeo, pIND(SP1), pREP8 (all available from Invitrogen, Carlsbad, CA) (see Example 1), and the like. Antibody genes may be expressed via viral or retroviral vectors, which may be exemplified by MLV based vectors, vaccinia virus based vectors, *etc.* Similarly, antibody genes may be expressed using the pCOMB series of vectors on the surface of M13 phage, as two independent chains, which may be renatured to form the intact antibody. Alternatively, antibodies may be expressed as a single chain, including at least the variable regions. Genes may be used for gene therapy by introducing the genes into appropriate cells, such as lymphocytes, muscle cells, fibroblasts, and the like, where antibodies may be expressed and secreted, either constitutively or inductively, to provide a continuous or intermittent source of antibody over a predetermined period of time, based on the lifetime of the host cell. Genes in conjunction with a marker gene (*e.g.*, antibiotic resistance; fluorescent label; nutrient selection; *etc.*) may be introduced in cell cultures of cells taken from a subject, the modified cells selected by means of the marker and the marked cells returned to the host. DNA may be introduced into cells using various plasmid DNA, naked DNA, DNA virus constructs (*e.g.*, adenovirus, adeno-associated virus, or vaccinia virus), and/or RNA viruses (*e.g.*, *Vesicular stomatitis* virus, sindbis virus, and semiliki forest virus), to name but a few. In some embodiments, a DNA construct has a promoter for which transcription factors are present in the subject cells or can be induced or introduced and the genes under the transcriptional control of such promoter. Other regulatory sequences may also be present, such as leaders for secretion, enhancers, RNA stabilizing sequences, and the like.

[0086] In some embodiments, antibodies are of the IgG class. In some embodiments, antibodies are of the IgG1 or IgG2 class (Figure 3). In some embodiments, antibodies are of the IgG₁ class. In certain embodiments, antibodies are of the IgG_{1k} class. In some embodiments, antibodies are of the IgG_{1γ}, IgG_{1δ}, or IgG_{1λ} class. In some embodiments, antibodies are of the IgG₂, IgG₃, or IgG₄ class. In some embodiments, antibodies are of the IgA, IgD, IgE, or IgM class. In some embodiments, antibodies are of the IgA₁ or IgA₂ class.

[0087] Antibodies may be used in their native form or may be truncated to provide Fab or F(ab')₂ fragments. Genes encoding heavy and light chains may be isolated and modified in a number of different manners. Conveniently, using RT-PCR, cDNA may be obtained for the genes in a convenient construction for further manipulation. Nucleotide sequences of variable regions of heavy and light chains may be isolated and joined, either directly or indirectly or through a chain of 3n nucleotides, where n is at least 1 and not more than about 60, usually not more than about 40, to provide a linker of amino acids between the two variable regions. The length of the chain can be determined empirically to provide the optimum affinity and other properties, *e.g.*, linkage through mercapto, carboxy, or amino groups, for chelation, bonding to a surface or other molecule, or the like.

[0088] Labels or tags may be attached to the gene encoding the antibody to provide for specific affinity isolation methods for the expressed antibody, attachment to a surface, labels or tags for identification, *etc.* Labels or tags may otherwise improve the utility of the isolated antibody gene. In some embodiments, labels or tags are associated with antibodies via linkers, such as cleavable arms, protease sites, *etc.* In some embodiments, labels or tags are directly associated (*e.g.*, covalently or non-covalently) to an antibody, a gene encoding an antibody, or fragments thereof.

[0089] Labels may include enzymes, chelating groups, ligands for binding to ligand binding proteins, *e.g.*, biotin/streptavidin, digoxigenin/antidigoxigenin, *etc.*, green fluorescent protein, and the like. The biotinylation sequence of *E. coli* biotin carboxylase carrier protein (BCCP) can be used for *in vivo* biotinylation of proteins expressed in *E. coli* or introduced in a lysate of *E. coli*. A sequence of six histidines or a sequence of alternating histidines and aspartic acids that are suitable for allowing binding of the antibody to a column containing immobilized divalent cations can be used. Sequences encoding high affinity epitopes may be employed, such as the FLAG epitope DYKDDDDK (SEQ ID NO: 1), the T7 tag sequence MASMTGGQMG (SEQ ID NO: 2), the S-tag sequence KETAAAKFERQHMDs (SEQ ID NO: 3), or any other sequence that confers high affinity binding to its correlative binding member or a protein reagent. Fusion proteins, besides the ones indicated above, include glutathione-S-transferase, luciferase, ligands to cell surface receptors found on hepatocytes, T-cells or other desirable cellular target, and the like. Such fusions are usually joined via a linker sequence of 3-50 amino acids that promotes the bi-functionality of the protein.

[0090] In some embodiments, labels include fluorescent, radioactive, luminescent, and/or enzymatically detectable moieties. Alternatively, antibodies may be linked to chelated toxic heavy metals or radioactive isotopes (*e.g.*, technetium, radioactive iodine, *etc.*). Antibodies may be chemically linked to fluorophores or chemiluminescent molecules. Chemical coupling may involve biotinylation using the activated carboxylic acid group or biotin-C11-hydroxysuccinimide ester, which will react with cysteines; coupling through the use of CNBr activation of various beads (sepharose, agarose, magnetic, polystyrene, *etc.*) or surfaces to link the antibodies, and the like; any number of other methods generally involving bridging the antibody to a useful chemical moiety, usually accomplished by modifying lysine or other basic residues or through use of reagents specific for free sulfhydryl groups.

[0091] In some embodiments, a label may provide cysteines for forming thioethers with maleimide groups, polyhistidine/cysteines or polyhistidines/aspartic acids for chelating metals, polylysines for reacting with aldehydes in reductive amination reactions, *etc.*

[0092] In some embodiments, a label may be a toxin, such as diphtheria toxin, ricin, abrin, ribosome inactivating proteins, apoptosis signaling proteins, pore forming proteins (*e.g.*, perforin), *etc.*

[0093] Genes for antibody heavy and light chain variable regions (particularly the hypervariable regions of the variable regions) may be mutated in accordance with known ways to enhance antibody binding affinity or to broaden reactivity. One may use *in vitro* selection to identify the optimum binding antibodies using phage display methodologies, random or directed mutagenesis of sequences, or other similar methodologies. Alternatively, one may use an alanine or glycine walk of the hypervariable regions to identify essential amino acids and then vary the amino acids at those or other sites to identify improved binding of the epitope. Other techniques known in the art may be employed to provide the mutagenized antibodies. See, *e.g.*, Gram *et al.*, 1992, *Proc. Natl. Acad. Sci., USA*, 89:3576-80; Griffiths *et al.*, 1993, *EMBO J.*, 12:725-34; de Kruif *et al.*, 1995, *J. Mol. Biol.*, 248:97-105; Low *et al.*, 1996, *J. Mol. Biol.*, 260:359-368; Hanes *et al.*, 2000, *Nat. Biotechnol.*, 18:1287-1292; Boder *et al.*, 2000, *Proc. Natl. Acad. Sci., USA*, 97:10701-10705; Graff *et al.*, 2004, *Protein Eng. Des. Sel.*, 17:293-304; Cumbers *et al.*, 2002, *Nat. Biotechnol.*, 20:1129-1134; Chowdhury and Pastan, 1999, *Nat. Biotechnol.*, 17:568-572; Neuberger and Milstein, 1995, *Curr. Opin. Immunol.*, 7:248-254; Jolly *et al.*, 1996, *Semin. Immunol.*, 8:159-168; Neuberger *et al.*, 1998, *Immunol. Rev.*, 162:107-116; Neuberger, 2002,

Biochem. Soc. Trans., 30:341-350; Beers *et al.*, 2000, *Clin. Cancer Res.*, 6:2835-2843; and Salvatore *et al.*, 2002, *Clin. Cancer Res.*, 8:995-1002; all of which are incorporated herein by reference.

[0094] References relating to production of human antibodies include Fountas *et al.*, 1990, *J. Immunol. Methods*, 70:83-90; and Zimmermann *et al.*, 1990, *J. Immunol. Methods*, 134:43-50; both of which are incorporated herein by reference. References relating to production of modified antibodies using combinatorial libraries, Burton and Barbas, Dixon, FJ (Ed.) *Advances in Immunology*, Vol. 57, Vi+391 p. *Academic Press, Inc.*, San Diego, CA, 191-280, 1994; Plaisant *et al.*, 1997, *Res. Virol.*, 148-169; and Barbas and Burton, *Monoclonal Antibodies from Combinatorial Libraries. Cold Spring Harbor Laboratory Course Manual*, Cold Spring Harbor, NY, 1994; all of which are incorporated herein by reference. Modified antibodies can be produced by mutagenesis followed by *in vitro* selection for a desirable property, such as increased affinity to a target antigen or broader or narrower specificity. Antibodies can also be modified by a toxin or other bioactive molecule. An assay for antibodies binding to HCV E2 is described by Rosa *et al.* (1996, *Proc. Natl. Acad. Sci., USA*, 93:1759-1763; incorporated herein by reference).

Conformational Epitopes

Characterization of Epitopes

[0095] Antibodies may be used to identify the structural epitopes on E2 protein that they bind. Two basic approaches may be employed using the monoclonal antibodies for identifying conformational epitopes. In the first, natural variants or mutation analysis of HCV isolates may be used to identify regions, and ultimately individual amino acids that are involved in the epitopes recognized by the monoclonal antibodies (Schwartz *et al.*, 1999, *J. Mol. Biol.*, 287:983-999; incorporated herein by reference). Antibodies are screened against a number of different HCV E2 isolates, identifying isolates that are selectively non-reactive with individual antibodies. "Chimeric" E2 envelope proteins are then constructed in which portions of the chimera are derived from E2 protein from one HCV genotype and other portions are derived from E2 protein of another HCV genotype. Chimeric E2 proteins are constructed by PCR amplifying overlapping fragments, and/or by using restriction sites common to both E2 proteins. An alternative method is DNA shuffling as pioneered by the biotechnology company MaxyGen

(reviewed in Cohen, 2001, *Science*, 293:237; Locher *et al.*, 2005, *DNA Cell Biol.*, 24: 256-263; Locher *et al.*, 2004, *Expert Opin. Biol. Ther.*, 4:589-597; Locher *et al.*, 2004, *Curr. Opin. Mol. Ther.*, 6:34-39; Kurtzman *et al.*, 2001, *Curr. Opin. Biotech.*, 12:361-370; and Minshull and Stemmer, 1999, *Curr. Opin. Chem. Biol.*, 3: 284-290; all of which are incorporated herein by reference). By surveying the observed binding reactivities of different chimeric E2 proteins with different monoclonal antibodies, amino acid regions in the E2 proteins involved in forming conformational epitopes are identified. Once the relevant regions are identified, individual amino acids that differ between the different genotypes are mutated to compose a reactive E2 sequence. Mutants that restore full reactivity identify amino acids that are involved in forming the epitope.

[0096] A second basic approach to defining a conformational epitope is to synthesize a series of overlapping peptides 10 – 15 residues in length that encode the desired sequence of HCV E2 (see, *e.g.*, Petit *et al.*, 2003, *J. Biol. Chem.*, 278:44385-44392; Moskalenko *et al.*, 2000, *J. Virol.*, 74:1761-1766; Pettersson, 1992, *Mol. Biol. Rep.*, 16:149-53; Gerlofs-Nijland *et al.*, 2003, *Nephron Exp. Nephrol.*, 94:e25-34; all of which are incorporated herein by reference). Peptides are then screened against the antibodies using high concentrations of antibody (often 100 µg/ml or higher). Individual regions that comprise the full conformational epitope often retain residual binding activity with the antibody that can be detected. Once these regions are identified, they can be confirmed using mutational studies involving the 10 – 15 residues of the peptide, either in the context of the peptide or by substituting into a conformationally intact HCV E2 protein. A variation of this methodology is described in Reineke *et al.* (1999, *Nat. Biotech.*, 17:271-275; incorporated herein by reference).

[0097] In some embodiments, the present invention provides definition of conformational epitopes in HCV E2 protein, and further provides compositions and compounds containing such epitopes. For example, the present invention provides mimicking agents (*e.g.*, proteins, peptides, small molecules, carbohydrates, lipids, nanoparticles, nucleic acids, mimotope, organic compound, organometallic compound, inorganic compound, *etc.*) comprising conformational epitopes of HCV E2 protein. Peptide agents may contain one or more epitopes recognized by the antibodies of the present invention. In certain embodiments, proteins are strings of concatenated peptides at least one of which contains a conformational epitope of HCV E2. The peptides of the string may contain different conformational epitopes of HCV or the peptides may contain the

same epitope. The peptides of the string should preferably fold properly in order to display the conformational epitope substantially as it appears in nature. Such proteins and peptides may be used in formulating vaccines or may be used in diagnostic tests.

[0098] The present invention provides a method for stratifying patients based on their immunological response to HCV and of identifying those patients likely to respond well to HCV immunotherapy. For example, a patient's serum may be used to test for the presence of antibodies directed against a particular epitope of HCV E2. If the patient does not have adequate levels of antibodies directed to such an epitope, human monoclonal antibodies directed against the epitope may be administered to the patient.

[0099] In formulating vaccines to HCV, any agent that mimics at least one conformational epitope of HCV E2 protein may be used. In some embodiments, epitopes represented in a vaccine include ones that are conserved among different genotypes of the virus or among different strains of the virus. In some embodiments, peptides or proteins that contain the conformationally-defined epitopes of E2 of HCV are used in the formulation of a vaccine to prevent an infection by HCV or to treat an HCV infection. In some embodiments, peptide or protein mimics may be less than 500, less than 200, less than 100, less than 50, less than 40, less than 30, or less than 20 amino acids in length. In some embodiments, peptides to be used in formulating a vaccine are peptide fragments of E2 protein of HCV. In some embodiments, a peptide folds in a manner similar to its fold in the native E2 protein thus preserving the three-dimensional structure of the conformational epitope.

[00100] A vaccine may also contain proteins that represent concatenated peptides that have the conformational epitope to which antibodies are desired. Several different peptides making up the multimer may be used so that each peptide contains a different epitope, or the same peptide may be used more than once in the multimer.

[00101] Peptides may be synthesized using any method known in the art including Merrifield solid phase chemistry. The peptides may also be obtained by cleavage of E1 or E2 protein and purification. The peptides may be made recombinantly and produced in *E. coli*, yeast (e.g., *S. cerevisiae*), insect cells (e.g., Sf9 cells), or mammalian cells (e.g., CHO cells) using any available techniques in the art (Sambrook *et al.*; Miller & Calos, eds., *Gene Transfer vectors for Mammalian Cells*, 1987; Ausubel *et al.*, eds., *Current Protocols in Molecular Biology*, 1987; each of which is incorporated herein by reference). Peptides may be modified to increase their

immunogenicity, solubility in aqueous solution, or to increase their propensity to fold correctly. For example, peptides may be glycosylated, farnesylated, hydroxylated, reduced, oxidized, *etc.*

[00102] In some embodiments, a peptide comprises amino acids 657-698 of the E2 protein of HCV. In some embodiments, a peptide comprises one or more of amino acids D658, F679, L692, I696, or D698 of the E2 protein of HCV. As would be appreciated by one of ordinary skill in this art, analogous amino acid sequences of E2 proteins from any genotype of HCV may be used. Analogous sequences may be determined by aligning multiple sequences of the E2 protein from different strains or genotypes of HCV. Homologous sequences that preserve the desired epitope may also be used in the formulation of vaccines. In some embodiments, sequences are at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or more homologous to the native sequence from HCV E2 protein.

[00103] In some embodiments, full-length HCV E2 proteins, as described herein, may have a sequence that is derived from a wild type HCV polyprotein. In some embodiments, full-length HCV E2 proteins, as described herein, may have a sequence that is derived from a non-wild type HCV polyprotein. Amino acid sequences of a variety of different HCV polyproteins (*e.g.*, from different genotypes, subtypes, isolates, *etc.*) are known in the art and are available in public databases such as GenBank. Exemplary HCV polyprotein sequences of multiple HCV genotypes, subtypes, and/or isolates are provided in Table 1 below.

Table 1: Exemplary HCV Polyprotein Sequences

GenBank Accession	Genotype & Subtype	HCV Polyprotein Sequence			
AAB67038	1a (H77)	MSTNPKPQRK	TKRNTNRRPQ	DVKFPGGGQI	VGGVYLLPRR
		KTSEERSQPRG	RRQPIPKARR	PEGRTWAQPG	YPWPLYGNEG
		RGSRPSWGPT	DPRRRSRNLG	KVIDTLTCGF	ADLMGYIPLV
		LAHGVRVLED	GVNYATGNLP	GCSFSIFLLA	LLSCLTVPAS
		YHVTND CPNS	SIVYEAADAI	LHTPGCVPCV	REGNASRCWV
		GKLPTTQLRR	HIDLLVGSAT	LCSALYVGDL	CGSVFLVGQL
		TQDCNCSIYP	GHITGHRMAW	DMMNWSPTA	ALVVAQLLRI
		AHWGVLGIA	YFSMVGNWAK	VLVVL L LFAG	VDAETHVTGG
		GLLTPGAKQN	IQLINTNGSW	HINSTALNCN	ESLNTGWL AG
		GCPERLTSCR	RLTDF AQWG	PISYANGSGL	DERPYCWHYP
		SVCGPVYCF	PSPVVVGTTD	RSGAPTYSWG	ANDTDV FVLN
		GCTWMNSTGF	TKVCGAPPCV	IGGVGNNTLL	CPTDCFRKHP
		PWITPRCMVD	YPYRLWHYPC	TINYTIFKVR	MYVGGVEHRL
		RCDLED RDRS	ELSPLLLSTT	QWQVLPCSFT	TLPALSTGLI
		YLYGVGSSIA	SWAIKWEYV	LLFLLLADAR	VCSC L WMMLL
		LVILNAASLA	GTHGLVSFLV	FFCF AWYLKG	RWVPGAAYAF
		LALPQRAYAL	DTEVAASCGG	VVLVGLMALT	LSPYYKRYIS
		TRVEAQLHVW	VPPLNVRGGR	DAVILLMCVV	HPTLVFDITK
		ILQASLLKVP	YFVRVQGLLR	ICALARKIAG	GHYVQMAIIK
		NHLTPLRDWA	HNGLRDLAVA	VEPVVFSRME	TKLITWGADT
				AACGDI INGL	

		<p> PVSARRGQEI LLGPADGMVS KGWRLQAPIT AYTTQQTRGLL GCIITSLTGR DKNQVEGEVQ IVSTATQTFL ATCINGVCWT VYHGAGTRTI ASPKGPVIQM YTNVDQDLVG WPAPQGSRSI APCTCGSSDL YLVTRHADVI PVRRRGDSRG SLLSPRPISY LKGSSGGPLL CPAGHAVGLF RAAVCTRGA KAVDFIPVEN LGTTRSPVF TDNSSPPAVP QSFQVAHLHA PTGSGKSTKV PAAYAAQGYK VLVLNPSVAA TLGFGAYMSK AHGVDPNIRT GVRTITTGSP ITYSTYKFL ADGGCSGGAY DIIICDECHS TDATSILGIG TVLDQAETAG ARLVVLATAT PPGSVTVSHP NIEEVALSTT GEIPFYGKAI PLEVIKGRH LIFCHSKKCC DELAALKVAL GINAVAYYRG LDVSVIPTSG DVVVVSTDAL MTGFTGDFDS VIDCNTCVTQ TVDFSLDPTF TIETTTLPQD AVSRTQRRGR TGRGKPGIYR FVAPGERPSG MFDSSVLCEC YDAGCAWYEL TPAETTVRLR AYMNTPGLPV CQDHLEFWEG VFTGLTHIDA HFLSQTKQSG ENFPYLVAYQ ATVCARAQAP PPSWDQMWKC LIRLKP TLHG PTPLLYRLGA VQNEVTLTHP ITKYIMTCMS ADLEVVTSTW VLVGGVLAAL AAYCLSTGCV VIVGRIVLSG KPAIIPDREV LYQEFDEMEE CSQHLPIYEQ GMMMLAEQFKQ KALGLLQTAS RHAEVITPAV QTNWQKLEVF WAKHMWNFIS GIQYLAGLST LPGNPAIASL MAFTAAVTSP LTTGQTLLEN ILGGWVAAQL AAPGAATAFV GAGLAGAAIG SVGLGKVLVD ILAGYGAGVA GALVAFKIMS GEVPSTEDLV NLLPAILSPG ALVVGVCVAA ILRRHVGPGG GAVQWMNRLI AFASRGNHVS PTHYVPESDA AARVTAILSS LTVTQLLRRL HQWISSECTT PCSGSWLRDI WDWICEVLSD FKTWLKAKLM PQLPGIPFVS CQRGYRGVWR GDGIMHTRCH CGAEITGHVK NGTMRIVGPR TCRNMWSGTF PINAYTTGPC TPLPAPNYKF ALWRVSAEEY VEIRRVGDFH YVSGMTTDLN KCPCQIPSPF FTELDGVRL HRFAPPCKPL LREEVSFRVG LHEYPVGSQ LCEPEPDVAV LTSMLTDP SH ITAEAAAGRRL ARGSPPSMAS SSASQLSAPS LKATCTANHD SPDAELIEAN LLWRQEMGGN ITRVESENKV VILDSFDPLV AEEDEREVS PAEILRKSRR FARALPVWAR PDYNPPLVET WKKPDYEPV VHGCPLPPR SPPVPPPRK RTVVLTESTL STALAEATK SFGSSSTSGI TGDNTTSSE PAPSGCPPDS DVESYSSMPP LEPEGDPDL SDGSWSTVSS GADTEDVCC SMSYSWTGAL VTPCAAEEQK LPINALSNSL LRHNLVYST TSRSACQRQK KVTFDRLQVL DSHYQDVLKE VKAAASKVKA NLLSVEEACS LTPPHSAKSK FGYGAKDVRC HARKAVAHIN SVWKDLLEDS VTPIDTTIMA KNEVFCVQPE KGGRKPARLI VFPDLGVRVC EKMALYDVVS KLPLAVMGSS YGFQYSPGQR VEFLVQAWKS KKTMPMGFSYD TRCFDSTVTE SDIRTEEAII QCCDLDPQAR VAIKSLTERL YVGGPLTNSR GENCGYRRCR ASGVLTTSCG NTLTCYIKAR AACRAAGLQD CTMLVCDDDL VVICESAGVQ EDAANLRAFT EAMTRYSAPP GDPPQPEYDL ELITSCSSNV SVAHDGAGKR VYYLTRDPTT PLARAAWETA RHTPVNSWL NIIMFAPTLW ARMILMTHFF SVLIARDQLE QALNCEIYGA CYSIEPLDLP PIIQRLHGLS AFSLSHSYSPG EINRVAACLR KLGVPPLRAW RHRARSVRAR LLSRGGRAAI CGKYLFWNAV RTKLKLTPIT AAGRLDLGSW FTAGYSGGDI YHSVSHARPR WFWFCLLLLL AGVGIIYLLPN R (SEQ ID NO: 4) </p>
AAK08509	1b	<p> MSTNPKPQRK TKRNTNRRPQ DVKFPGGGQI VGGVYLLPRR GPRLGVRATR KTSERSQPRG RRQPIPKARR PEGRTWAQPG YPWPLYGNEG MGWAGWLLSP RGSRPSWGPT DPRRRSRNLG KVIDTLTCGF ADLMGYIPLV GAPLGGAARA LAHGVRVLED GVNATGNLP GCSFSIFLLA LLSCLTIPAS AYEVRNVSGI YHVTNDCSNS SIVYEAADMI MHTPGCVPCV RESNFSRCWV ALTPTLAARN SSIPTTTIRR HVDLLVGAAA LCSAMYVGDL CGSVFLVSQ LFTFSPRRYET VQDCNCSIYP GHVSGHRMAW DMMMNWSPTT ALVVSQLLRI PQAVVDMVAG AHWGVLAGLA YYSMVGNWAK VLVMLLFFAG VDGHVHTGG RVASSTQSLV SWLSQGPSQK IQLVNTNGSW HINRTALNCN DSLQTGFIAA LFYAHRFNAS GCPERMASCR PIDKFAQGWG PITHVVPNIS DQRPYCWHYA PQPCGIVPAS QVCGPVYCFT PSPVVVGTTD RSGVPTYSWG ENETDVLLLN NTRPPQGNWF GCTWMNSTGF TKTCGGPPCN IGGVGNNTLI CPTDCFRKHP EATYTKCGSG PWLTPRCLVD YPYRLWHYPC TINFITFKVR MYVGGVEHRL NAACNWTGRGE RCDLEDRDRS ELSPLLLSTT EWQVLPCSFT TLPALSTGLI HLHQNIQVQ YLYGVGSVVV SVVIKWEYVL LLFLLLADAR VCACLWMMLL IAQAEATLEN LVVLNAASVA GAHGLLSFLV FFCAAWYIKG RLVPGAAYAL YGVWPLLLLL </p>

		LALPPRAYAM DREMAASCGG AVFVGLVLLT LSPYYKVFLA RLIWWLQYFI TRAEHLQVW VPPLNVRGGR DAIILLTCAV HPELIFDITK LLLAILGPLM VLQAGITRVP YFVRAQGLIH ACMLVRKVAG GHYVQMAFMK LGALTGTYYIY NHLTPLRDWA HAGLRDLAVA VEPVVFSDME TKIITWGADT AACGDIILGL PVSARRGKEI LLGPADSLEG RGWRL LAPIT AYSQQTRGLL GCIITSLTGR DKNQVEGEVQ VVSTATQSFL ATCVNGVCWT VYHGAGSKTL AGPKGPITQM YTNVDQDLVG WQAPPGARSL TPCTCGSSDL YLVTRHADVI PVRRRGDSRG SLLSPRPVSY LKGSSGGPLL CPSGHAVGIF RAAVCTRGA KAVDFVPVES METTMRSPVF TDNSSPPAVP QSFQVAHLHA PTGSGKSTKV PAAYAAQGYK VLVLNPSVAA TLGFGAYMSK AHGIDPNIRT GVRTITTGAP VTYSTYGFKL ADGGCSGGAY DIIICDECHS TDSTTILGIG TVLDQAETAG ARLVVLATAT PPGSVTVPHP NIEEVALSNT GEIPFYGKAI PIEAIRGGRH LIFCHSKKKC DELAAKLSGL GINAVAYYRG LDVSVIPTIG DVVVATDAL MTGYTGFDS VIDCNTCVTQ TVDFS LDPTF TIETTTVPQD AVSRSQRRGR TGRGRRGIYR FVTPGERPSG MFDSSVLCEC YDAGCAWYEL TPAETSVRLR AYLNTPGLPV CQDHLEFWES VFTGLTHIDA HFLSQTKQAG DNFPYLVAYQ ATVCARAQAP PPSWDQMWK LIRLKPTLHG PTPLLYRLGA VQNEVTLLTHP ITKYIMACMS ADLEVVTSTW VLVGGVLAAL AAYCLTTGVS VIVGRIILSG RPAIVPDREL LYQEFDEMEE CATHLPYIEQ GMQLAEQFKQ KALGLLQTAT KQAEAAAPVV ESKWRALETF WAKHMWNFIS GIQYLAGLST LPGNPAIASL MAFTASITSP LTTQSTLLFN ILGGWVAAQL APPSAASAFV GAGIAGAAVG SIGLGKVLVD ILAGYGAGVA GALVAFKVMS GEMPSTEDLV NLLPAILSPG ALVVGVCVAA ILRRHVGPGE GAVQWMNRLI AFASRGNHVS PTHYVPESDA AARVTQILSS LTITQLLKRL HQWINEDCST PCSGSWLRDV WDWICTVLTD FKTWLQSKLL PQLPGVPFFS CQRGYKGVWR GDGIMQTTCP CGAQITGHVK NGSMRIVGPK TCSNTWHGTF PINAYTTGPC TPSPAPNYSR ALWRVAAEEY VEVTRVGDFH YVTGMTTDNV KCPCQVPAPPE FFSEVDGVRL HRYAPACRPL LREEVTFQVG LNQYLVGSQ L PCEPEPDVAV LTSMLTDP SH ITAETAKRRL ARGSPPSLAS SSASQLSAPS LKATCTTHHV SPDADLIEAN LLWRQEMGGN ITRVESENKV VVLDSDFDPLR AEEDEREVS PAEILRKS K FPAAMPIWAR PDYNPPLLES WKDPDYVPPV VHGCPLPPIK APPIPPPRRK RTVVLTESSV SSALAEATK TFGSSESSAV DSGTATALPD QASDDGDKGS DVESYSSMPP LELEGPDPL SDGSWSTVSE EASEDVCCS MSYTWTGALI TPCAAEESKL PINALSNSLL RHHNMVYATT SRSAGLRQKK VTFDRLQVLD DHYRDVLKEM KAKASTVKAK LLSVEEACKL TPPHSAKSKF GYGAKDVRNL SSKAVNHIHS VWKDLLEDTV TPIDTTIMAK NEVFCVQPEK GGRKPARLIV FPDLGVRVCE KMALYDVVST LPQVVMGSSY GFQYSPGQRV EFLVNTWKSK KNPMGFSYDT RCFDSTVTEN DIRVEESIYQ CCDLAPEARQ AIKSLTERLY IGGPLTNSKG QNCGYRRCRA SGVLTTS CGN TLTCYLKASA ACRAAKLQDC TMLVNGDDL V VICESAGTQE DAASLRVFTE AMTRYSAPPG DPPQPEYDLE LITSCSSNVS VAHDASGKRV YYLTRDPTTP LARAAWETAR HTPVNSWLGN IIMYAPTLWA RMILMTHFFS ILLAQEQLEK ALDCQIYGAC YSIEPLDLPQ IIERLHGLSA FSLHSYSPGE INRVASCLRK LGVPPLRVWR HRARSVRARL LSQGGRAATC GKYLFWNAVK TKLKLTPIPA ASQLDLSGWF VAGYSGGDIY HSLSRARPRW FMLCLLLLSV GVGIYLLPNR (SEQ ID NO: 5)
BAB32872	2a (JFH-1)	MSTNPKPQRK TKRNTNRRPE DVKFPGGGQI VGGVYLLPRR GPRLGVRTTR KTSERSQPRG RRQPIPKDRR STGKAWGKPG RPWPLYGNEG LGWAGWLLSP RGSRPSWGPT DPRHRSRNVG KVIDTLTCGF ADLMGYIPV GAPLSGAARA VAHGVRVLED GVNYATGNLP GFPPSIFLLA LLSCITVPVS AAQVKNTSSS YMVTNDCSND SITWQLEAAV LHVP GCVPCE RVGNTRSRCW PVSPNMAVRQ PGALTQGLRT HIDMVMSAT FCSALYVGDL CGGVMLAAQV FIVSPQYHWF VQECNCSIYP GTITGHRMAW DMMMNWSPTA TMILAYVMRV PEVIIDIVSG AHWGVMFGLA YFSMQGAWAK VIVILLLLAAG VDAGTTTVGG AVARSTNVIA GVFSHGPPQN IQLINTNGSW HINRTALNCN DSLNTGFLAA LFYTNRFNSS GCPGRLSACR NIEAFRIGWG TLQYEDNVTN PEDMRPYCWH YPPKPCGVVP ARSVCGPVYC FTPSPVVVGT TDRRGVPTYT WGENETDVFL LNSTRPPQGS WFGCTWMNST GFTKTCGAPP CRTRADFNAS TDLLCPTDCF RKHPDATYIK

		CGSGPWLTPK CLVHYPYRLW HYPCTVNFTI FKIRMYVGGV EHRLTAACNF TRGDRCLED RDRSQLSPLL HSTTEWAILP CTYSIDLPLS TGLLHLHQNI VDVQYMYGLS PAITKYVVRW EWVLLFLLL ADARVCACW MLILLGQAEA ALEKLVVLHA ASAANCHGLL YFAIFFVAW HIRGRVPLT TYCLTGLWPF CLLLMALPRQ AYAYDAPVHG QIGVGLLLI TLFTLTGPYK TLLGQCLWWL CYLLTLGEAM IQEWVPMQV RGGRDGIWA VTIFCPGVVF DITKWLLALL GPAYLLRAAL THVPYFVRAH ALIRVCALVK QLAGGRYVQV ALLALGRWTG TYIYDHLTPM SDWAASGLRD LAVAVEPIIF SPMEKKVIVW GAETAACGDI LHGLPVSARL GQEILLGPAD GYTSKGWKLK APITAYAQQT RGLLGAIVVS MTGRDRTEQA GEVQILSTVS QSFLGTTISG VLWTVYHGAG NKTLAGLRGP VTQMYSSAEG DLVGWPSPPG TKSLEPCKCG AVDLYLVRN ADVIPARRG DKRGALLSPR PISTLKSSG GPVLCPRGHV VGLFRAAVCS RGVAKSIDFI PVETLDVVTR SPTFSDNSTP PAVPQTYQVG YLHAPTGSKG STKVPVAYAA QGYKVLVLNP SVAATLGFGA YLSKAHGINP NIRTGVRTVM TGEAIVSTY GKFLADGGCA SGAYDIIICD ECHAVDATSI LGIGTVLDQA ETAGVRLTVL ATATPPGSVT TPHPDIEEVG LGREGEIPFY GRAIPLSCIK GGRHLIFCHS KKKCEDELAAL LRGMLNVA YRGLDVSII PAQGDVVVA TDALMTGYTG DFDSVIDCNV AVTQAVDFSL DPTFTITTQT VPQDAVSRQ RRGRTGRGRQ GTTRYVSTGE RASGMFDSVV LCECYDAGAA WYDLTPAETT VRLRAYFNTP GLPVCQDHLE FWEAVFTGLT HIDAHLFSQT KQAGENFAYL VAYQATVCAR AKAPPPSWDA MWKCLARLKP TLAGPTPLLY RLGPITNEVT LTHPGTKYIA TCMQADLEVMT TSTWVLAGGV LAAVAAYCLA TGCVSIIGRL HVNQRVVVAP DKEVLYEAFD EMEECASRAA LIEEGQRIAE MLKSKIQGLL QQASKQAQDI QPAMQASWPK VEQFWARHMM NFISGIQYLA GLSTLPGNPA VASMMAFSAA LTSPLSTSTT ILLNIMGGWL ASQIAPPAGA TGFVVSGLVG AAVGSIGLGK VLVDILAGYG AGISGALVAF KIMSGEKPSM EDVINLLPGI LSPGALVVG ICAILRRHV GPGEAVQWM NRLIAFASRG NHVAPTHYVT ESDASQVQTQ LLGSLTITSL LRRLHNWITE DCPICSGSW LRDVWDWVCT ILTDFKNWLT SKLFPKLPGL PFISCQKGYK GVWAGTGIMT TRCPCGANIS GNVRGLSMRI TGPKTCMNTW QGTFPINCYT EGQCAPKPPT NYKTAIWRVA ASEYAEVTQH GSYSYVTGLT TDNLKIPCQL PSPEFFSWVD GVQIHRFAPT PKPFRDEVS FCVGLNSYAV GSQLPCEPEP DADVLRSLMT DPPHITAETA ARRLARGSP SEASSSVSQL SAPSLRATCT THSNTYDVM V DANLLMEGG VAQTEPESRV PVLDFLEPMA EEESDLEPSI PSECMLPRSG FPRALPAWAR PDYNPLPVS WRRPDYQPPT VAGCALPPPK KAPTPPRRR RTVGLSESTI SEALQQLAIK TFGQPPSSGD AGSSTGAGAA ESGGPTSPGE PAPSETGSAS SMPPLEGEPG DPDLESDQVE LQPPPQGGGV APGSGSGSW TCSEEDTTV CCSMSYSWTG ALITPCSPEE EKLPIINPLSN SLLRYHNKVY CTTSKSASQR AKKVTFDRTQ VLDAHYSVL KDIKLAASKV SARLLTLEEA CQLTPPHSAR SKYGFGAKEV RSLSGRAVNH IKSVMKDLLE DPQTPIPTTI MAKNEVFCVD PAKGGKKPAR LIVYPDLGVR VCEKMALYDI TQKLPQAVMG ASYGFQYSPA QRVEYLLKAW AEKKDPMGFS YDTRCFDSTV TERDIRTEES IYQACSLPEE ARTAIHSLTE RLYVGGPMFN SKGQTCGYRR CRASGVLTS MGNTITCYVK ALAACKAAGI VAPTMLVCGD DLVVISSESG TEEDERNLRA FTEAMTRYSA PPGDPPRPEY DLELITSCSS NVSVALGPRG RRRYYLTRDP TTPLARAWE TVRHSPINSW LGNIIQYAPT IWVRMVLMT HFSILMVQDT LDQNLNFEMY GSVYSVNPLD LPAIIERLHG LDAFSMHTYS HHELTRVSA LRKLGAPPLR VWKSRARAVR ASLISRGGA AVCGRYLFNW AVKTKLKLTP LPEARLLDLS SWFTVGAGGG DIFHSVSRAR PRSLLFGLLL LFVGVGLFLL PAR (SEQ ID NO: 6)
BAB08107	2b (JPUT971017)	MSTNPKPQK TKNRNTNRRPQ DVKFPGGGQI VGGVYLLPRR GPRLGVRATR KTSERSQPRG RRQPIPKDRR STGKSWGKPG YPWPLYGNEG CGWAGWLLSP RGSRPWTGPS DPRHRSRNLG RVIDTITCGF ADLMGYIPVV GAPVGGVARA LAHGVRVLED GINYATRNLG GCSFSIFLLA LLSCVTVPVS SVEIRNISTS YYATNDCSNN SITWQLTNAV LHLPGCVPE NDNGTLRCWI QVTPNVAVKH RGALTHNLRA HVDVIVMAAT VCSALYVGDV CGAVMIVSQA LIVSPERHNF TQECNCSIY GHITGQMAW DMMLNWSPTL TMILAYAARV PELVLEIVFG GHWGVVFGLA YFSMQGAWAK VIAILLVAG VDATTYSTGA TVGRTVGSFA

		GLFKLGAQQN VQLINTNGSW HINRTALNCN DSLHTGFMAA LFYANKFNSS GCPERLSSCR GLDDFRIGWG TLEYETNVN VEDMRPYCWH YPPKPCGIVP AQSVCGPVYC FTPSPVVVGT TDRQGVPTYN WGDNETDVFL LNSTRPPRGA WFGCTWMNGT GFTKTCGAPP CRIRKDFNST LDLLCPTDCF RKHPDATYVK CGAGPWLTPR CLIDYPYRLW HYPCTVNFTI FKVRMYVGGV EHRFSAACNF TRGDRCRLED RDRGQQSPLL HSTTEWAVLP CSFSDLPAL S TGLLHLHQNI VDVQYLYGLS PAVTKYIVKW EWVLLFLLL ADARICACLW MLIILGQAEA ALEKLIILHS ASAASANGPL WFFIFFTAAW YLKGRRVPA TYSVLGLWSF LLLVLALPQQ AYALDAAEQG ELGLVILMII SIFTLTPAYK ILLSRSVWWL SYMLVLAEAQ VQQWVPPLEA RGGRDGIIWV AVILHPLHVF EVTKWLLAIL GSAYLLKASL LRVFYFVRAH ALLRVCTLRV HLAGARYIQM LLITMGRWTG TYIYDHLSP L STWAAQGLRD LAVAVEPVVF SPMEKKVIVW GAETVACGDI LHGLPV SARL GREVLLGPAD GYTSKGWKL APITAYTQQT RGLLGAIVS LTGRDKNEQA GOVQVLSSVT QSFLGTSISG VLWTVYHGAG NKTLPASPRG VTQMYTSAEG DLVGWPSPPG TKS LDPCTCG AVDLYLVTRN ADVIPVRRKD DRRGALLSPR PLSTLKGSSG GPVLCPRGHA VGLFRAAVCA RGVAKSIDFI PVESLDIARR TPSFSDNSTP PAVPQTYQVG YLHAPTSGSK STKVPAAYTS QGYKVLV LNP SVAATLGFGA YMSKAHGINP NIRTGVRTVT TGDSITYSTY GKFLADGGCS AGAYDIIICD ECHSVDATTI LGIGTVLDQA ETAGVRLVVL ATATPPGTVT TPHANIEEVA LGHEGEIPFY GKAIPLASIK GGRHLIFCHS KKKCDLAAA LRGMGVNAVA YYRGLDVSVI PTQGDVVVVA TDALMTGYTG DFDSVIDCNV AVTQIVDFSL DPTFTITTQT VPQDAVSR SQ RRGRTGRGRL GTTRYVSSGE RPSGMFDSVV LCECYDAGAA WYELTPAETT VRLRAYFNTP GLPVCQDHLE FWEAVFTGLT HIDAHLFSQT KQGGDNFAYL TAYQATVCAR AKAPPPSWDV MWKCLTRLKP TLTGPTPLLY RLGAVTNEIT LTHPVTKYIA TCMQADLEVM TSTWVLAGGV LAAVAAYCLA TGCISIIIGRI HLNDQVVVAP DKEILYEA FD EMEECASKAA LIEEGQRM AE MLKSKILGLL QQATKQAQDI QPAMQSSWPK IEQFWARHMW NFISGIQYLA GLSTLPGNPA VASMMAFSAA LTSPLPTSTT ILLNIMGGWL ASQIAPPAGA TGFVVSGLVG AAVGSIGLGK ILVDVLAGYG AGISGALVAF KIMS GEKPSV EDVVNLLPAI LSPGALVVG ICAAILRRHV GQEGAVQWM NRIAFA SRG NHVAPTHYVA ESDASLRVTQ VLSSLTITSL LRRLHAWITE DCPVPCSGSW LRDIEWEWC S ILTDFKNWLS AKLLPKMPGL PFISCQKGYR GVWAGTG VMT TRCSCGANIS GHVRLGTMKI TGPKTCLNMW QGTFPINCYT EGPCVPKPPP NYKTAIWRVA ASEYVEVTQH GSFSYVTGLT SDNLKVPCQV PAPEFFSWVD GVQIHRFAPT PGPFRRDEV FTVGLNSLVV GSQ LPCDPEP DTEVLASMLT DPHSHITAETA ARRLARGSP SQASSASQL SAPSLKATCT THKTAYDCDM VDANLFMGGD VTRIESDSKV IVLDSLDSMT EVEDDREPSV PSEYLTRRRK FPPALPPWAR PDYNPPVIET WKRPDYEPPT VLGALPPTP QAPVPPPRRR RARVLTQDNV EGVLEMA DK VLSPLQDTND SGHSTGADTG GDSVQQPSGE TAASDAGSLS SMPPLEGEPG DPDLEFEPAR SAPPSEGECE VIDSDSKSWS TVSDQEDSVI CCSMSYSWTG ALITPCGPEE EKLPISPLSN SLMRFHNKVY STTSRSASLR AKKVTFDRVQ VLDAYDSVL QDVKRAASKV SARLLSVEEA CALTPPHSAK SRYGFGAKEV RSLSRGAVNH IRSVWEDLLE DQHTPIDTTA MAKNEVFCID PAKGGKKPAR LIVYPDLGVR VCEKMALYDI AQKLPKAIMG PSYGFQYSPA ERVDFLLKAW GSKKDPMGFS YDTRCFDSTV TERDIRTEES IYQACSLPQE ARTVIHSITE RLYVGGPMTN SKGQSCGYR CRASGVFTTS MGNMTMTCYIK ALAACKAAGI VDPTMLVCGD DLVVIS ESQ NEEDERNLRA FTEAMTRYSA PGDLPRPEY DLELITSCSS NVSVALDSRG RRRYFLTRDP TTPITRAAWE TVRHSPVNSW LGNI IQYAPT IWVRMVIMTH FFSILLAQDT LNQNLFEMY GAVYSVNPLD LPAIIERLHG LDAFSLHTYS PHELSRVAAT LRKLGA PLR AWKSRARAVR ASLI IQGGRA ATCGRYLFNW AVKTKLKLTP LPEASRLDLS GWFTVGAGGG DIFHSVSHAR PRLLLCLLL LSVGVGIFLL PAR (SEQ ID NO: 7)
CAA72338	4 (ED43)	MSTNPKPQRK TKRNTNRRPM DVKFPGGGQI VGGVYLLPRR GPRLGVRATR KTSESRQPRG RRQPIPKARR PEGRSWAQPG YPWPLYGNEG CGWAGWLLSP RGSRPSWGN DPRGRSRNLG KVIDTLTCGF ADLMGYIPLV GAPVGSVARA LAHGVRAL ED GINYATGNLP GCSFSIFLLA LLSCLTVPAS AVNYRNVSGI

		YHVTNDPCNS	SIVYEADHHI	MHLPGCVPCV	REGNQSRCWV	ALTPTVAAPY
		IGAPLESIRS	HVDLMVGAAT	VCSGLYIGDL	CGGLFLVGQM	FSFRPRRHWT
		TQDCNCISIYT	GHITGHRMAW	DMMMNWSPTT	TLVLAQVMRI	PTTLVDLLSG
		GHWGVLVGVA	YFSMQANWAK	VILVLFLFAG	VDAETHVSGA	AVGRSTAGLA
		NLFSSGSKQN	LQLINSNGSW	HINRTALNCN	DSLNTGFLAS	LFYTHKFNSS
		GCSERLACCK	SLDSYGQGWG	PLGVANISGS	SDDRPYCWHY	APRPCGIVPA
		SSVCGPVYCF	TPSPVVVGTT	DHVGVPPTYTW	GENETDVFL	NSTRPPHGAW
		FGCVWMNSTG	FTKTCGAPPC	EVNTNNGTWH	CPTDCFRKHP	ETTYAKCGSG
		PWITPRCLID	YPYRLWHFPC	TANFSVFNIR	TFVGGIEHRM	QAACNWTGRG
		VCGLEHRDRV	ELSPLLLTTT	AWQILPCSFT	TLPALSTGLI	HLHQNIQVQ
		YLYGVGSAVV	SWALKWEYVV	LAFLLLADAR	VSAYLWMMFM	VSQVEAALSN
		LININAASAA	GAQGFYAIL	FICIVWHVKG	RFPAAAAAYAA	CGLWPCFLLL
		LMLPERAYAY	DQEVAGSLGG	AIVVMLTILT	LSPHYKLWLA	RGLWWIQYFI
		ARTEAVLHVY	IPSFNVRGPR	DSIVVLAVLV	CPDLVFDITK	YLLAILGPHL
		ILQASLLRIP	YFVRAQALVK	ICSLLRGVVY	GKVFQMVVLK	SRGLTGTYIY
		DHLTPMSDWP	PYGLRDLAVA	LEPVVFTPME	KKVIVWGADT	AACGDIIRGL
		PVSARLGNEI	LLGPADTETS	KGWRL LAPIT	AYAQQTRGLF	STIVTSLTGR
		DTNENCGEVQ	VLSTATQSFL	GTAVNGVMWT	VYHGAGAKTI	SGPKGPVNQM
		YTNVDQDLVG	WPAPPGVRS	APCTCGSADL	YLVTRHADVI	PVRRRGDTRG
		ALLSPRPISI	LKGSSGGPLL	CPMGHRAGIF	RAAVCTRGA	KAVDFVPVES
		LETTMRSPVF	TDNSTPPAVP	QTYQVAHLHA	PTGSGKSTKV	PAAHAAQGYK
		VLVLNPSVAA	TLGFGVYMSK	AYGIDPNIRS	GVRTITTGAP	ITYSTYGKFL
		ADGGCSGGAY	DIIICDECYS	TDSTTILGIG	TVLDQAETAG	VRLTVLATAT
		PPGSVTTPHS	NIEEVALPTT	GEIPFYGKAI	PLELIKGRH	LIFCHSKKKC
		DELARQLTSL	GLNAVAYYRG	LDVSVIPTSG	DVVVCATDAL	MTGFTGDFDS
		VIDCNTSVIQ	TVDFSLDPTF	SIEITTVPQD	AVSRSQRRGR	TGRGRLGTYR
		YVTPGERPSG	MFDTAELCEC	YDAGCAWYEL	TPAETTTTLK	AYFDTPLGPV
		CQDHLEFWES	VFTGLTHIDG	HFLSQTKQSG	ENFPYLVAYQ	ATVSAKVWLA
		PPSWDTMWKC	LIRLKPTLHG	PTPLLYRLGS	VQNEVVLTHP	ITKYIMACMS
		ADLEVVTSTW	VLVGGVLAAL	AAAYCLSVGSV	VIVGRVVLGS	QPAVIPDREV
		LYQQFDEMEE	CSKHLPLVEH	GLQLAEQFKQ	KALGLNLFAG	KQAQEATPVI
		QSNFAKLEQF	WANDMWNFIS	GIQYLAGLST	LPGNPAIASL	MSFTAAVTSP
		LTTQQTLLFN	ILGGWVASQI	RDSASTAFV	VSGLAGAAGV	SVGLGKILVD
		ILPGYGAGVR	GAVVTFKIMS	GEMPSTEDLV	NLLPAILSPG	ALVVEVVC
		ILRRHVGPGE	GAVQWMNRLI	AFASRGNHVS	PTHYVPESDA	ARRVTILSS
		LTVTSLRLRL	HKWINEDCST	PCAESWLWEV	WDWVLHVLSD	FKTCLKAKFV
		PLMPGIPLLS	WPRGYKGEWR	GDGVMHTTCP	CGADLAGHIK	NGSMRITGPK
		TCSNTWHGTF	PINAYTTGPG	VPIAPPNYKF	ALWRVSAEDY	VEVRRVGDFH
		YVTGVTQDNI	KFPCQVPAP	LFTEVDGIRI	HRHAPKCKPL	LRDEVSFVSG
		LNSFVVGSQL	PCEPEPDVAV	LTSMMLTDPH	ITAESARRRL	ARGSRPSLAS
		SSASQLSPRL	LQATCTAPHD	SPGTDLLEAN	LLWGSTATRV	ETDEKVIILD
		SFESCVAEQN	DDREVSVAAE	ILRPTKKFPP	ALPIWARPDY	NPPLTETWKQ
		QDYQAPT VH	CALPPAKQPP	VPSPRRKRTV	QLTESVVSTA	LAELAAKTFG
		QSEPSSDRDT	DLTTPTEETD	SGPIVVDDAS	DDGSYSMPPI	LEGE PGDPDL
		TSDSWSTVSG	SEDVCCSMS	YSWTGALVTP	CAAEESKLPI	SPLSNSLLRH
		HNMVYATTTR	SAVTRQKKVT	FDRLQVVDST	YNEVLKEIKA	RASRVKPRLL
		TTEEACDLTP	PHSARSKFGY	GKKDVRSHSR	KAINHISSVW	KDLLDDNNT
		IPTTIMAKNE	VFAVNPAGKG	RKPARLIVYP	DLGSRVCEKR	ALHDVIKKTA
		LAVMGAAYGF	QYSPAQRVEF	LLTAWKSKND	PMGFSYDTRC	FDSTVTEKDI
		RVEEEVYQCC	DLEPEARKVI	TALTDRLYVG	GPMHNSKGDL	CGYRRCRATG
		VYTTSFGNTL	TCYLKATAAI	RAAALRDCTM	LVCGDDLVVI	AESDGV EEDN
		RALRAFTEAM	TRYSAPPGDA	PQPAYDLELI	TSCSSNVSV	HDVTGKKVYY
		LTRDPETPLA	RAVWETVRHT	PVNSWLGNII	VYAPTIWVRM	ILMTHFFSIL
		QSQEALEKAL	DFDMYGV TYS	ITPLDLPAII	QRLHGLSAFT	LHGYSPEHLN
		RVAGALRKLG	VPPLRAWRHR	ARAVRAKLIA	QGGRAKICGI	YLFNWAVKTK
		LKLTPLPAAA	KLDLSGWFTV	GAGGGDIYHS	MSHARPRYLL	LCLLILTGVG
		GIFLLPAR	(SEQ ID NO: 8)			

ABE98159	6a	MSTLPKPQRK	TKRNTNRRPM	DVKFPGGGQI	VGGVYLLPRR	GPRLGVRATR
		KTSESRQPRG	RRQPIPKARQ	PQGRHWAQPG	YPWPLYGNEG	CGWAGWLLSP
		RGSRPHWGP	DPRRRSRNLG	KVIDTLTCGF	ADLMGYIPVV	GAPLGGVAAA
		LAHGVRATED	GINYATGNLP	GCSFSIFLLA	LLSCLTTPAS	ALTYGNSSGL
		YHLTNDPCNS	SIVLEADAMI	LHLPGCLPCV	RVDNQSTCWH	AVSPTLAIPN
		ASTSATGFRR	HVDLLAGAAV	VCSSLYIGDL	CGSLFLAGQL	FTFQPRRHWT
		VQDCNCSIYT	GHVTGHRMAW	DMMMNWSPTT	TLVLSSILRV	PEICASVIFG
		GHWGILIAVA	YFGMAGNWLK	VLAVLFLFAG	VEATTTIGRE	MGSTTAGLVR
		FLAPGPKQNL	QLINTNGSWH	INRTALNCND	SLQTGFIAASL	FYAHTFNSSG
		CPERMAACRP	LADFRQGWGQ	ITYKDNISGP	SDDRPYCWHY	APRPCSVVPA
		STVCGPVYCF	TPSPVVVGTT	DRRGNPTYTW	GENETDVFML	GSLRPPTGGW
		FGCTWMNSTG	FTKTCGAPPC	QIVPGDYNSS	ANELLCPTDC	FRKHPEATYQ
		RCGSGPWLTP	RCLVHYPYRL	WHYPCTINFT	VHKVRMFVGG	IEHRFDAACN
		WTRGERCELH	DRDRIEMSPL	LFSTTQLSIL	PCSFSTMPAL	STGLIHLHQN
		IVDVQYLYGV	SSSVTSWVVK	WEYIVLMFLV	LADARICTCL	WLMLLISNVE
		AAVERLVVLN	AASAAGTAGW	WWAVLFLCCV	WYVKGRLLVA	CTYTALGMWP
		LLLTLALPR	RAYAMDNEQA	ASLGAVGLLV	ITIFTITPMY	KKLLTCFIWW
		NQYFLARAEA	MIHEWVPDLR	VRGGRDSIIL	LTCLLHPQLG	FEVTKILLAI
		LAPLYILQYS	LLKVPYFVRA	HILLRACLLV	RRLAGGKYVQ	ACLLRLGAWT
		GTYVYDHLAP	LSDWASDGLR	DLAVAVEPVI	FSPMEKKIIT	WGADTAACGD
		ILSGLPV SAR	LGNLVLLGPA	DDMQRGGWKL	LAPITAYAQQ	TRGLVGTIVT
		SLTGRDKNEV	EGEVQVVSTA	TQSFLATSIN	GVMWTVYHGA	GSKTLAGPKG
		PVCQMYTNVD	QDLVGWPSPP	GARSLTPCTC	GSNDLYLVTR	EADVIPARRR
		GDSRAALLSP	RPISTLKGSS	GGPIMCPSGH	VVGLFRAAVC	TRGVAKSLDF
		IPVENMETTM	RSPSFTDNST	PPAVPQTYQV	GYLHAPTGS	KSTRVPAAYA
		SQGYKVLVLN	PSVAATLSFG	SYMRQAYGVE	PNVRTGVRTI	TTGGAITYST
		YGKFLADGGC	SGGAYDIIIC	DECHSTDPTT	VLGIGTVLDQ	AETAGVRLTV
		LATATPPGSV	TVPHPNITET	ALPTTGEIPF	YGKAIPLEIY	KGGRHLIFCH
		SKKKCDELAL	KLKSLGLNAV	AFYRGVDVSV	IPITSGDVVVC	ATDALMTGYT
		GDFDSVIDCN	VAVTQVVD FS	LDPTFSIETT	TVPQDAVSRS	QRRGRTRGRK
		PGVYRFVSQ	ERPSGMFDTV	VLCEAYDTGC	AWYELTPSET	TVRLRAYLNT
		PGLPVCQDHL	EFWEGVFTGL	THIDAHFLSQ	TKQGGENFAY	LVAYQATVCA
		RAKAPPPSWD	TMWKCLIRLK	PTLTGPTPLL	YRLGAVQNEI	ITTHPITKYI
		MTCMSADLEV	ITSTWVIVGG	VLAALAAYCL	SVGCVVICGR	ITLTGKPVVV
		PDREVLVQQF	DEMEECSRHI	PYLAEGQQIA	EQFRQKVLGL	LQASAKQAE
		LKPAVHSAWP	RVEEFWRKHM	WNFVSGIQYL	AGLSIWPGNP	AVASLMSFTA
		SLTSPLRTSQ	TLLLNLGGW	IATQVAPPPA	STAFVVSGLA	GATVGSIGLG
		RVLVDVLAGY	GAGVSGALVA	FKIMSGECPS	TEDMVNLLPA	LLSPGALVVG
		VVCAAILRRH	VGPAEGANQW	MNRLIAFASR	GNHVSPTHYV	PETDASKNVT
		QILTSLTITS	LLRRLHQWVT	EDTATPCATS	WLRD VWDWVC	TVLSDFK VWL
		KAKLLPRLPG	IPFLSCQ TGY	RGVWAGDGVC	HTTCTCGAVI	AGHVKN GSKM
		ITGPKTCSNT	WHGTFFINAT	TTGPSTPRPA	PNYQALWVRV	SAEDYVEVRR
		LGDCHYV VGA	TAEGLKCP CQ	VPAP EFFTEV	DGVRIHRYAP	PCKPLL RDEV
		TFSVGLSTYA	IGSQLPCEPE	PDVT VVTSML	TDPHTHIT AET	AARRLKRGSP
		PSLASSSASQ	LSAPSLKATC	TTSKDHPDME	LIEANLLWRQ	EMGGNITRVE
		SENKV VILDS	FEPLTADYDE	REISVSAECH	RPPRHKFPPA	LPIWARPDYN
		PPLIQAWQMP	GYEPPVVS GC	AVAPPKPAPI	PPRRKR LVH	LDESTVSHAL
		AQLADKV FVE	SSDGP GPSSD	SGLSITSPVP	PTPTTPDDAC	SEAESYSSMP
		PLEGEPGD PD	LSSGSWSTVS	DQDDV VCCSM	SYSWTGALIT	PCAAEE EKLP
		INPLSNSLIR	HHNMVYSTTS	RSASLRQKKV	TFDRVQVFDQ	HYQEV LKEIK
		LRASTVQAKL	LSIEEACDLT	PSHSARSKYG	YGAKDVRS HA	SKAVDHIRSV
		WEDLLEDSDT	PIPTTIMAKN	EVFCVDPSKG	GRKPARLIVF	PDLGVRVCEK
		MALYDVTRKL	PQAVMGPAYG	FQYSPNQ RVE	YLLKMWR SKK	VPMGFSYDTR
		CFDSTVTERD	IRTENEIYQS	CQLDPMARKA	VSSLTERLYV	GGPMVNSKGQ
		SCGYRRCRAS	GVLPTSMGNT	LTCYLKAQAA	CRAANIKDYD	MLVCGDDL VV
		ICESAGVQED	TASLRAFTDA	MTRYSAAPPD	APQPTYDLEL	ITSCSSNVSV
		AHDGNGK RYY	YLTRDCTT PL	ARAAWETARH	TPVNSWLGNI	IMFAPTIWVR

		MVLMTHFFSI LQSQEQLEKA LDFDIYGVTY SVSPLDLPAL IQR LHGMAAF SLHGYSFVEL NRVGACLRKL GAPPLRAWRH RARAVRAKLI AQQGKAAICG KYLFWAVKT KLKLTPLASA SKLDLSDWFV AGYDGGDIYH SVSLARPLL LLGLLLLTVG VGIFLLPAR (SEQ ID NO: 9)
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[00104] While sequences of exemplary HCV polyproteins are provided herein, it will be appreciated that any sequence having characteristics of an epitope of E2 protein may be employed. In some embodiments, an HCV polyprotein for use in accordance with the present invention has an amino acid sequence which is about 60% identical, about 70% identical, about 80% identical, about 85% identical, about 90% identical, about 91% identical, about 92% identical, about 93% identical, about 94% identical, about 95% identical, about 96% identical, about 97% identical, about 98% identical, about 99% identical, or 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 4-9. In some embodiments, such an HCV polyprotein comprises a sequence that retains the ability to bind one or more HCV E2 antibodies.

[00105] In some embodiments, a portion of an HCV polyprotein has an amino acid sequence which comprises about 50 contiguous amino acids of a sequence selected from the group consisting of SEQ ID NOs: 4-9. In some embodiments, a portion of an HCV polyprotein has an amino acid sequence which is about 60% identical, about 70% identical, about 80% identical, about 85% identical, about 90% identical, about 91% identical, about 92% identical, about 93% identical, about 94% identical, about 95% identical, about 96% identical, about 97% identical, about 98% identical, about 99% identical, or 100% identical to a contiguous stretch of about 50 amino acids of a sequence selected from the group consisting of SEQ ID NOs: 4-9.

[00106] In some embodiments, a portion of an HCV polyprotein has an amino acid sequence which comprises about 42 contiguous amino acids of a sequence selected from the group consisting of SEQ ID NOs: 4-9. In some embodiments, a portion of an HCV polyprotein has an amino acid sequence which is about 60% identical, about 70% identical, about 80% identical, about 85% identical, about 90% identical, about 91% identical, about 92% identical, about 93% identical, about 94% identical, about 95% identical, about 96% identical, about 97% identical, about 98% identical, about 99% identical, or 100% identical to a contiguous stretch of about 42 amino acids of a sequence selected from the group consisting of SEQ ID NOs: 4-9.

[00107] In some embodiments, a portion of an HCV polyprotein has an amino acid sequence which comprises about 45, about 40, about 35, about 30, about 25, about 20, about 15, about 10, or about 5 contiguous amino acids of a sequence selected from the group consisting of SEQ ID

NOs: 4-9. In some embodiments, a portion of an HCV polyprotein has an amino acid sequence which is about 60% identical, about 70% identical, about 80% identical, about 85% identical, about 90% identical, about 91% identical, about 92% identical, about 93% identical, about 94% identical, about 95% identical, about 96% identical, about 97% identical, about 98% identical, about 99% identical, or 100% identical to a contiguous stretch of about 45, about 40, about 35, about 30, about 25, about 20, about 15, about 10, or about 5 contiguous amino acids of a sequence selected from the group consisting of SEQ ID NOs: 4-9.

[00108] One of skill in the art will recognize that the HCV polyproteins listed in Table 1 are merely representative examples of HCV polyproteins. Various genotypes, subtypes, and/or isolates of HCV exist and continue to be identified. It will be understood by one skilled in the art that the methods and compositions provided herein may be adapted to utilize sequences of additional genotypes, subtypes, and/or isolates. Such variation is contemplated and encompassed within the methods and compositions provided herein.

Mimotopes

[00109] A mimotope, in its broadest sense, refers to a macromolecule which mimics the structure of an epitope. In some embodiments, a mimotope elicits an antibody response identical or similar to that elicited by its corresponding epitope. In some embodiments, an antibody that recognizes an epitope also recognizes a mimotope which mimics that epitope. In some embodiments, a mimotope is a peptide. In some embodiments, a mimotope is a small molecule, carbohydrate, lipid, or nucleic acid.

[00110] Antibodies in accordance with the invention may be used for screening for mimotopes. Mimotopes may be prepared using phage display, and the peptides screened with the subject antibodies (Livnah *et al.*, 1996, *Science*, 273:464; and Prezzi *et al.*, 1996, *J. Immunol.*, 156:4504; both of which are incorporated herein by reference). In some embodiments, mimotopes are peptide or non-peptide mimotopes of conformationally-conserved HCV epitopes. Antibodies that recognize conformationally conserved HCV epitopes may be used as templates for the rational design of peptide or non-peptide structural mimics of the conformational epitope or mimotopes.

[00111] In some embodiments, by mimicking the structure of the conformationally defined viral epitope, a mimotope interferes with the ability of HCV virus particles to bind to its natural binding partners (*e.g.*, HCV target receptor), *e.g.*, by binding to the natural binding partner itself.

For example, analysis of a solved crystal structure defining the interface between a monoclonal antibody and tumor necrosis factor (TNF) enabled the rational design of a non-peptide mimetic capable of antagonizing the biological function of TNF by binding to the TNF receptor (Takasaki *et al.*, 1997, *Nat. Biotech.*, 15:1266-1270; incorporated herein by reference). Computational techniques that may be employed to rationally deduce protein folding from a primary amino acid sequence for use in designing a peptide structural mimetic are reviewed in Teichmann *et al.* (1999, *Curr. Opin. Struct. Biol.*, 9:390-399; incorporated herein by reference). The practical application of computer programs for use in structurally modeling conformationally conserved epitopes is described by Schwartz *et al.* (1999, *J. Mol. Biol.* 287:983; incorporated herein by reference). An alternative method for rationally creating a peptide structural mimic of an antibody epitope involves systematic permutations of synthetic peptides designed to be a linear representation of a discontinuous antibody-binding site (Reineke *et al.*, 1999, *Nat. Biotech.*, 17:271; incorporated herein by reference).

[00112] Peptides or other small molecules may have specific affinity for a monoclonal antibody and may be competitive with an epitope of a conformationally intact E2 protein. Alternatively or additionally, peptides or other small molecules may have specific affinity for a monoclonal antibody and may be competitive with an epitope of E2 complexed with E1. Such peptides may be used as vaccines, in diagnostic assays, for immunization for the production of antibodies to a specific HCV epitope, in competitive assays for defining genotype and/or subtype, and the like. See, for example, Puntoriero *et al.* (1998, *EMBO J.*, 17:3521-3533; incorporated herein by reference), Meola *et al.* (1995, *J. Immunol.*, 154:3162-3172; incorporated herein by reference), and Tafi *et al.*, (1997, *Biol. Chem.*, 378:495-502; incorporated herein by reference).

[00113] Another approach to effectively create structural mimetics of conformationally conserved HCV epitopes is to produce anti-idiotypic antibodies to conformationally dependent HCV antibodies. Anti-idiotypics may effectively block the binding of native virus with its target receptor (Chanh *et al.*, 1987, *Proc. Natl. Acad. Sci., USA*, 84:3891-3895; Kopecky *et al.*, 1999, *Interviol.*, 42:9-16; and Xue *et al.*, 1993, *J. Gen. Virol.*, 74:73-79; all of which are incorporated herein by reference). Anti-idiotypic antibodies recognizing the conformational binding sites of any one of HCV antibodies HC-1, HC-3, HC-11, and CBH-23 could prevent viral infectivity by

interfering with E2 binding to a target cellular protein, or even by interfering with virion attachment to the target cell.

[00114] In some embodiments, epitope mimics (*e.g.*, mimotopes) can serve as tools for drug discovery. To give but one example, an epitope mimic (*e.g.*, peptide, small molecule, *etc.*) that mimics the binding of E2 protein to an HCV antibody could be used to screen for other agents (*e.g.*, peptides, proteins, antibodies, small molecules, *etc.*) that bind to the epitope. In some embodiments, such identified agents may exhibit similar binding specificity and/or affinity for the E2 epitope than an HCV E2 antibody does. In some embodiments, such identified agents may exhibit increased binding specificity and/or affinity for the E2 epitope than an HCV E2 antibody does.

Applications

[00115] In some embodiments, HCV antibodies in accordance with the invention may be used for prophylactic, therapeutic, and/or diagnostic purposes. In some embodiments, HCV antibodies in accordance with the invention may be used to treat (*e.g.*, alleviate, ameliorate, relieve, delay onset of, inhibit progression of, reduce severity of, and/or reduce incidence of one or more symptoms or features of) HCV infection, HCV-mediated liver disease, and/or any other HCV-associated condition. In some embodiments, HCV antibodies may be used for a variety of therapeutic purposes, *e.g.*, immunotherapy. In some embodiments, HCV antibodies may be used for a variety of prophylactic purposes and/or passive immunization, *e.g.*, for development of vaccines for HCV. In some embodiments, HCV antibodies may be used for a variety of diagnostic purposes, *e.g.*, for capturing and/or identifying HCV virions and/or E2 protein. These and other uses for HCV antibodies are described in further detail in the sections below. In some embodiments, therapeutic, diagnostic, and/or prophylactic applications utilize HCV antibodies and/or pharmaceutical compositions thereof, as described herein. It will be appreciated that antibodies can be directed to conformational epitopes, as described herein.

[00116] In some embodiments, HCV E2 antibodies can be directed to E2 epitopes of a single HCV genotype, two HCV genotypes, three HCV genotypes, four HCV genotypes, five HCV genotypes, six HCV genotypes, seven HCV genotypes, eight HCV genotypes, nine HCV genotypes, ten HCV genotypes, eleven HCV genotypes, or more than eleven HCV genotypes, should new HCV genotypes be discovered in the future.

[00117] In some embodiments, HCV E2 antibodies can be directed to E2 epitopes of a single HCV subtype, two HCV subtypes, three HCV subtypes, four HCV subtypes, five HCV subtypes, six HCV subtypes, seven HCV subtypes, eight HCV subtypes, nine HCV subtypes, ten HCV subtypes, 11 HCV subtypes, 12 HCV subtypes, 13 HCV subtypes, 14 HCV subtypes, 15 HCV subtypes, 16 HCV subtypes, 17 HCV subtypes, 18 HCV subtypes, 19 HCV subtypes, 20 HCV subtypes, 21 HCV subtypes, or more than 21 HCV subtypes, should new HCV subtypes be discovered in the future.

[00118] In some embodiments, HCV E2 antibodies can be directed to E2 epitopes of a single HCV strain, two HCV strains, three HCV strains, four HCV strains, five HCV strains, six HCV strains, seven HCV strains, eight HCV strains, nine HCV strains, ten HCV strains, 20 HCV strains, 30 HCV strains, 40 HCV strains, 50 HCV strains, 75 HCV strains, 100 HCV strains, or more than 100 HCV strains, should new HCV strains be discovered in the future.

Therapeutic Applications

[00119] The present invention provides systems and methods for treating patients suffering from, susceptible to, and/or displaying symptoms of HCV infection. In some embodiments, the invention provides systems and methods useful for stratifying patients suffering from, susceptible to, and/or displaying symptoms of HCV infection.

[00120] In some embodiments, therapeutic applications comprise administering a therapeutically effective amount of at least one HCV antibody in accordance with the invention to a subject in need thereof. In some embodiments, administration of HCV antibodies to a subject may alleviate, ameliorate, relieve, delay onset of, inhibit progression of, reduce severity of, and/or reduce incidence of one or more signs, symptoms, and/or features of HCV infection.

[00121] In some embodiments, administration of HCV antibodies reduces the level of HCV virions circulating in a subject (*e.g.*, HCV virions that are capable of infecting new cells). In some embodiments, administration of HCV antibodies reduces the level of HCV virions circulating in a subject by about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99%, or about 100% relative to non-treated controls.

[00122] In some embodiments, HCV antibodies may be used *in vitro* to reduce viral load in a subject. For reducing viral load of a body component, particularly a body component of a patient infected with HCV, a patient's blood is passed through a device comprising antibodies bound to

a surface or solid support for capturing HCV virions (see, for example, U.S. Patent Numbers 5,698,390 and 4,692,411; both of which are incorporated herein by reference). Various other devices found in the literature can be used with the subject antibodies to achieve a similar result. A body component can be a biological fluid (*e.g.*, blood, serum, *etc.*), a tissue, an organ, such as the liver, and the like.

[00123] In some embodiments, the “level of HCV virions circulating in a subject” refers to an absolute number of virions circulating in a subject. In some embodiments, the “level of HCV virions circulating in a subject” refers to the number of virions per unit volume (*e.g.*, milliliter, liter, *etc.*) of the subject’s blood. In some embodiments, the “level of HCV virions circulating in a subject” refers to viral load and/or HCV RNA levels.

[00124] In some embodiments, administration of HCV antibodies inhibits replication of HCV virions in a subject, for example, by blocking infection of uninfected cells. In some embodiments, administration of HCV antibodies inhibits replication of HCV virions in a subject by about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 10-fold, about 50-fold, about 100-fold, about 500-fold, about 1000-fold, about 10,000-fold, or greater than about 10,000-fold relative to non-treated controls.

[00125] In some embodiments, administration of HCV antibodies kills and/or inactivates HCV virions in a subject. In some embodiments, administration of HCV antibodies kills and/or inactivates about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99%, or about 100% of HCV virions in a subject relative to non-treated controls.

[00126] In some embodiments, administration of HCV antibodies inhibits binding of virus to cellular target proteins. In some embodiments, administration of HCV antibodies inhibits binding of virus to at least one cellular target protein by about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 10-fold, about 50-fold, about 100-fold, about 500-fold, about 1000-fold, about 10,000-fold, or greater than about 10,000-fold relative to non-treated controls. To give but one example, administration of HCV antibodies may inhibit binding of HCV virus to CD81. In some embodiments, administration of HCV antibodies inhibits binding of HCV to CD81 by about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 10-fold, about 50-fold, about 100-fold, about 500-fold, about 1000-fold, about 10,000-fold, or greater than about 10,000-fold relative to non-treated controls.

[00127] In some embodiments, administration of HCV antibodies inhibits virus-mediated fusion with a target cell. In some embodiments, administration of HCV antibodies inhibits virus-mediated fusion with a target cell by about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 10-fold, about 50-fold, about 100-fold, about 500-fold, about 1000-fold, about 10,000-fold, or greater than about 10,000-fold relative to non-treated controls.

[00128] In some embodiments, administration of HCV antibodies inhibits conformational changes of one or more proteins associated with virus entry. In some embodiments, administration of HCV antibodies inhibits conformational changes of one or more proteins associated with virus entry by about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 10-fold, about 50-fold, about 100-fold, about 500-fold, about 1000-fold, about 10,000-fold, or greater than about 10,000-fold relative to non-treated controls.

[00129] In some embodiments, administration of HCV antibodies promotes antibody-mediated complement activation. In some embodiments, administration of HCV antibodies promotes antibody-mediated complement activation by about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 10-fold, about 50-fold, about 100-fold, about 500-fold, about 1000-fold, about 10,000-fold, or greater than about 10,000-fold relative to non-treated controls.

[00130] In some embodiments, administration of HCV antibodies promotes antibody-mediated aggregation of virions, which leads to clearance by phagocytic cells. In some embodiments, administration of HCV antibodies promotes antibody-mediated aggregation of virions by about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 10-fold, about 50-fold, about 100-fold, about 500-fold, about 1000-fold, about 10,000-fold, or greater than about 10,000-fold relative to non-treated controls.

[00131] The chimpanzee is an accepted animal model for screening HCV vaccines and therapeutics (see, for example, Farci *et al.*, 1996, *Proc. Natl. Acad. Sci., USA*, 93:15394-15399; Farci *et al.*, 1994, *Proc. Natl. Acad. Sci., USA*, 91:7792-7796; Farci *et al.*, 1992, *Science*, 258:135-140; Krawczynski *et al.*, 1996, *J. Infect. Dis.*, 173:822-828; and Bassett *et al.*, 1998, *J. Virol.*, 72:2589-2599; all of which are incorporated herein by reference). Antibody effectiveness can be determined by monitoring for the presence and titer of HCV RNA using quantitative PCR methods. A successful reduction of viral load, or prevention of infection in a test animal or subject is reflected as a reduction or elimination of HCV RNA in serum. Enzymatic tests such as measurement of alanine aminotransferase or other liver enzymes and/or use of sequential punch

needle liver biopsies also may also be used to test effectiveness, where improvement in the rating of either would indicate a reduction in viral-induced liver damage.

[00132] In some embodiments, administration of HCV antibodies results in interference with conformational changes in the viral envelope proteins necessary for cell infectivity. For example, administered HCV antibodies may bind to such viral envelope proteins, thereby sterically blocking an envelope protein's ability to recognize and/or interact with cellular surfaces (*e.g.*, with proteins, *e.g.*, CD81; lipids; carbohydrates; receptors; *etc.* on cell surfaces). In some embodiments, administered HCV antibodies may bind to such viral envelope proteins, thereby changing the three-dimensional conformation of the envelope protein in such a way that renders the envelope protein incapable of recognizing and/or interacting with cellular surfaces (*e.g.*, with proteins, lipids, carbohydrates, receptors, *etc.* on cell surfaces).

[00133] In some embodiments, treatment regimens are particularly tailored for the individual being treated. Briefly, a patient to be treated is provided, and a sample of serum is taken from the patient. The serum sample is then analyzed for the presence of particular antibodies, to give but a few examples, neutralizing antibodies, antibodies that bind to a particular region or epitope of a protein of HCV, *etc.* Any method known in the art, including but not limited to those described in this application, may be used to determine the presence of the antibodies to be detected (*e.g.*, ELISA, competition assay, virus neutralization-of-binding assay, *etc.*). Based on the level of antibodies in the patient's serum, treatment can be designed for the patient. For example, a patient who does not have antibodies known to interfere with the binding of virions to their natural receptor may be treated with monoclonal antibodies of this type. In some embodiments, serum from a patient (*e.g.*, a patient suffering from, susceptible to, infected with, and/or displaying symptoms of HCV infection, HCV-mediated liver disease, and/or any other HCV-associated condition) is considered positive for the presence of a competing antibody if 50% or greater inhibition of E2 binding is obtained at a dilution of the patient's serum of 1/100 or greater, 1/200 or greater, 1/300 or greater, 1/400 or greater, 1/500 or greater, 1/600 or greater, 1/700 or greater, 1/800 or greater, 1/900 or greater, or 1/1000 or greater.

[00134] In some embodiments, methods such as those described above may be advantageous because the treatment is tailored to the particular individual being treated, for example; only those antibodies that are needed and not produced naturally by the patient are administered. This avoids or reduces the risk of adverse reactions from administering therapeutics that are not

needed. Such methods eliminate the expense of treating patients who would not benefit from such treatment. For example, if a patient were already producing therapeutic levels of an antibody to a particular epitope of E2, there may be no need to administer a human monoclonal antibody directed against the epitope exogenously.

[00135] Treatment of a subject with HCV antibodies may be given alone, during the course of another treatment, and/or after the cessation of treatment of other antiviral compounds.

Alternatively or additionally, HCV antibodies may be physically conjugated (*e.g.*, covalently or non-covalently) to known toxins or proteins capable of inducing apoptosis or other cell death processes. Modified HCV antibodies can be administered to individuals suffering from, susceptible to, infected with, and/or displaying symptoms of HCV infection, HCV-mediated liver disease, and/or any other HCV-associated condition as a means of killing HCV infected cells.

Prophylactic Applications

[00136] In some embodiments, HCV antibodies in accordance with the invention may be utilized for prophylactic applications. In some embodiments, prophylactic applications involve systems and methods for preventing, inhibiting progression of, and/or delaying the onset of HCV infection, HCV-mediated liver disease, and/or any other HCV-associated condition in individuals susceptible to and/or displaying symptoms of HCV infection, HCV-mediated liver disease, and/or any other HCV-associated condition. In some embodiments, prophylactic applications involve systems and methods for preventing, inhibiting progression of, and/or delaying the onset of chronic liver disease. In some embodiments, prophylactic applications involve systems and methods for preventing, inhibiting progression of, and/or delaying the onset of liver disease. In some embodiments, prophylactic applications involve systems and methods for preventing, inhibiting progression of, and/or delaying the onset of liver failure.

[00137] In some embodiments, vaccines to HCV may be utilized for passive immunization (*i.e.*, immunization wherein antibodies are administered to a subject). In some embodiments, vaccines to HCV for passive immunization may comprise HCV antibodies, such as any of the compositions described herein. In some embodiments, passive immunization occurs when antibodies are being transferred from mother to fetus during pregnancy. In some embodiments, antibodies are administered directly to an individual (*e.g.*, by injection, orally, *etc.*).

[00138] In some embodiments, prophylactic applications may include administering vaccines. In some embodiments, vaccination is tailored to the individual patient. For example, as

described above, serum may be collected from a patient and tested for presence of HCV antibodies. In some embodiments, a vaccine may be designed to induce production of antibodies that have been found to be lacking in the patient. In some embodiments, it is desirable for vaccine compositions to comprise antigens that have a native conformation, mediate a protective response (*e.g.*, complement activation, virus neutralization, *etc.*), and/or can induce a strong antibody response. In some embodiments, a vaccine contains an epitope or mimotope thereof to which antibodies are not being produced naturally in the individual. For example, synthetic peptide mimotopes isolated with HCV antibodies (*e.g.*, HCV antibodies recognizing multiple genotypes and/or subtypes) have the potential to induce a potent immune response similar to the antibody used in the original isolation of the mimotope. Administration of such a vaccine might induce a patient's immune system to start producing a set of antibodies directed against the administered epitope. It will be appreciated that the mimotopes (or epitopes) in accordance with the invention can be used alone or in combination with recombinant proteins, inactivated HCV virus, killed HCV virus, and/or as a cocktail of several different mimotopes.

[00139] In some embodiments, vaccines to HCV may be utilized for active immunization (*i.e.*, immunization wherein microbes, proteins, peptides, epitopes, mimotopes, *etc.* are administered to a subject). In some embodiments, vaccines to HCV may comprise any agent that mimics at least one conformational epitope of HCV E2 protein may be used. For example, the agent may be a peptide, protein, glycopeptide, glycoprotein, small molecule, mimotope, organic compound, lipid, saccharide, organometallic compound, inorganic compound, *etc.* In some embodiments, epitopes represented in a vaccine include those against which antibodies known to prevent infection are directed. In some embodiments, epitopes represented in a vaccine in accordance with the invention include ones that are conserved among different genotypes and/or subtypes of the virus or among different strains of virus. In some embodiments, peptides or proteins that contain conformationally defined epitopes of E2 of HCV are used in formulations of a vaccine to prevent, delay onset of, treat, ameliorate symptoms of, and/or reduce severity of infection by HCV. In some embodiments, HCV E2 epitopes may be linear epitopes. In some embodiments, E2 epitopes may be a mixture of linear and conformational epitopes. In some embodiments, E2 epitopes may be conformational epitopes. In some embodiments, peptide epitopes are less than 100 amino acids in length. In certain embodiments, peptide epitopes are less than 50, less than 40, less than 30, less than 20, or less than 10 amino acids in length. In some embodiments,

peptides to be used in formulating a vaccine are peptide fragments of E2 protein of HCV.

Typically, a peptide is used that folds in a manner similar to its three-dimensional fold in the native E2 protein, thus preserving the three-dimensional structure of the conformational epitope.

[00140] In some embodiments, a vaccine may contain proteins that represent concatenated peptides that have one or more conformational epitopes to which antibodies are desired. Several different peptides making up a multimer may be used so that each peptide contains a different epitope, or the same peptide may be used more than once in the multimer.

[00141] Peptides in accordance with the invention may be synthesized using any method known in the art, including Merrifield solid phase chemistry (see, e.g., Atherton and Sheppard, 1989, *Solid Phase Peptide Synthesis: A Practical Approach*, ILR Press, Oxford, England; Stewart and Young, 1984, *Solid Phase Peptide Synthesis*, 2nd ed., Pierce Chemical Company, Rockford; Merrifield, 1963, *J. Am. Chem. Soc.*, 85:2149-2154; all of which are incorporated herein by reference). Alternatively or additionally, peptides may be obtained by cleavage of E2 protein and optional purification of cleavage products. In some embodiments, peptides may be made recombinantly and produced in *E. coli*, yeast (e.g., *S. cerevisiae*), insect cells (e.g., Sf9 cells), and/or mammalian cells (e.g., CHO cells) using any available techniques in the art (e.g., Sambrook *et al.*; Miller & Calos, eds., *Gene Transfer vectors for Mammalian Cells*, 1987; and Ausubel *et al.*, eds., *Current Protocols in Molecular Biology*, 1987; both of which are incorporated herein by reference). In some embodiments, peptides may be modified to increase their immunogenicity, solubility in aqueous solution, and/or to increase their propensity to fold correctly. For example, peptides may be glycosylated, farnesylated, hydroxylated, reduced, oxidized, methylated, *etc.*

[00142] In certain embodiments, a peptide is or comprises amino acids 523 through 540 of the E2 protein of HCV subtype 1a, 1b, 2a, 2b, 3a, 4, 5, and/or 6. As would be appreciated by one of ordinary skill in this art, analogous, homologous, similar, and/or identical amino acid sequences of E2 proteins from other subtypes of HCV may be used. Analogous sequences may be determined by aligning multiple sequences of the E2 protein from different strains or subtypes of HCV. Homologous, similar, and/or identical sequences that preserve the desired epitope may also be used in the formulation of vaccines.

[00143] In some embodiments, the sequences are about 50% homologous to the native sequence from HCV 1a, 1b, 2a, 2b, 3a, 4, 5, and/or 6 E2 protein, about 60% homologous, about

70% homologous, about 80% homologous, about 90% homologous, about 95% homologous, about 99% homologous, or more than about 99% homologous. In some embodiments, the sequences are about 50% similar to the native sequence from HCV 1a, 1b, 2a, 2b, 3a, 4, 5, and/or 6 E2 protein, about 60% similar, about 70% similar, about 80% similar, about 90% similar, about 95% similar, about 99% similar, or more than about 99% similar. In some embodiments, the sequences are about 50% identical to the native sequence from HCV 1a, 1b, 2a, 2b, 3a, 4, 5, and/or 6 E2 protein, about 60% identical, about 70% identical, about 80% identical, about 90% identical, about 95% identical, about 99% identical, or more than about 99% identical.

[00144] In some embodiments, the sequences are at least 50% homologous to the native sequence from HCV 1a, 1b, 2a, 2b, 3a, 4, 5, and/or 6 E2 protein, at least 60% homologous, at least 70% homologous, at least 80% homologous, at least 90% homologous, at least 95% homologous, at least 99% homologous, or substantially 100% homologous. In some embodiments, the sequences are at least 50% similar to the native sequence from HCV 1a, 1b, 2a, 2b, 3a, 4, 5, and/or 6 E2 protein, at least 60% similar, at least 70% similar, at least 80% similar, at least 90% similar, at least 95% similar, at least 99% similar, or substantially 100% similar. In some embodiments, the sequences are at least 50% identical to the native sequence from HCV 1a, 1b, 2a, 2b, 3a, 4, 5, and/or 6 E2 protein, at least 60% identical, at least 70% identical, at least 80% identical, at least 90% identical, at least 95% identical, at least 99% identical, or substantially 100% identical.

[00145] In some embodiments, a vaccine composition comprises at least one adjuvant. Any adjuvant may be used in accordance with the present invention. A large number of adjuvants are known; a useful compendium of many such compounds is prepared by the National Institutes of Health and can be found on the internet

(www.niaid.nih.gov/daids/vaccine/pdf/compendium.pdf). See also Allison (1998, *Dev. Biol. Stand.*, 92:3-11; incorporated herein by reference), Unkeless *et al.* (1998, *Annu. Rev. Immunol.*, 6:251-281; incorporated herein by reference), and Phillips *et al.* (1992, *Vaccine*, 10:151-158; incorporated herein by reference). Hundreds of different adjuvants are known in the art and could be employed in the practice of the present invention. Exemplary adjuvants that can be utilized in accordance with the invention include, but are not limited to, cytokines, gel-type adjuvants (*e.g.*, aluminum hydroxide, aluminum phosphate, calcium phosphate, *etc.*); microbial adjuvants (*e.g.*, immunomodulatory DNA sequences that include CpG motifs; endotoxins such as

monophosphoryl lipid A; exotoxins such as cholera toxin, *E. coli* heat labile toxin, and pertussis toxin; muramyl dipeptide, *etc.*); oil-emulsion and emulsifier-based adjuvants (*e.g.*, Freund's Adjuvant, MF59 [Novartis], SAF, *etc.*); particulate adjuvants (*e.g.*, liposomes, biodegradable microspheres, saponins, *etc.*); synthetic adjuvants (*e.g.*, nonionic block copolymers, muramyl peptide analogues, polyphosphazene, synthetic polynucleotides, *etc.*); and/or combinations thereof. Other exemplary adjuvants include some polymers (*e.g.*, polyphosphazenes; described in U.S. Patent 5,500,161, which is incorporated herein by reference), Q57, QS21, squalene, tetrachlorodecaoxide, *etc.*

[00146] Pharmaceutically acceptable excipients are described in further detail below in the section entitled "*Pharmaceutical Compositions.*"

Diagnostic Applications

[00147] In some embodiments, HCV antibodies in accordance with the invention are used for diagnostic applications. For example, by virtue of the variety of binding profiles of HCV antibodies, diagnostic assays may be employed which will detect a plurality of HCV genotypes and/or subtypes, so as to provide a pan-HCV antibody, while at the same time being able to dissect individual genotypes and/or subtypes by subtractive analysis.

[00148] For diagnostic purposes, antibodies may be used in a wide variety of formats for detecting E2 protein, discerning HCV genotypes and/or subtypes, detecting virions and antibodies (see, *e.g.*, U.S. Patent Number 5,695,390; incorporated herein by reference).

Antibodies may be used individually or in combination with other antibodies of the subject group or other antibodies or with lectins which bind to the glycosyl groups present on HCV envelope proteins (*i.e.*, E1 or E2) and/or other proteins with which HCV E2 complexes (*e.g.*, a HCV E1:HCV E2 complex). For diagnostic purposes, a wide variety of labels may be employed, which for the most part have been mentioned previously. These include, but are not limited to, fluorophores, chemiluminescent moieties, radioisotopes, enzymes, particles (*e.g.*, colloidal carbon particles, gold particles, latex particles, *etc.*) ligands for which there are high affinity receptors, and prolabels, which can be activated to provide a detectable signal.

[00149] In some embodiments, a surface is coated with a protein, which can bind to HCV antigens as free protein (*e.g.*, circulating proteins) or as part of an intact or partially intact virion. One may use antibodies of the subject invention which bind to multiple HCV genotypes and/or subtypes or to lectins (*e.g.*, *Galanthus nivalis* lectin; "GNA"). One may also use antibodies in

accordance with the invention which bind to HCV subtypes 1, 2, 3, 4, 5, and 6. In some embodiments, antibodies in accordance with the invention bind to at least one, two, three, four, five, or more than five different genotypes and/or subtypes.

[00150] In some embodiments, assays may involve contacting a surface with a medium, which may contain free or HCV-associated protein(s), where the medium may be the sample or a solution of known E2 of one or more genotypes and/or subtypes. After incubation and washing to remove non-specifically bound protein, the assay may proceed in various manners depending upon what is being assayed. Where a blood sample suspected of being seropositive is being assayed, the sample may be applied to the layer of E2 protein, incubated, and washed, and the presence of human antibodies bound to the protein layer determined. One may use labeled α -human antibodies (other than against the isotype of the subject antibodies, where the subject antibodies have been initially used). In assays for antibodies in seropositive subjects, subject antibodies may be used as controls with the same reagent used to detect any human anti-HCV antibodies in the sera of such subjects. The specificity of the antibodies in the sample can be confirmed by using the subject antibodies, which are differentially labeled from the anti-human antibodies and determine whether they are blocked by the antibodies in the sample.

[00151] Where the sample is assayed for HCV E2 protein, detection employs labeled subject antibodies, the selection depending upon whether one is interested in genotyping or detection of E2 protein. After washing away non-specifically bound antibody, the presence of labeled antibodies is determined by detecting the presence of the label in accordance with known techniques. Alternatively or additionally, where the subject antibodies are bound to a surface, a labeled lectin for E2 may be employed to detect the presence of E2 protein.

[00152] Antibodies in accordance with the invention can be used to measure the reactivity of other antibodies, including antibodies in sera, monoclonal antibodies, antibodies expressed as a result of genetic engineering, *etc.* In some embodiments, intact virions are used. In some embodiments, conformationally conserved envelope proteins are used. For virion capture, see, for example, Kimura *et al.*, 1998, *J. Med. Virology*, 56:25-32; Morita *et al.*, 1996, *Hepato-Gastroenterology*, 43:582-585; Sata *et al.*, 1993, *Virology*, 196:354-357; and Hijikata *et al.*, 1993, *J. Virol.*, 67:1953-1958; all of which are incorporated herein by reference. One protocol involves steps of coating a solid support with a lectin (*e.g.*, GNA) and then contacting the surface with a medium (*e.g.*, serum of a seropositive patient) comprising intact HCV virions. Additives

which might destroy virions should usually be avoided (*e.g.*, detergents). After incubating the medium and washing to remove non-specifically bound components of the medium, virions may be contacted with antibodies in accordance with the invention and antibodies of the sample. This may be performed concurrently or consecutively, where the sample is added first. An amount of the subject antibody is used which is sensitive to displacement by another antibody. Such amount may be determined empirically, and one may wish to use different amounts of the subject antibody in a series of tests. By knowing the signal, which is obtained in the absence and presence of the sample, one can determine the reactivity or binding affinity of the antibodies in the sample. Various techniques may be used to determine the amount of a subject antibody bound to the virions. Where the subject antibodies are labeled, *e.g.*, with biotin or digoxigenin, streptavidin or anti-digoxigenin labeled with a fluorophore or enzyme whose substrate produces a detectable signal can serve to determine the amount of the subject antibodies.

[00153] Labeled subject antibodies may be used in assaying for the presence of HCV from biopsy material. Labeled antibody may be incubated with immobilized biopsy material, such as a liver slice, with a solution of one or more of the subject labeled antibodies. After washing away non-specifically bound antibodies, the presence of the antibodies bound to the cells of the biopsied tissue may be detected in accordance with the nature of the label.

[00154] In some embodiments, HCV antibodies in accordance with the invention can be used to identify HCV receptors. Those skilled in the art will appreciate the multitude of ways this can be accomplished (Sambrook J., Fritsch E. and Maniatis T. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Press, Cold Spring Harbor, NY, 1989; and Ausubel *et al.*, eds., *Current Protocols in Molecular Biology*, 1987; both of which are incorporated herein by reference). Typically, protein and peptide receptors can be identified by determining whether an antibody to E2 can inhibit attachment of HCV virions to a cell susceptible to HCV infection. Thus, receptors for HCV E2 proteins and peptides can be identified in this manner. A susceptible cell can be incubated in the presence of HCV and anti-HCV E2 antibody, and a cell-binding assay can be utilized to determine whether attachment is decreased in the presence of the antibody.

[00155] Cells expressing putative receptors for HCV and/or libraries of putative receptors for HCV may be screened for their abilities to bind HCV. For example, cells expressing a putative HCV receptor (*e.g.*, a receptor for HCV E2) can be contacted with an HCV protein or peptide in

the presence of an antibody for a time and under conditions sufficient to allow binding of the HCV protein or peptide to putative receptor on the surface of the cell. Alternatively or additionally, HCV proteins, peptides, or virions can be pre-incubated with antibody prior to contacting the putative receptor on the cell surface. Binding can be detected by any means known in the art, *e.g.*, flow cytometry *etc.* (see Ausubel *et al.* or Sambrook *et al.*, *supra*). A decrease in binding to the surface of the cell in the presence of antibody compared to binding in the absence of the cell in the absence of the antibody indicates the identification of an HCV receptor.

[00156] In some embodiments, methods of identifying HCV receptors (*e.g.*, such as E2 receptors) include the use of solid supports, such as beads, columns, and the like. For example, receptors for HCV proteins and peptides (*e.g.*, E2 proteins and/or fragments thereof) and/or HCV virions can be identified by attaching an HCV antibody to a solid support and then contacting the antibody with an HCV protein or peptide for a time sufficient for the HCV protein or peptide to bind to the antibody. This provides an HCV protein ligand for putative HCV receptors that can be contacted with the antibody:ligand complex on the solid support for a time and under conditions sufficient to allow binding of a receptor to the HCV protein or peptide. Proteins can be expressed from a library or provided as a cell extract or purified protein preparation from natural or recombinant cells. Once specific binding complexes between the HCV protein peptide are formed, unbound HCV proteins or peptides, *e.g.*, library proteins or peptide that did not bind specifically to the HCV proteins or peptides, are removed, *e.g.*, by standard washing steps. Bound proteins are then eluted and identified, *e.g.*, by gel electrophoresis.

Administration

[00157] HCV antibodies in accordance with the invention and pharmaceutical compositions thereof in accordance with the present invention may be administered using any amount and any route of administration effective for treatment.

[00158] The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the infection, the particular composition, its mode of administration, its mode of activity, and the like. HCV antibodies are typically formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of the compositions of the present invention will

be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject or organism will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific HCV antibody employed; the specific pharmaceutical composition administered; the half-life of the composition after administration; the age, body weight, general health, sex, and diet of the subject; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors, well known in the medical arts.

[00159] Pharmaceutical compositions of the present invention may be administered by any route. In some embodiments, pharmaceutical compositions of the present invention are administered by a variety of routes, including oral (PO), intravenous (IV), intramuscular (IM), intra-arterial, intramedullary, intrathecal, subcutaneous (SQ), intraventricular, transdermal, interdermal, intradermal, rectal (PR), vaginal, intraperitoneal (IP), intragastric (IG), topical (*e.g.*, by powders, ointments, creams, gels, lotions, and/or drops), mucosal, intranasal, buccal, enteral, vitreal, sublingual; by intratracheal instillation, bronchial instillation, and/or inhalation; as an oral spray, nasal spray, and/or aerosol, and/or through a portal vein catheter.

[00160] In general, the most appropriate route of administration will depend upon a variety of factors including the nature of the agent being administered (*e.g.*, its stability in the environment of the gastrointestinal tract), the condition of the subject (*e.g.*, whether the subject is able to tolerate a particular mode of administration), *etc.* In specific embodiments, HCV antibodies in accordance with the present invention and/or pharmaceutical compositions thereof may be administered intravenously, for example, by intravenous infusion. In specific embodiments, HCV antibodies in accordance with the present invention and/or pharmaceutical compositions thereof may be administered by intramuscular injection. In specific embodiments, HCV antibodies in accordance with the present invention and/or pharmaceutical compositions thereof may be administered by subcutaneous injection. In specific embodiments, HCV antibodies in accordance with the present invention and/or pharmaceutical compositions thereof may be administered via portal vein catheter. However, the invention encompasses the delivery of HCV antibodies in accordance with the present invention and/or pharmaceutical compositions thereof

by any appropriate route taking into consideration likely advances in the sciences of drug delivery.

[00161] In certain embodiments, HCV antibodies in accordance with the present invention and/or pharmaceutical compositions thereof in accordance with the invention may be administered at dosage levels sufficient to deliver from about 0.001 mg/kg to about 100 mg/kg, from about 0.01 mg/kg to about 50 mg/kg, from about 0.1 mg/kg to about 40 mg/kg, from about 0.5 mg/kg to about 30 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 0.1 mg/kg to about 10 mg/kg, or from about 1 mg/kg to about 25 mg/kg of subject body weight per day to obtain the desired therapeutic effect. The desired dosage may be delivered more than three times per day, three times per day, two times per day, once per day, every other day, every third day, every week, every two weeks, every three weeks, every four weeks, every two months, every six months, or every twelve months. In certain embodiments, the desired dosage may be delivered using multiple administrations (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations).

[00162] It will be appreciated that HCV antibodies in accordance with the present invention and/or pharmaceutical compositions thereof can be employed in combination therapies. The particular combination of therapies (*e.g.*, therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will be appreciated that the therapies employed may achieve a desired effect for the same purpose (for example, HCV antibodies useful for treating, preventing, and/or delaying the onset of HCV infection may be administered concurrently with another agent useful for treating, preventing, and/or delaying the onset of HCV infection), or they may achieve different effects (*e.g.*, control of any adverse effects). The invention encompasses the delivery of pharmaceutical compositions in combination with agents that may improve their bioavailability, reduce and/or modify their metabolism, inhibit their excretion, and/or modify their distribution within the body.

[00163] Pharmaceutical compositions in accordance with the present invention may be administered either alone or in combination with one or more other therapeutic agents. By “in combination with,” it is not intended to imply that the agents must be administered at the same time and/or formulated for delivery together, although these methods of delivery are within the scope of the invention. Compositions can be administered concurrently with, prior to, or

subsequent to, one or more other desired therapeutics or medical procedures. It will be appreciated that therapeutically active agents utilized in combination may be administered together in a single composition or administered separately in different compositions. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent.

[00164] In general, it is expected that agents utilized in combination will be utilized at levels that do not exceed the levels at which they are utilized individually. In some embodiments, the levels utilized in combination will be lower than those utilized individually.

[00165] In some embodiments, HCV E2 antibodies in accordance with the invention (*e.g.*, HC-1, HC-3, HC-11, and CBH-23) may be administered with interferon, with ribavirin, or with both interferon and ribavirin.

[00166] In some embodiments, compositions for combination therapy can comprise a plurality of antibodies directed to a single conformational epitope. In some embodiments, compositions for combination therapy can comprise a plurality of antibodies that recognize distinct conformational epitopes (*e.g.*, on the same viral envelope protein or on different viral envelope proteins), thereby simultaneously interfering with multiple mechanisms in the infectious process.

[00167] In certain embodiments, compositions in accordance with the invention comprise exactly one antibody to E2 (*e.g.*, HC-1, HC-3, HC-11, and CBH-23). In certain embodiments, compositions include exactly two, exactly three, exactly four, exactly five, exactly six, or more than six HCV E2 antibodies. In some embodiments, compositions comprise all possible permutations and combinations of HC-1, HC-3, HC-11, and CBH-23. Exemplary compositions comprising 1, 2, 3, or 4 antibodies selected from the group consisting of HC-1, HC-3, HC-11, and CBH-23 are shown in Table 2:

Table 2: Exemplary Compositions Comprising HCV Antibodies

One Antibody	Two Antibodies	Three Antibodies	Four Antibodies
HC-1	HC-1 and HC-3	HC-1, HC-3, and HC-11	HC-1, HC-3, HC-11, and CBH-23
HC-2	HC-1 and HC-11	HC-1, HC-3, and CBH-23	
HC-11	HC-1 and CBH-23	HC-1, HC-11, and CBH-23	
CBH-23	HC-3 and HC-11	HC-3, HC-11, and CBH-23	
	HC-3 and CBH-23		
	HC-11 and CBH-23		

[00168] In some embodiments, hybridoma cell lines that secrete anti-HCV E2 antibodies have been deposited in the American Type Culture Collection (ATCC). For example, hybridoma cell lines that secrete human monoclonal antibody HC-1 has been deposited under Accession number PTA-9416; human monoclonal antibody HC-3 secreted by the hybridoma cell line has been deposited in the ATCC under Accession number PTA-9417; human monoclonal antibody HC-11 secreted by the hybridoma cell line has been deposited in the ATCC under Accession number PTA-9418; and human monoclonal antibody CBH-23 secreted by the hybridoma cell line has been deposited in the ATCC under Accession number PTA-9419.

[00169] It will be appreciated by one of skill in the art that any permutation or combination of HC-1, HC-3, HC-11, and CBH-23 can be combined with any other antibody (*e.g.*, antibodies that recognize E1, E2, and/or other envelope proteins) to formulate compositions comprising a plurality of different antibodies. In certain embodiments, HC-1, HC-3, HC-11, and/or CBH-23 can be combined with any of the following previously described antibodies against E2: CBH-2, CBH-4G, CBH-5, CBH-7, CBH-8C, CBH-8E, CBH-9, CBH-11, fragments thereof, and/or combinations thereof (see U.S. Patent 6,692,908; and U.S. Patent Publications 2006/0104980 and 2006/0188511; all of which are incorporated herein by reference). In certain embodiments, HC-1, HC-3, HC-11, and/or CBH-23 can be combined with H-111, H-114 (both of which are previously described antibodies against E1), fragments thereof, and/or combinations thereof (see U.S. Patent Publication 2003/180284; incorporated herein by reference). In certain embodiments, HC-1, HC-3, HC-11, and/or CBH-23 can be combined with humanized AP33 (Owsianka *et al.*, 2005, *J. Virol.*, 79:11095-104; incorporated herein by reference); Fab e137 (Perotti *et al.*, 2008, *J. Virol.*, 82:1047-52; incorporated herein by reference, 2008); mAbs 1:7 and A8 (Johansson *et al.*, 2007, *Proc. Natl. Acad. Sci., USA*, 104:16269-74; incorporated herein by reference); AR3 human MAbs (Law *et al.*, 2008, *Nat. Med.*, 14:25-27; incorporated herein by reference); fragments thereof; and/or combinations thereof.

Pharmaceutical Compositions

[00170] The present invention provides HCV antibodies and pharmaceutical compositions comprising at least one HCV antibody and at least one pharmaceutically acceptable excipient. Such pharmaceutical compositions may optionally comprise one or more additional therapeutically active substances. In accordance with some embodiments, methods of

administering a pharmaceutical composition comprising administering HCV antibodies to a subject in need thereof are provided. In some embodiments, pharmaceutical compositions are administered to humans. For the purposes of the present disclosure, the phrase “active ingredient” generally refers to an HCV antibody in accordance with the invention. In certain embodiments, an HCV antibody is an antibody that recognizes E2 envelope protein. In certain embodiments, an HCV antibody is an antibody that recognizes a conformational epitope of E2 envelope protein.

[00171] Pharmaceutical compositions for administration of HCV E2 antibodies may be provided in a sterile injectible form (*e.g.*, a form that is suitable for subcutaneous injection or intravenous infusion). For example, in some embodiments, antibodies are provided in a liquid dosage form that is suitable for injection. In some embodiments, antibodies are provided as lyophilized sterile powders, optionally under vacuum, which are reconstituted with an aqueous diluent (*e.g.*, water, buffer, salt solution, *etc.*) prior to injection. In some embodiments, antibodies are diluted and/or reconstituted in water, sodium chloride solution, sodium acetate solution, benzyl alcohol solution, phosphate buffered saline, *etc.* In some embodiments, powder should be mixed gently with the aqueous diluent (*e.g.*, not shaken).

[00172] In some embodiments, antibody formulations comprise one or more pharmaceutically acceptable excipients (*e.g.*, preservative, inert diluent, dispersing agent, surface active agent and/or emulsifier, buffering agent, *etc.*). In some embodiments, antibody formulations comprise one or more preservatives. In some embodiments, antibody formulations comprise no preservative.

[00173] In some embodiments, antibodies are provided in a form that can be refrigerated and/or frozen. In some embodiments, antibodies are provided in a form that cannot be refrigerated and/or frozen. In some embodiments, reconstituted antibody solutions and/or antibody liquid dosage forms may be stored for a certain period of time after reconstitution (*e.g.*, 2 hours, 12 hours, 24 hours, 2 days, 5 days, 7 days, 10 days, 2 weeks, a month, two months, or longer). In some embodiments, storage of antibody formulations for longer than the specified time results in antibody degradation.

[00174] Liquid dosage forms and/or reconstituted antibody solutions may comprise particulate matter and/or discoloration prior to administration. In general, a solution should not be used if discolored or cloudy and/or if particulate matter remains after filtration.

[00175] Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with an excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping and/or packaging the product into a desired single- or multi-dose unit.

[00176] A pharmaceutical composition in accordance with the invention may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a “unit dose” is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

[00177] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the invention will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

[00178] Pharmaceutical formulations of the present invention may additionally comprise a pharmaceutically acceptable excipient, which, as used herein, includes any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's *The Science and Practice of Pharmacy*, 21st Edition, A. R. Gennaro, (Lippincott, Williams & Wilkins, Baltimore, MD, 2006) discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional excipient medium is incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention.

[00179] In some embodiments, the pharmaceutically acceptable excipient is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% pure. In some embodiments, the excipient is approved for use in humans and for veterinary use. In some embodiments, the

excipient is approved by United States Food and Drug Administration. In some embodiments, the excipient is pharmaceutical grade. In some embodiments, the excipient meets the standards of the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), the British Pharmacopoeia, and/or the International Pharmacopoeia.

[00180] Pharmaceutically acceptable excipients used in the manufacture of pharmaceutical compositions include, but are not limited to, inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and/or oils. Such excipients may optionally be included in the formulations. Excipients such as cocoa butter and suppository waxes, coloring agents, coating agents, sweetening, flavoring, and/or perfuming agents can be present in the composition, according to the judgment of the formulator.

[00181] Exemplary diluents include, but are not limited to, calcium carbonate, sodium carbonate, calcium phosphate, dicalcium phosphate, calcium sulfate, calcium hydrogen phosphate, sodium phosphate lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, inositol, sodium chloride, dry starch, cornstarch, powdered sugar, *etc.*, and/or combinations thereof

[00182] Exemplary granulating and/or dispersing agents include, but are not limited to, potato starch, corn starch, tapioca starch, sodium starch glycolate, clays, alginic acid, guar gum, citrus pulp, agar, bentonite, cellulose and wood products, natural sponge, cation-exchange resins, calcium carbonate, silicates, sodium carbonate, cross-linked poly(vinyl-pyrrolidone) (crospovidone), sodium carboxymethyl starch (sodium starch glycolate), carboxymethyl cellulose, cross-linked sodium carboxymethyl cellulose (croscarmellose), methylcellulose, pregelatinized starch (starch 1500), microcrystalline starch, water insoluble starch, calcium carboxymethyl cellulose, magnesium aluminum silicate (VEEGUM[®]), sodium lauryl sulfate, quaternary ammonium compounds, *etc.*, and/or combinations thereof.

[00183] Exemplary surface active agents and/or emulsifiers include, but are not limited to, natural emulsifiers (*e.g.*, acacia, agar, alginic acid, sodium alginate, tragacanth, chondrux, cholesterol, xanthan, pectin, gelatin, egg yolk, casein, wool fat, cholesterol, wax, and lecithin), colloidal clays (*e.g.*, bentonite [aluminum silicate] and VEEGUM[®] [magnesium aluminum silicate]), long chain amino acid derivatives, high molecular weight alcohols (*e.g.*, stearyl alcohol, cetyl alcohol, oleyl alcohol, triacetin monostearate, ethylene glycol distearate, glyceryl

monostearate, and propylene glycol monostearate, polyvinyl alcohol), carbomers (*e.g.*, carboxy polymethylene, polyacrylic acid, acrylic acid polymer, and carboxyvinyl polymer), carrageenan, cellulosic derivatives (*e.g.*, carboxymethylcellulose sodium, powdered cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose), sorbitan fatty acid esters (*e.g.*, polyoxyethylene sorbitan monolaurate [TWEEN[®]20], polyoxyethylene sorbitan [TWEEN[®]60], polyoxyethylene sorbitan monooleate [TWEEN[®]80], sorbitan monopalmitate [SPAN[®]40], sorbitan monostearate [SPAN[®]60], sorbitan tristearate [SPAN[®]65], glyceryl monooleate, sorbitan monooleate [SPAN[®]80]), polyoxyethylene esters (*e.g.*, polyoxyethylene monostearate [MYRJ[®]45], polyoxyethylene hydrogenated castor oil, polyethoxylated castor oil, polyoxymethylene stearate, and SOLUTOL[®]), sucrose fatty acid esters, polyethylene glycol fatty acid esters (*e.g.*, CREMOPHOR[®]), polyoxyethylene ethers, (*e.g.*, polyoxyethylene lauryl ether [BRIJ[®]30]), poly(vinyl-pyrrolidone), diethylene glycol monolaurate, triethanolamine oleate, sodium oleate, potassium oleate, ethyl oleate, oleic acid, ethyl laurate, sodium lauryl sulfate, PLURONIC[®]F 68, POLOXAMER[®]188, cetrimonium bromide, cetylpyridinium chloride, benzalkonium chloride, docusate sodium, *etc.* and/or combinations thereof.

[00184] Exemplary binding agents include, but are not limited to, starch (*e.g.*, cornstarch, starch paste, *etc.*); gelatin; sugars (*e.g.*, sucrose, glucose, dextrose, dextrin, molasses, lactose, lactitol, mannitol, *etc.*); natural and synthetic gums (*e.g.*, acacia, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapol husks, carboxymethylcellulose, methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, cellulose acetate, poly(vinyl-pyrrolidone), magnesium aluminum silicate [VEEGUM[®]], larch arabogalactan, *etc.*); alginates; polyethylene oxide; polyethylene glycol; inorganic calcium salts; silicic acid; polymethacrylates; waxes; water; alcohol; *etc.*; and combinations thereof.

[00185] Exemplary preservatives may include, but are not limited to, antioxidants, chelating agents, antimicrobial preservatives, antifungal preservatives, alcohol preservatives, acidic preservatives, and/or other preservatives. Exemplary antioxidants include, but are not limited to, alpha tocopherol, ascorbic acid, acorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, monothioglycerol, potassium metabisulfite, propionic acid, propyl gallate, sodium ascorbate, sodium bisulfite, sodium metabisulfite, and/or sodium sulfite. Exemplary

chelating agents include ethylenediaminetetraacetic acid (EDTA), citric acid monohydrate, disodium edetate, dipotassium edetate, edetic acid, fumaric acid, malic acid, phosphoric acid, sodium edetate, tartaric acid, and/or trisodium edetate. Exemplary antimicrobial preservatives include, but are not limited to, benzalkonium chloride, benzethonium chloride, benzyl alcohol, bronopol, cetrimide, cetylpyridinium chloride, chlorhexidine, chlorobutanol, chlorocresol, chloroxylonol, cresol, ethyl alcohol, glycerin, hexetidine, imidurea, phenol, phenoxyethanol, phenylethyl alcohol, phenylmercuric nitrate, propylene glycol, and/or thimerosal. Exemplary antifungal preservatives include, but are not limited to, butyl paraben, methyl paraben, ethyl paraben, propyl paraben, benzoic acid, hydroxybenzoic acid, potassium benzoate, potassium sorbate, sodium benzoate, sodium propionate, and/or sorbic acid. Exemplary alcohol preservatives include, but are not limited to, ethanol, polyethylene glycol, phenol, phenolic compounds, bisphenol, chlorobutanol, hydroxybenzoate, and/or phenylethyl alcohol. Exemplary acidic preservatives include, but are not limited to, vitamin A, vitamin C, vitamin E, beta-carotene, citric acid, acetic acid, dehydroacetic acid, ascorbic acid, sorbic acid, and/or phytic acid. Other preservatives include, but are not limited to, tocopherol, tocopherol acetate, deteroxime mesylate, cetrimide, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ethylenediamine, sodium lauryl sulfate (SLS), sodium lauryl ether sulfate (SLES), sodium bisulfite, sodium metabisulfite, potassium sulfite, potassium metabisulfite, GLYDANT PLUS[®], PHENONIP[®], methylparaben, GERMALL[®] 115, GERMABEN[®] II, NEOLONE[™], KATHON[™], and/or EUXYL[®].

[00186] Exemplary buffering agents include, but are not limited to, citrate buffer solutions, acetate buffer solutions, phosphate buffer solutions, ammonium chloride, calcium carbonate, calcium chloride, calcium citrate, calcium gluconate, calcium gluceptate, calcium gluconate, D-gluconic acid, calcium glycerophosphate, calcium lactate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate, phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine, magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, *etc.*, and/or combinations thereof.

[00187] Exemplary lubricating agents include, but are not limited to, magnesium stearate, calcium stearate, stearic acid, silica, talc, malt, glyceryl behenate, hydrogenated vegetable oils, polyethylene glycol, sodium benzoate, sodium acetate, sodium chloride, leucine, magnesium lauryl sulfate, sodium lauryl sulfate, *etc.*, and combinations thereof.

[00188] Exemplary oils include, but are not limited to, almond, apricot kernel, avocado, babassu, bergamot, black current seed, borage, cade, camomile, canola, caraway, carnauba, castor, cinnamon, cocoa butter, coconut, cod liver, coffee, corn, cotton seed, emu, eucalyptus, evening primrose, fish, flaxseed, geraniol, gourd, grape seed, hazel nut, hyssop, isopropyl myristate, jojoba, kukui nut, lavandin, lavender, lemon, litsea cubeba, macademia nut, mallow, mango seed, meadowfoam seed, mink, nutmeg, olive, orange, orange roughy, palm, palm kernel, peach kernel, peanut, poppy seed, pumpkin seed, rapeseed, rice bran, rosemary, safflower, sandalwood, sasquana, savoury, sea buckthorn, sesame, shea butter, silicone, soybean, sunflower, tea tree, thistle, tsubaki, vetiver, walnut, and wheat germ oils. Exemplary oils include, but are not limited to, butyl stearate, caprylic triglyceride, capric triglyceride, cyclomethicone, diethyl sebacate, dimethicone 360, isopropyl myristate, mineral oil, octyldodecanol, oleyl alcohol, silicone oil, and/or combinations thereof.

[00189] Liquid dosage forms for oral and parenteral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and/or elixirs. In addition to active ingredients, liquid dosage forms may comprise inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, oral compositions can include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and/or perfuming agents. In certain embodiments for parenteral administration, compositions are mixed with solubilizing agents such as CREMOPHOR[®], alcohols, oils, modified oils, glycols, polysorbates, cyclodextrins, polymers, and/or combinations thereof.

[00190] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing agents,

wetting agents, and/or suspending agents. Sterile injectable preparations may be sterile injectable solutions, suspensions, and/or emulsions in nontoxic parenterally acceptable diluents and/or solvents, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P., and isotonic sodium chloride solution. Sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. Fatty acids such as oleic acid can be used in the preparation of injectables.

[00191] Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, and/or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[00192] In order to prolong the effect of an active ingredient, it is often desirable to slow the absorption of the active ingredient from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

[00193] Compositions for rectal or vaginal administration are typically suppositories which can be prepared by mixing compositions with suitable non-irritating excipients such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active ingredient.

[00194] Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active ingredient is mixed with at least one inert, pharmaceutically acceptable excipient such as sodium citrate or dicalcium phosphate and/or

fillers or extenders (*e.g.*, starches, lactose, sucrose, glucose, mannitol, and silicic acid), binders (*e.g.*, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia), humectants (*e.g.*, glycerol), disintegrating agents (*e.g.*, agar, calcium carbonate, potato starch, tapioca starch, alginic acid, certain silicates, and sodium carbonate), solution retarding agents (*e.g.*, paraffin), absorption accelerators (*e.g.*, quaternary ammonium compounds), wetting agents (*e.g.*, cetyl alcohol and glycerol monostearate), absorbents (*e.g.*, kaolin and bentonite clay), and lubricants (*e.g.*, talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate), and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may comprise buffering agents.

[00195] Solid compositions of a similar type may be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally comprise opacifying agents and can be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. Solid compositions of a similar type may be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

[00196] Dosage forms for topical and/or transdermal administration of a compound in accordance with this invention may include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants and/or patches. Generally, the active ingredient is admixed under sterile conditions with a pharmaceutically acceptable excipient and/or any needed preservatives and/or buffers as may be required. Additionally, the present invention contemplates the use of transdermal patches, which often have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms may be prepared, for example, by dissolving and/or dispensing the compound in the proper medium. Alternatively or additionally, the rate may be controlled by either providing a rate controlling membrane and/or by dispersing the compound in a polymer matrix and/or gel.

[00197] Suitable devices for use in delivering intradermal pharmaceutical compositions described herein include short needle devices such as those described in U.S. Patents 4,886,499; 5,190,521; 5,328,483; 5,527,288; 4,270,537; 5,015,235; 5,141,496; and 5,417,662. Intradermal compositions may be administered by devices which limit the effective penetration length of a needle into the skin, such as those described in PCT publication WO 99/34850 and functional equivalents thereof. Jet injection devices which deliver liquid vaccines to the dermis via a liquid jet injector and/or via a needle which pierces the stratum corneum and produces a jet which reaches the dermis are suitable. Jet injection devices are described, for example, in U.S. Patents 5,480,381; 5,599,302; 5,334,144; 5,993,412; 5,649,912; 5,569,189; 5,704,911; 5,383,851; 5,893,397; 5,466,220; 5,339,163; 5,312,335; 5,503,627; 5,064,413; 5,520,639; 4,596,556; 4,790,824; 4,941,880; 4,940,460; and PCT publications WO 97/37705 and WO 97/13537. Ballistic powder/particle delivery devices which use compressed gas to accelerate vaccine in powder form through the outer layers of the skin to the dermis are suitable. Alternatively or additionally, conventional syringes may be used in the classical mantoux method of intradermal administration.

[00198] Formulations suitable for topical administration include, but are not limited to, liquid and/or semi liquid preparations such as liniments, lotions, oil in water and/or water in oil emulsions such as creams, ointments and/or pastes, and/or solutions and/or suspensions. Topically administrable formulations may, for example, comprise from about 1% to about 10% (w/w) active ingredient, although the concentration of the active ingredient may be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

[00199] A pharmaceutical composition in accordance with the invention may be prepared, packaged, and/or sold in a formulation suitable for pulmonary administration via the buccal cavity. Such a formulation may comprise dry particles which comprise the active ingredient and which have a diameter in the range from about 0.5 nm to about 7 nm or from about 1 nm to about 6 nm. Such compositions are conveniently in the form of dry powders for administration using a device comprising a dry powder reservoir to which a stream of propellant may be directed to disperse the powder and/or using a self propelling solvent/powder dispensing container such as a device comprising the active ingredient dissolved and/or suspended in a low-boiling propellant in a sealed container. Such powders comprise particles wherein at least 98% of the particles by

weight have a diameter greater than 0.5 nm and at least 95% of the particles by number have a diameter less than 7 nm. Alternatively, at least 95% of the particles by weight have a diameter greater than 1 nm and at least 90% of the particles by number have a diameter less than 6 nm. Dry powder compositions may include a solid fine powder diluent such as sugar and are conveniently provided in a unit dose form.

[00200] Low boiling propellants generally include liquid propellants having a boiling point of below 65 °F at atmospheric pressure. Generally the propellant may constitute 50% to 99.9% (w/w) of the composition, and the active ingredient may constitute 0.1% to 20% (w/w) of the composition. The propellant may further comprise additional ingredients such as a liquid non-ionic and/or solid anionic surfactant and/or a solid diluent (which may have a particle size of the same order as particles comprising the active ingredient).

[00201] Pharmaceutical compositions in accordance with the invention formulated for pulmonary delivery may provide the active ingredient in the form of droplets of a solution and/or suspension. Such formulations may be prepared, packaged, and/or sold as aqueous and/or dilute alcoholic solutions and/or suspensions, optionally sterile, comprising the active ingredient, and may conveniently be administered using any nebulization and/or atomization device. Such formulations may further comprise one or more additional ingredients including, but not limited to, a flavoring agent such as saccharin sodium, a volatile oil, a buffering agent, a surface-active agent, and/or a preservative such as methylhydroxybenzoate. The droplets provided by this route of administration may have an average diameter in the range from about 0.1 nm to about 200 nm.

[00202] Formulations described herein as being useful for pulmonary delivery are useful for intranasal delivery of a pharmaceutical composition. Another formulation suitable for intranasal administration is a coarse powder comprising the active ingredient and having an average particle from about 0.2 μm to 500 μm . Such a formulation is administered in the manner in which snuff is taken, *i.e.*, by rapid inhalation through the nasal passage from a container of the powder held close to the nose.

[00203] Formulations suitable for nasal administration may, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of the active ingredient, and may comprise one or more of the additional ingredients described herein. A pharmaceutical composition in accordance with the invention may be prepared, packaged, and/or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets and/or

lozenges made using conventional methods, and may, for example, 0.1% to 20% (w/w) active ingredient, the balance comprising an orally dissolvable and/or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder and/or an aerosolized and/or atomized solution and/or suspension comprising the active ingredient. Such powdered, aerosolized, and/or aerosolized formulations, when dispersed, may have an average particle and/or droplet size in the range from about 0.1 nm to about 200 nm, and may further comprise one or more of the additional ingredients described herein.

[00204] A pharmaceutical composition in accordance with the invention may be prepared, packaged, and/or sold in a formulation suitable for ophthalmic administration. Such formulations may, for example, be in the form of eye drops including, for example, a 0.1/1.0% (w/w) solution and/or suspension of the active ingredient in an aqueous or oily liquid excipient. Such drops may further comprise buffering agents, salts, and/or one or more other of the additional ingredients described herein. Other ophthalmically-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form and/or in a liposomal preparation. Ear drops and/or eye drops are contemplated as being within the scope of this invention.

[00205] General considerations in the formulation and/or manufacture of pharmaceutical agents may be found, for example, in *Remington: The Science and Practice of Pharmacy* 21st ed., Lippincott Williams & Wilkins, 2005.

Kits

[00206] The invention provides a variety of kits for conveniently and/or effectively carrying out methods in accordance with the present invention. Kits typically comprise one or more HCV antibodies in accordance with the invention (e.g., HC-1, HC-3, HC-11, and CBH-23). In some embodiments, kits comprise a collection of different HCV antibodies to be used for different purposes (e.g., diagnostics, treatment, and/or prophylaxis). Typically kits will comprise sufficient amounts of HCV antibodies to allow a user to perform multiple administrations to a subject(s) and/or to perform multiple experiments. In some embodiments, kits are supplied with or include one or more HCV antibodies that have been specified by the purchaser.

[00207] In certain embodiments, kits for use in accordance with the present invention may include one or more reference samples; instructions (*e.g.*, for processing samples, for performing tests, for interpreting results, for solubilizing HCV antibodies, for storage of HCV antibodies, *etc.*); buffers; and/or other reagents necessary for performing tests. In certain embodiments kits can comprise panels of antibodies. Other components of kits may include cells, cell culture media, tissue, and/or tissue culture media.

[00208] Kits may comprise instructions for use. For example, instructions may inform the user of the proper procedure by which to prepare a pharmaceutical composition comprising HCV antibodies and/or the proper procedure for administering pharmaceutical compositions to a subject.

[00209] In some embodiments, kits include a number of unit dosages of a pharmaceutical composition comprising HCV antibodies. A memory aid may be provided, for example in the form of numbers, letters, and/or other markings and/or with a calendar insert, designating the days/times in the treatment schedule in which dosages can be administered. Placebo dosages, and/or calcium dietary supplements, either in a form similar to or distinct from the dosages of the pharmaceutical compositions, may be included to provide a kit in which a dosage is taken every day.

[00210] Kits may comprise one or more vessels or containers so that certain of the individual components or reagents may be separately housed. Kits may comprise a means for enclosing the individual containers in relatively close confinement for commercial sale, *e.g.*, a plastic box, in which instructions, packaging materials such as styrofoam, *etc.*, may be enclosed.

[00211] In some embodiments, kits are used in the treatment, diagnosis, and/or prophylaxis of a subject suffering from and/or susceptible to HCV. In some embodiments, such kits comprise (i) at least one HCV antibody; (ii) a syringe, needle, applicator, *etc.* for administration of the at least one HCV antibody to a subject; and (iii) instructions for use.

[00212] In some embodiments, kits are used in the treatment, diagnosis, and/or prophylaxis of a subject suffering from and/or susceptible to HCV. In some embodiments, such kits comprise (i) at least one HCV antibody provided as a lyophilized powder; and (ii) a diluent for reconstituting the lyophilized powder. Such kits may optionally comprise a syringe, needle, applicator, *etc.* for administration of the at least one HCV antibody to a subject; and/or instructions for use.

[00213] The present invention provides kits containing reagents for the generation of vaccines comprising at least one HCV antibody. In some embodiments, such kits may include cells expressing HCV antibodies, characteristic portions thereof, and/or biologically active portions thereof; (ii) media for growing the cells; and (iii) columns, resin, buffers, tubes, and other tools useful for antibody purification. In some embodiments, such kits may include (i) plasmids containing nucleotides encoding HCV antibodies, characteristic portions thereof, and/or biologically active portions thereof; (ii) cells capable of being transformed with the plasmids, such as mammalian cell lines, including but not limited to, Vero and MDCK cell lines; (iii) media for growing the cells; (iv) expression plasmids containing no nucleotides encoding HCV antibodies as negative controls; (v) columns, resin, buffers, tubes, and other tools useful for antibody purification; and (vi) instructions for use.

[00214] In some embodiments, kits are used to detect the presence of HCV in one or more samples. Such samples may be pathological samples, including, but not limited to, blood, serum/plasma, peripheral blood mononuclear cells/peripheral blood lymphocytes (PBMC/PBL), sputum, urine, feces, throat swabs, dermal lesion swabs, cerebrospinal fluids, cervical smears, pus samples, food matrices, and tissues from various parts of the body such as brain, spleen, and liver. Such samples may be environmental samples, including, but not limited to, soil, water, and flora. Other samples that have not been listed may also be applicable. In some embodiments, such kits comprise (i) at least one HCV antibody; (ii) a sample known to contain HCV, as a positive control; and (iii) a sample known not to contain HCV, as a negative control; and (iv) instructions for use.

[00215] In some embodiments, kits are used to neutralize HCV in one or more samples. Such kits may provide materials needed to treat an HCV-containing sample with at least one HCV antibody and to test the ability of the treated sample to infect cultured cells relative to untreated sample. Such kits may include (i) at least one HCV antibody; (ii) cells capable of being cultured and infected with HCV; (iii) an antibody that is incapable of binding to and neutralizing HCV, as a negative control; (iv) an antibody that is capable of binding to and neutralizing HCV, as a positive control; (v) a sample known not to contain HCV, as a negative control; (vi) a sample known to contain HCV, as a positive control; and (vii) instructions for use.

[00216] These and other aspects of the present invention will be further appreciated upon consideration of the following Examples, which are intended to illustrate certain particular embodiments of the invention but are not intended to limit its scope, as defined by the claims.

Examples

Example 1: Definition of a Conserved Immunodominant Domain on HCV E2 Glycoprotein by Neutralizing Human Monoclonal Antibodies

Materials and Methods

Cells and culture conditions

[00217] 293T and CHO-K1 cells were obtained from ATCC. Huh7 cells were obtained from Dr. Michael Lai (University of Southern California) and Huh7.5 cells were obtained from Dr. Charles Rice (Rockefeller University). Cells were grown in Dulbecco's modified minimal essential medium (DMEM, Invitrogen, Carlsbad, CA) supplemented with 10% fetal calf serum (FCS, Sigma-Aldrich Co. St. Louis, MO) and 2 mM glutamine. CHO-K1 cells were grown in F-12 Kaighn's medium (Invitrogen 21127-022) containing L-glutamine and supplemented with 10% FCS.

Viruses and virus models

[00218] Production and purification of HCVpp (genotype 1a) have been previously described (Bartosch *et al.*, 2003, *J. Exp. Med.*, 197:633-42; and Keck *et al.*, 2005, *J. Virol.*, 79:13199-208; both of which are incorporated herein by reference). Briefly, 293T cells were transfected with HCVpp plasmids using the calcium-phosphate method. After 48 hours of growth, cell-free supernatant was collected by filtration using a 0.45 μ m pore-size filter.

[00219] For production of infectious genotype 2a JFH-1 virus, HCVcc (Wakita *et al.*, 2005, *Nat. Med.*, 11:791-96; incorporated herein by reference), XbaI linearized pJFH-1 plasmid was *in vitro* transcribed (MEGAscript; Ambion, Austin, TX) and electroporated into Huh7.5 cells. Briefly, 10 μ g of *in vitro* transcribed JFH-1 RNA was mixed with 0.4×10^6 Huh7.5 cells in a 4-mm cuvette in calcium-free PBS containing 10 μ g calf liver tRNA and pulsed at 0.27 kV and 960 μ F using a Bio-Rad Gene Pulser system. Electroporated cells were seeded into 10 cm cell culture dishes with 10 ml complete DMEM containing 10 % FCS. Expression of HCV E2 was confirmed by indirect immunofluorescent assay (IFA) at each passage of the cells. For JFH-1 virus production, transfected cells were passaged at 4- to 5-day intervals with a 1:4 to 1:5 split

into fresh culture flasks. Virus harvests placed in small aliquots were stored at -80 °C.

Production of 1a HJ3-5 HCVcc has been described previously (Yi *et al.*, 2006, *Proc. Natl. Acad. Sci., USA*, 103:2310-15; and Yi *et al.*, 2007, *J. Virol.*, 81:629-38; both of which are incorporated herein by reference).

Antibodies and reagents

[00220] CBH-4G, an antibody to HCV E2 has been previously described (Hadlock *et al.*, 2000, *J. Virol.*, 74:10407; and Keck *et al.*, 2005, *J. Virol.*, 79:13199; both of which are incorporated herein by reference). Murine MAb to *c-myc* was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). CD81 LEL was obtained from Dr. Shoshana Levy (Stanford University). FITC-conjugated goat anti-human IgG, Fc γ fragment specific and R-phycoerythrin-conjugated F(ab')₂ fragment goat anti-mouse IgG (H+L) were obtained from Jackson Immuno Research (West Grove, PA). Alkaline phosphatase conjugated goat anti-human IgG (H+L) and alkaline phosphatase conjugated goat anti-mouse IgG (H+L) were purchased from Promega (Madison, WI). *Galanthus nivalis* lectin (GNA) and *p*-nitrophenyl phosphate disodium hexa-hydrate (phosphatase substrate) were purchased from Sigma (St. Louis, MO).

Monoclonal antibody production and purification

[00221] Generation of new HCV antibodies from peripheral blood B cells was performed essentially as described in Hadlock *et al.* (2000, *J. Virol.*, 74:10407; incorporated herein by reference). Specific HCV antibodies were selected by indirect immunofluorescent assay (IFA) using a subtype 1a or 1b HCVpp infected cell lysates as the target antigen (GenBank accession no AF348705). Monoclonality was achieved by limiting dilution cloning and confirmed by DNA sequencing (Sequetech, Mountain View, CA) of the IgG genes. Cloning and analyzing the V_L and V_H domains of the IgG genes were performed as previously described (Keck *et al.*, 2004, *J. Virol.*, 78:7257; incorporated herein by reference). HCV antibody production and purification were performed essentially as described in Hadlock *et al.* (2000, *J. Virol.*, 74:10407; incorporated herein by reference) and biotinylation of the antibodies was carried out according to the manufacturer's instructions (Pierce Biotechnology, Inc, Rockford, IL).

IgG subclass typing

[00222] IgG subclass typing was conducted using the Human IgG Subclass SD Combi BINDARDID Kit (The Binding Site Inc., San Diego, CA) in accordance with the manufacturer's protocol.

Indirect immunofluorescent assay

[00223] 293T cells were transfected with the constructs bearing E1E2 sequences of genotypes 1 to 6, 40-48 hours post-transfection cells were fixed onto HTC Super Cured 24-spot slides (Cell Line Associates, Newfield, N.J.) with 100% acetone for 10 minutes at room temperature. Fixed cells were incubated with antibodies as indicated for 30 minutes at 37 °C and washed for 5 minutes with PBS, pH 7.4. Slides were then incubated for 30 minutes at 37 °C with a 0.001% solution of Evan's blue counterstain and fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). Bound antibody to fixed cells was revealed by fluorescence microscopy as described (Hadlock *et al.*, 1997, *J. Virol.*, 71:5828-40; incorporated herein by reference).

Antibody sequencing

[00224] Human monoclonal antibodies V_L and V_H domain CDR sequences was performed as described (Keck *et al.*, 2004, *J. Virol.*, 78:7257-63; incorporated herein by reference). Essentially, the entire variable regions of heavy and light chain from H-111 were sequenced to identify the FRs and CDRs, the germline V gene counterparts, and to determine the maturational status of the V domains. V_L and V_H domains of HC-1, HC-3, HC-11 and CBH-23 were amplified by RT-PCR from total cellular RNA isolated from the corresponding hybridoma cells (the RNeasy mini kit, Qiagen, Valencia, CA) using 5' family-specific V leader primers and 3' J region primers, as described previously (Campbell *et al.*, 1992, *Mol. Immunol.*, 29:193-203; and Chan *et al.*, 2001, *Blood*, 97:1023-26; both of which are incorporated herein by reference). Sequences were analyzed using Vector NTI (Invitrogen, San Diego, CA) and aligned with germline sequences using V-QUEST and VBASE database (Cook and Tomlinson, 1995, *Immuno. Today*, 16:237-42; incorporated herein by reference).

Competition assay

[00225] Antibody cross-competition studies were performed as previously described (Keck *et al.*, 2004, *J. Virol.*, 78:9224; incorporated herein by reference). Briefly, 1b HCVpp cell lysate was captured onto 96-well plates coated with GNA in PBS for 1 hour at 37 °C. After washing and blocking, competing antibodies at 20 µg/ml were added to each well and incubated for 30 minutes at room temperature, followed by adding the biotinylated test antibody at 2 µg/ml. After 1.5 hour incubation at room temperature, test antibody was detected using alkaline phosphatase-conjugated streptavidin (R & D Systems, Minneapolis, MN), followed by incubation of *p*-

nitrophenyl phosphate for color development. Absorbance was measured with a multiwell plate reader (Molecular Devices, Sunnyvale, CA) at 405 nm and subtracting the background reading at 570 nm. Mean OD values, as measured with biotinylated test HCV antibody to E2 in the presence of competing antibody, were divided by signals measured from biotinylated test HCV antibodies to E2 without competing antibody followed by multiplying by 100 to obtain the percent of test antibody bound to E2. Relatedness of the new HCV antibodies to other antibodies was determined by a modified approach of unweighted pair-group method using arithmetic averages, as previously described (Keck *et al.*, 2004, *J. Virol.*, 78:9224; incorporated herein by reference). This method assumes the extent of bidirectional inhibition as the extent of epitope overlap by the competing antibodies. Unidirectional inhibition or enhancement is interpreted as proximal but not overlapping epitopes.

Immunoprecipitation

[00226] 293T cells producing 1b HCVpp were lysed with a buffer containing 10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% NP-40, 20 mM α -Iodoacetamide, and protease inhibitors. This was used as the source of antigen, and approximately 225 ng/ml E2 was used per immunoprecipitation. 2 μ g/ml HCV antibodies was incubated with antigen for 1.5 hours at 4 °C, followed by incubation with immobilized protein A (Pierce, Rockford, IL) for an additional 1.5 hours at 4 °C. Between each step, beads were washed once with IP lysis buffer. After the last step, they were washed 3 times with IP lysis buffer and once with distilled water. Precipitates were heated at 70 °C for 5 minutes in SDS-PAGE sample buffer, run on a 10% SDS-polyacrylamide gel, and transferred to a nitrocellulose membrane. The membrane was immunoblotted with murine MAb AP33 (Owsianka *et al.*, 2005, *J. Virol.*, 79:11095; incorporated herein by reference), followed by incubation with HRP-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and detection using ECL Plus Western blotting detection system from GE Healthcare.

Antibody affinity measurement

[00227] Antibody affinity measurements were performed with 1a HCVpp cell lysate containing 1 μ g/ml E2 glycoproteins. Microtiter plates were prepared by coating each well with 500 ng of GNA followed by blocking of the wells with BLOTTO consisting of 2.5% non-fat dry milk and 2.5% normal goat serum in TBST (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Tween-20). After blocking, E2 in cell lysate was captured by GNA on plate and later bound by a

range of 0.01 µg/ml – 200 µg/ml of HCV antibodies. Bound HCV antibodies were incubated with alkaline phosphatase-conjugated goat anti-human IgG (Promega, Madison, WI), followed by incubation of *p*-nitrophenyl phosphate for color development. Absorbance was measured at 405 nm and 570 nm. Data were analyzed by nonlinear regression to measure antibody disassociation constant, K_d , and maximum binding, B_{max} (OD), using Prism software (GraphPad).

HCV RNA transfection and virus production

[00228] *Xba*I linearized pJFH-1 plasmid was *in vitro* transcribed (MEGAscript; Ambion, Austin, TX) and electroporated into Huh7.5 cells. Briefly, 10 µg of *in vitro* transcribed JFH-1 RNA was mixed with 0.4×10^6 Huh7.5 cells in a 4-mm cuvette in calcium-free PBS containing 10 µg calf liver tRNA and pulsed at 0.27 kV and at 960 µF using Bio-Rad Gene Pulser system. Electroporated cells were seeded into 10 cm cell culture dishes with 10 ml complete DMEM containing 10% FCS. Expression of HCV E2 was confirmed by IFA at each passage of the cells. For JFH-1 virus production, transfected cells were passaged at 4- to 5-day intervals with a 1:4 to 1:5 split into fresh culture flasks. Pooled medium virus titer was measured as foci forming units (FFU) as described below, placed in small aliquots and kept at -80 °C.

[00229] A second infectious HCV virus is an inter-genotypic chimeric virus produced by replacing the core-NS2 segment of the JFH-1 virus genome with the comparable segment of the subtype 1a H77 virus (Yi *et al.*, 2007, *J. Virol.*, 81:629; incorporated herein by reference). This chimeric virus, H-[NS2/NS3]-J/Y361H/Q1251L (hereinafter referred to as “HJ3-5,” contains two compensatory mutations that promote its growth in cell culture, one of which is within the E1 sequence (polyprotein residue 361) as shown (Yi *et al.*, 2007, *J. Virol.*, 81:629; incorporated herein by reference). Virus stocks were produced in FT3-7 cells (Blight *et al.*, 2002, *J. Virol.*, 76:13001; incorporated herein by reference).

HCVpp neutralization assay

[00230] Neutralization of HCVpp was performed as described previously (Keck *et al.*, 2007, *J. Virol.*, 81:1043-47; and Keck *et al.*, 2005, *J. Virol.*, 79:13199-208; both of which are incorporated herein by reference). Briefly, Huh-7 cells were seeded at 8×10^3 cells per well in a white nontransparent 96 well plate 24 hours before infection. The infection medium was incubated with various concentrations of antibodies for 60 minutes at 37 °C before adding to Huh-7 cells using PBS as a no antibody control. After 15 hours of incubation, HCVpp medium

was replaced with fresh complete medium and incubated for an additional 72 hours. Antibody neutralization activity was determined by the percent reduction of luciferase activity compared with the infection medium containing PBS.

HCVcc infectivity assay

[00231] Neutralization of HCVcc was assessed by NS3 expression reduction in infected cells as monitored by immunoblotting. 350 µl aliquot of 2a JFH-1 virus (10^5 FFU/ml) or 1a HJ3-5 HCVcc infected cells culture supernatants were incubated with antibodies at 20 µg/ml for 1 hour at 37 °C prior to inoculation onto naïve Huh 7.5 cells seeded 24 hours previously into 24-well plates at cell density of 32,000 per well. At 3 hours post-infection (hpi), HCV/antibody-containing medium was removed, and cells were washed with PBS and replaced with fresh complete DMEM. Cells were harvested for Western blotting analysis at 72 hpi. Samples were heated at 70°C for 5 minutes in SDS-PAGE sample buffer and run on a 10% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. Membranes were immunoblotted with anti-NS3 antibody, followed by incubation with HRP-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and detection using ECL Plus Western blotting detection system from GE Healthcare. Images were captured with a Bio-Rad gel doc system. Percent neutralization was compared to no antibody control. RO4 is an isotype-matched monoclonal antibody to HCMV and was used as a negative control.

Focus-forming unit (FFU) reduction assay

[00232] A 60 µl aliquot of stock HJ3-5 or JFH-1 virus (approximately 100 FFU) was mixed with an equal volume of diluted antibody and incubated at 37 °C for 1 hour prior to inoculation of 100 µl of the virus/antibody mixture onto Huh7.5 cells seeded 24 hours previously into 8-well chamber slides (Nalge Nunc Rochester, NY). Cultures were placed in a 5% CO₂ environment at 37 °C for 24 hours, fed with an additional 200 µl of media, and then re-incubated for an additional 48 hours. Supernatant fluids were removed, cells were washed once with PBS, and cells were fixed with 1:1 methanol-acetone prior to labeling with 1:300 MAb C7-50 specific for the core protein (Affinity BioReagents, Golden, CO) as described (Yi *et al.*, 2007, *J. Virol.*, 81:629; incorporated herein by reference). Following extensive washing, secondary labeling with FITC-conjugated goat anti-mouse IgG (1010-02, Southern Biotech, Birmingham, AL) at a 1:100 dilution, nuclei were counter-stained with Bisbenzimidazole H (Hoechst, Frankfurt am Main, Germany), and slides were mounted and examined under a Zeiss UV fluorescence microscope.

Foci of antigen-positive cells were counted in each individual slide, with each cluster of infected cells staining positively for core antigen considered to constitute a single infectious focus-forming unit (FFU), as described (Yi *et al.*, 2007, *J. Virol.*, 81:629; and Yi *et al.*, 2006, *Proc. Natl. Acad. Sci., USA*, 103:2310; both of which are incorporated herein by reference). Percent neutralization was calculated as the reduction in FFU compared to virus incubated with an irrelevant control antibody, RO4.

Blocking E2 binding to CD81

[00233] Genotype 1b E1E2 expressed in 293T containing 1 µg/ml E2 was incubated with each HCV antibody as indicated at 10 µg/ml for 1 hour at room temperature, and the antibody-antigen complex was then added onto CD81 pre-coated wells. Wells were washed and incubated with 5 µg/ml biotinylated CBH-4D for 1 hour. Bound CBH-4D was detected with alkaline phosphatase-conjugated streptavidin (R&D Systems; Minneapolis, MN), followed by incubation of *p*-nitrophenyl phosphate for color development. Absorbance was measured at 405 nm using plate reader Spectra Max 190 from Molecular Devices (Sunnyvale, CA). CBH-5 was used as a positive control and RO4 as a negative control.

Epitope mapping by site-directed mutagenesis

[00234] Alanine scanning mutagenesis was conducted using the QuickChange II Site-Directed Mutagenesis Kit (Stratagen, La Jolla, CA) in accordance with the protocol provided. Mutations were introduced into E2 of genotype 1a H77c strain (GenBank accession number AF009606). All mutants were sequenced (Sequetech, Mountain View, CA) to ensure that the clones possessed only the expected mutation. Mutated E2 proteins were expressed in 293T cells and analyzed by ELISA. Briefly, microtiter plates were coated with 500 ng per well of *Galanthus nivalis* lectin (GNA), followed by blocking with BLOTTO, consisting of 2.5% nonfat dry milk and 2.5% normal goat serum in TBST (20mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Tween-20). After blocking, transfected cell lysates diluted in BLOTTO were added to the plate. Bound mutant E2 proteins were then incubated with HC-1 or HC-11, followed by alkaline phosphatase-conjugated goat anti-human IgG. Samples were then visualized by adding *p*-nitrophenyl phosphate disodium hexa-hydrate and reading the absorbance at 405 nm. Results are shown in Figure 7. Derived values for the mutant E2 proteins were then displayed as a percent of that observed for the wt H77c E2. Mutated amino acids are depicted on the *y*-axis. The number in the between two substituted amino acids correspond to the position in the polyprotein of

reference strain H (GenBank accession number AF009606). HC-1 and HC-11 HCV antibodies binding to each mutant is expressed as the percent of binding value normalized by the binding of CBH-17, an antibody to a linear epitope on E2, and divided by HC antibody binding to the wild-type on the *x*-axis.

Binding of HCV Antibodies to E2 mutants by ELISA

[00235] ELISA was performed to measure antibody binding to the mutant E2 glycoproteins. Microtiter plates were coated with 500 ng GNA per well, followed by blocking with BLOTTO (2.5% nonfat dry milk and 2.5% normal goat serum in TBST [20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Tween-20]) for 1 hour. Wild type and mutated E2 glycoproteins expressed in 293T cell lysates were captured onto GNA coated plates. Bound E2 was detected by each antibody with the amount of E2 in each well normalized by using an anti-*c-myc* mouse MAb. Bound antibodies were incubated with alkaline phosphatase-conjugated goat anti-human IgG and followed by incubation of *p*-nitrophenyl phosphate for color development. Absorbance was measured at 405 nm and 570 nm. Derived values for the mutant E2 proteins were then displayed as a percent of that observed for the wild-type E2.

HCVpp neutralization time course

[00236] Antibody-mediated neutralization of HCVpp infection at different time intervals after attachment was performed essentially as described (Evans et al., 2007, *Nature*, 446:801-5; incorporated herein by reference) with modifications. Culture supernatants collected from 293T cells containing HCVpp was filtered using a 0.45 μ m filter and cooled to 4 °C. Huh 7.5 cells were seeded at 3.2×10^4 per well in poly-L-lysine coated 24 well tissue culture plates 24 hours prior to infection. After pre-cooling of Huh 7.5 cells at 4 °C and at *t* = -120 minutes, media was replaced with 200 μ l of HCVpp supernatant containing 4 μ g/ml polybrene and 50 mM Hepes, with or without antibody as indicated. The plates were centrifuged at 2100 rpm for 120 minutes at 4 °C. At *t* = 0 minutes, plates were washed twice with 1 ml cold PBS and then fresh 37 °C media containing 4 μ g/ml polybrene, with or without antibodies, was added and followed by placing the plates in a 37°C CO₂ incubator. At different time intervals, antibodies were added to media at the indicated concentrations. At 18 hours post infection, culture medium was changed to fresh medium without antibodies. Cells were harvested 3 days post-infection in 100 μ l cell culture lysis buffer and the expression of the luciferase reporter was measured with the addition of 100 μ l Bright Glo reagent from Promega (Madison, WI).

Cushion pellet virus assay

[00237] HCV pseudoparticles (HCVpp) were produced as described (Bartosch *et al.*, 2003, *J. Exp. Med.*, 197:633-42; and Keck *et al.*, 2005, *J. Virol.*, 79:13199-208; both of which are incorporated herein by reference) by co-transfection of 293T cells with plasmids containing the *env*-defective proviral genome and an expression plasmid encoding the HCV glycoproteins (gps). Here, HCVpp carries each of six mutated residues (R657A, D658a, F679A, L692A, I696A, D698A) involved in HC-3 epitope binding. Wild type H77c and/or E431A (a residue located outside of HC-3 epitope) are positive controls. Cells expressing HCV E1E2 and virus containing extracellular media were collected separately 48 hours post transfection. Cell lysate was prepared by resuspending cells expressing HCV E1E2 using lysis buffer (150 mM NaCl, 20 mM Tris [pH 7.5], 0.5% deoxycholate, 1.0% Nonidet-P40, 1 mM EDTA, 0.5 mg/ml Pefabloc, 2 µg/ml aprotinin, 2 µg/ml leupeptin, 1 µg/ml pepstatin). Nuclei were pelleted by centrifugation at $18,000 \times g$ at 4°C for 10 minutes, and resulting cytoplasmic extracts were collected for the Western blot analysis. Extracellular medium containing virus was first separated from cell debris by passing through a 0.45 µm filter and was further concentrated by processing 30 ml of filtered supernatant through a 20% sucrose cushion by ultracentrifugation using a Beckman Coulter SW 28 rotor (25,000 rpm, 2 hours at 4 °C). Cushion pelleted virus was resuspended in 150 µl of NTE buffer (150 mM NaCl, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4). For Western blotting, equal volumes of 20% sucrose cushion pellet suspension or 293T cell lysates were mixed with an equal volume of 2x SDS sample buffer and denatured by heating at 95 °C for 5 minutes. All samples were resolved by 10% SDS-PAGE using Tris-HCl Criterion gels (Bio-Rad Laboratories, Hercules, CA). Proteins on the gel were transferred onto a nitrocellulose filter. The filter was blocked with 5% dry-milk in 0.1% T-TBS (20 mM Tris-HCl, pH 7.5, containing 150 mM NaCl and 0.1% Tween-20).

[00238] The following pairs of primary and secondary antibodies were used to detect the amount of proteins in the samples: for E1 detection, H111 (Keck *et al.*, 2004, *J. Virol.*, 78:7257-63; incorporated herein by reference) at 2 µg/ml and anti-human IgG HRP (1:5000, GE Healthcare); for E2 detection, AP33 (Owsianka *et al.*, 2005, *J. Virol.*, 79:11095-104; incorporated herein by reference) at 2 µg and goat anti-mouse IgG (1:2000, Santa Cruz Biotechnologies); goat anti-HIV p24 (1:2500, ViroStat, Portland, OR) and bovine anti-goat IgG HRP (1:5000, Santa Cruz Biotechnologies). GAPDH and P24 proteins were used as internal

controls for cellular protein and HCV particles production, respectively, and were detected by mouse anti-GAPDH and goat anti-mouse IgG HRP (Santa Cruz Biotechnologies). The filter was incubated with ECL plus and chemiluminescent images were capture using a chemi-doc image system from Bio-Rad.

HCVpp infectivity assay

[00239] The amount of virus in virus-containing medium was first normalized by p24 expression using QuickTiter lentivirus titer kit (Cell Biolabs Lab, San Diego, CA) in accordance with the manufacturer's instructions. Subsequently, equal amount of virus was added to pre-seeded Huh-7.5 cells (8×10^3 cells/well) in a white nontransparent 96-well plate and infections were centrifuged at $730 \times g$ for 2 hours at room temperature before placing in a humidified cell culture chamber containing 5% CO₂ at 37°C. Unbound virus was replaced with fresh complete medium incubated for a total of 72 hours. After adding 100 µl of reconstituted Bright-Glo (Promega) to each well followed by mixing for 2 minutes at room temperature, luciferase activity was measured by a Veritas microplate luminometer (Turner Biosystems). Virus infectivity is shown as luciferase activity (RLU).

Results

Isolation of new domain B HCV Antibodies to HCV E2 glycoprotein

[00240] HCV E2 contains at least three immunogenic conformational domains with distinct properties and biological functions (Keck *et al.*, 2007, *J. Virol.*, 81:1043; Keck *et al.*, 2005, *J. Virol.*, 79:13199; and Keck *et al.*, 2004, *J. Virol.*, 78:9224; all of which are incorporated herein by reference). Each domain contains multiple overlapping epitopes having similar properties and function. To expand the panel of neutralizing antibodies, a group of human monoclonal antibodies from peripheral B-cells of three HCV infected individuals having high serum neutralization titers were produced and characterized. Peripheral B cells from individuals with either HCV 1a or 1b infection and high serum antibody neutralization activities were used to produce and characterize a panel of human monoclonal antibodies.

[00241] The initial screening made use of a genotype 1b E2 protein for a 1a infected donor, or 1a E2 protein for a 1b infected donor. B cells were activated by Epstein-Barr virus and used to produce human hybridomas as described (Hadlock *et al.*, 2000, *J. Virol.*, 74:10407; incorporated herein by reference). Four hybridomas, labeled as HC-1, HC-3, HC-11, and CBH-23, were

selected secreting antibodies that bound to subtype 1a HCVpp and 1b E2 but not 1b E1 glycoproteins by indirect immunofluorescence assay (IFA; Figure 1). This screening emphasized selecting HCV antibodies to conserved epitopes as previously shown and showed that these antibodies are to E2 (Keck *et al.*, 2004, *J. Virol.*, 78:9224; incorporated herein by reference).

[00242] Two rounds of single cell cloning established monoclonality of the hybridomas that was confirmed by sequencing the IgG genes as described (Keck *et al.*, 2004, *J. Virol.*, 78:7257; incorporated herein by reference). In addition, the four antibodies were sequenced, and the complementarity determining regions (CDRs) of each antibody's heavy and light chains were determined (Figure 2). In addition, IgG subclasses of each of all four antibodies were determined (Figure 3).

[00243] To find out the extent of epitope conservation among different HCV genotypes and/or subtypes, HC-1, HC-3, HC-11, and CBH-23 were tested by IFA against subtypes 1a, 1b, 2a, 2b, 3a, 4, 5 and 6 HCVpp infected Huh7.5 cells (Owsianka *et al.*, 2008, *J. Gen. Virol.*, 89:653-9; incorporated herein by reference; Figure 4).

[00244] As summarized in Figure 4, HCV antibodies HC-1 and HC-11 were able to neutralize all HCVpp genotypes except for genotype 4 for HC-1 and genotype 5 for HC-11. Both HC-1 and HC-11 neutralize HCVcc 1a and 2a. CBH-23 was able to neutralize HCVpp 1a and 1b infection, as well as HCVcc 2a infection. HC-3 was able to neutralize HCVpp 1a and HCVcc 2a. In contrast, the isotype-matched control RO4, a antibody to a CMV specific protein, was unable to bind to any HCVpp genotypes. All four antibodies were able to immunoprecipitate E2 (Figure 1) but did not detect E2 under reducing conditions by either ELISA or Western blot analysis, indicating that the HCV antibodies are to conformational epitopes on HCV E2 glycoprotein. HC-3, HC-11 and CBH-23 human hybridomas secrete IgG₁ antibodies, and HC-1 secretes IgG₂ (Figure 3). Antibody production was between 20 µg/ml – 60 µg/ml in spent supernatant. Sequence analysis of their Ig genes (V_L and V_H) showed that HC-1, HC-3, HC-11, and CBH-23 were derived from independent B cells expressing unique combinations of heavy and light chain CDR1, 2 and 3 regions (Figure 2).

[00245] To define the relationship of these new antibodies to the earlier panel, competition analysis with representative biotin-labeled domains A (CBH-4D), B (CBH-5), and C (CBH-7) HCV antibodies was performed (Figure 5). HC-1 and HC-11 showed minimum or no

competition with domains A and C antibodies, and 60% – 80% competition with CBH-5 (Figure 5), suggesting that epitopes recognized by these new antibodies are located within domain B. CBH-23 showed minimum or no competition with domains A and B antibodies, and >90% competition with CBH-7 suggesting that this epitope is located within domain C. HC-3 showed minimum or no competition with domains A, B, and C antibodies, suggesting that this antibody recognizes a new distinct domain.

Domain B epitopes elicit neutralizing antibodies

[00246] If the model of overlapping epitopes within a domain is to have similar properties and functions (Keck *et al.*, 2004, *J. Virol.*, 78:9224; incorporated herein by reference), HC-1, HC-11, and CBH-23 should also neutralize HCV as observed with earlier domains B and C HCV antibodies. Neutralization activities at 20 µg/ml of antibody were first measured using 1a and 1b HCVpp infection with each HCV antibody neutralizing both isolates. Average neutralization of the HCV antibodies against 1b HCVpp was comparable to CBH-5, at 64%. In contrast, an average of 80% neutralization for all HCV antibodies was observed in 1a HCVpp infection, compared with 60% with CBH-5. Indeed, more detailed neutralization analysis with HC-11 showed that this antibody has IC₅₀ of 0.9 µg/ml. This value is roughly 5 fold higher in 1a HCVpp neutralization potency than CBH-5 at 4.0 µg/ml. This may reflect differences in their epitopes between subtypes since CBH-5 was isolated from a 1b-infected patient and the new HCV antibodies were isolated from a 1a-infected patient. Neutralization was further tested with infectious subtype 1a and 2a HCVcc (Figure 4). For 2a HCVcc, neutralizing activity was measured by two assays, inhibition of NS3 protein expression in Huh7.5 infected cells by Western blot analysis and by FFU reduction. The effect of each antibody on 1a HCVcc infectivity was determined by FFU reduction. As shown in Figure 4, neutralization for 2a HCVcc was complete with HC-1, HC-3, and HC-11 with each HCV antibody at 10 µg/ml as measured by HCV NS3 expression. In contrast, infectivity with 2a HCVcc was unaffected by the negative controls (*i.e.*, RO4 antibody or no antibody (PBS) controls). Levels of β-actin protein used as an internal control were comparable between different samples. These results together are consistent with the model of overlapping conformational epitopes within a distinct immunogenic domain on E2 glycoprotein having similar functions. The successful isolation of HC-1 and HC-11 to domain B provides confirmation that domain B epitopes are highly immunogenic and elicit potent neutralizing antibodies.

Domain B antibodies inhibit E2 binding to CD81

[00247] The mechanism of neutralization with earlier domain B and C HCV antibodies is by inhibiting binding E2 to CD81. This was studied with HC-1, HC-3, HC-11, and CBH-23 in a CD81 capture assay. As shown in Figure 6, preincubation of E2 glycoproteins in the presence of 15 µg/ml of HCV antibodies HC-1, HC-11, CBH-23, or CBH-5 reduced by over 90% E2 binding to CD81 compared to the RO4 negative control. Similar to other domain B or C HCV antibodies, these HCV antibodies neutralize HCV by blocking E2 binding to CD81. In contrast, preincubation of E2 glycoproteins in the presence of HC-3 did not reduce E2 binding to CD81.

Epitope mapping of HC-1 and HC-12 by site-directed mutagenesis

[00248] Alanine scanning mutagenesis was performed to define the residues within the HCV antibody epitopes that engage in E2-CD81 interactions. A series of mutated proteins at amino acid sites ranging between 523 and 540 were obtained by site-directed mutagenesis. This region contains Tyr527, Trp529, Gly530, and Asp535 that are contact point residues for E2 binding to CD81 (Owsianka *et al.*, 2006, *J. Virol.*, 80:8695; incorporated herein by reference). Moreover, residues Gly523, Pro525, Gly530, Asp535 and Asn540 are involved in the CBH-5 epitope (Owsianka *et al.*, 2008, *J. Gen. Virol.*, 89:653-9; incorporated herein by reference). For these mutants, the effect on HC-1 binding was analyzed by using from transfected cells with mutant plasmids encoding E2-*c-myc* fusion protein. Mutant E2 proteins were assessed for their reactivity to HC-1 by ELISA (Figure 7). This assessment was confirmed by flow analysis. Since *c-myc* is a C-terminal tag, the amount of *c-myc* detected by PE-anti-*c-myc* was used as an internal control to normalize the level of expression of E2. To evaluate the percentage of HC-1 binding to mutant E2 proteins, E2/*c-myc* ratio was calculated for each mutant and compared to the ratio obtained with wild-type protein. CBH-4G, a nonneutralizing antibody, was used as a control to identify residues that are specific to HC-1. The lack of binding at substitution sites with both CBH-4G and a HCV antibody suggested either an overall change in E2 conformation or some degree of overlap between a neutralizing and a nonneutralizing epitopes. We previously noted some degree of cross-competition between neutralizing and nonneutralizing HCV antibodies to E2 suggesting spatial proximity of their immunogenic domains on E2 (Keck *et al.*, 2004, *J. Virol.*, 78:9224; incorporated herein by reference).

[00249] A significant decrease compared to wild type in HC-1 binding to 10% or less was noted when Trp529 (row 3), Gly530 (row 4), and Asn540 (row 12) were replaced by alanine, as

shown in Figure 7A. Because the Asn540 mutant also affected CBH-4G binding, it is unclear whether this residue participates in the HC-1 epitope. A mutation at position Asp535 (row 9) decreased HC-1 binding by >80%. For HC-11, a significant decrease of binding was observed when Gly530 and Asp535 were replaced by alanine, as shown in Figure 7B.

[00250] A mutation Gly530 (row 4) decreased HC-1 binding by >80%. In contrast to HC-1, a mutation at Trp529 (row 3) reduced HC-11 binding by only 44%, as compared to wild type. For both antibodies (Figures 7A and 7B), substitution at Gly523 (row 1) decreased by 60%.

Substitutions in Pro525 (row 2), Ala531 (row 5), Asn532 (row 6), Asp533 (row 7), Thr534 (row 8) had no or slight effect with +/- 20% on HC-1 and HC-12 binding. Substitutions at Val536 (row 10), Phe537 (row 11) and Asn540 (row 12) reduced HC-1 and HC-11 binding by 75-95%, but also reduced CBH-4G binding by 70-95%. Collectively, these results indicate that Trp529, Gly530 and Asp535 are contact residues for HC-1, and Gly530 and Asp535 are contact residues for HC-11. Thus, the present invention encompasses the recognition that HCV antibodies HC-1 and HC-11 neutralize HCVpp and HCVcc infections by directly blocking residues that are also contact points for E2 binding to CD81. The fact that no single substitution knock down binding by 100% is consistent with HCV antibodies HC-1 and -11 binding to discontinuous epitopes with other contact points to be defined that may or may not be directly involved in E2 binding to CD81.

[00251] Epitope mapping of HC-3 showed no involvement of residues between 530-535, but showed greater than 90% decrease in binding at residues R657, D658, F679, L692, I696, and D698 (Figure 8).

[00252] To approximate the step that HC-3 inhibits during the entry pathway, a time course study was performed compared to domain B and an antibody to CD81. Infectious HCVcc were incubated with 4 °C pre-cooled Huh-7.5 cells to permit virus attachment but no entry. Test antibody was then added at 15 minute intervals after placement of the virus-cell complex in a 37 °C incubator. As shown in Figure 9, there are similar patterns of progressive loss in blocking virus entry with both anti-CD81 and the domain B HCV antibody, HC-11. This is expected as domain B HCV antibodies inhibit virus entry by blocking E2 binding to CD81. The similarity in patterns with HC-3 with these two antibodies suggests that HC-3 inhibits virus entry at a temporal step near virus interaction with CD81. This includes the possible inhibition of E2 interaction with a different HCV co-receptor, such as SR-B1 or a step immediately following

CD81 engagement. The identification of contact residues for HC-3 at R657, D658, F679, L692, I696, and D698, and the lack of contact residues between 530-535, are consistent with these findings that HC-3 inhibits virus entry by a different step than blocking E2 binding to CD81.

[00253] The amino acids involved in HC-3 binding to E2 were tested in a cushion pellet assay to see if they are also involved in mediating E1E2 dimerization. In this assay, HCVpp carries each of six mutated residues (R657A, D658A and F679A, L692A, I696A, or D698A) that are part of the HC-3 E2 epitope. Alternatively, HCVpp carries wild type H77c or the E431A mutation (a residue located outside of the HC-3 epitope), as a positive control. Cells expressing HCV E1E2 were separated from extracellular medium (containing virus). Cell lysates and cushioned virus pellets were prepared and loaded on a gel for western blotting.

[00254] Compared to H77c, HCVpp mutants R657A and D658A (and to a lesser extent, F679A) showed significant reduction in E1 (Figure 10). This suggests that these residues are involved in heterodimer formation between E1 and E2. These residues have not yet been identified as being involved in E1E2 dimerization. Similar observations were observed with E1E2 from cell lysates (Figure 10). The E431A mutant serves as a control of a mutation not affecting heterodimer formation.

[00255] Infectivity of virus having mutations at a contact point for HC-3 (*i.e.*, infectivity of virus with a mutation at R657A, D658A and F679A, L692A, I696A, or D698A) was determined. Results of these experiments (Figure 11) indicate that each mutation is lethal to the virus. The control mutation at E431A showed no change. Together with the findings in Figure 10, these data suggest that HC-3 mediates neutralization by affecting the transition from a E1E2 heterodimer to a state that is necessary for internalization and subsequent release of the virus into the cytosol. The present invention encompasses the recognition that one or more of these amino acid residues could be a target for small molecules that would mimic the effect of HC-3 to affect this transition step.

Discussion

[00256] HCV has an extraordinary capacity to generate quasispecies leading to escape viral mutants that are pathogenic. Despite this ability to evade immune containment, the demonstration of animal protection with passive immunotherapy and findings that broadly neutralizing antibodies are found in infected individuals provide support for a vaccine approach

that includes the capability to induce these antibodies. Development of a successful vaccine will require characterization of immunodominant epitopes mediating virus neutralization that is broadly conserved among different genotypes, subtypes, and/or strains. In this example, the HCV antibodies (*i.e.*, HC-1, HC-3, HC-11, and CBH-23) are derived from a B cell donor infected with either subtype 1a or 1b. The antibodies were screened by IFA using subtype 1a or 1b E2 expressed in 293T cells to emphasize selecting HCV antibodies to conserved epitopes. Three of the four HCV antibodies are IgG₁, which is consistent with previous findings of the antibody response to HCV infection as mainly of the IgG₁ subclass (Chen *et al.*, 1999, *Gastroenterology*, 116:135; and Hadlock *et al.*, 2000, *J. Virol.*, 74:10407; both of which are incorporated herein by reference). The four HCV antibodies bind to epitopes conserved across subtypes 1-6 as detected by IFA and are to conformational epitopes as shown by their ability to immunoprecipitate HCVpp but not to detect E2 by western blot analysis. Elimination of binding to denatured antigens further proved the antibodies are to conformational epitopes. Cross-competition studies placed these antibodies within an earlier group of related HCV antibodies, labeled as domain B containing a cluster of tightly overlapping epitopes. Within the earlier set of domain B HCV antibodies, some as represented by CBH-5 bind and neutralized all subtypes and subtypes HCVpp while others as represented by CBH-8C are more restricted in not binding or neutralizing some subtypes/subtypes (Keck *et al.*, 2004, *J. Virol.*, 78:9224; and Owsianka *et al.*, 2008, *J. Gen. Virol.*, 89:653-9; both of which are incorporated herein by reference). Similar to CBH-5, HC-1 and HC-11 neutralize broadly as tested with 1a and 1b HCVpp, and 1a and 2a HCVcc. While the extent of neutralization achieved was similar to CBH-5 with 1b HCVpp, neutralization potency against 1a with some of the HCV antibodies was far greater than with CBH-5. This suggests some differences in their contact residues and is consistent with the fact that the donor of the B cells was infected with a 1a HCV isolate. Among the expanded panel of domain B HCV antibodies, neutralization potency was correlated with antibody binding affinity. With subtype 1a, CBH-5 has a lower antibody binding affinity of 2.2×10^{-7} M K_d (Keck *et al.*, 2004, *J. Virol.*, 78:9224; incorporated herein by reference) compared to the affinities of the HCV antibodies between $2.4\text{-}6.6 \times 10^{-9}$ M K_d.

[00257] In general, the primary mechanism of antibody-mediated virus neutralization is by inhibiting an early step of virus entry and appears to be mostly directed at the E2 glycoprotein. Compelling evidence showed that E2 interactions with glycosaminoglycans, the lipoprotein

receptor scavenger receptor class B type 1, SR-B1, and CD81 are involved in HCVpp and HCVcc entry [reviewed in Moradpour *et al.*, 2007, *Nat. Rev. Microbiol.*, 5:453; incorporated herein by reference)]. Whether E2 is also pivotal in later steps remains to be determined, such as interaction with claudin-1, a member in a large family of intercellular adhesion molecules and recently shown to be an essential step in entry that follows E2 binding to CD81 (Evans *et al.*, 2007, *Nature*, 446:801; incorporated herein by reference). Of the HCV antibodies, each inhibits E2 binding to CD81 as shown with other domain B HCV antibodies (Hadlock *et al.*, 2000, *J. Virol.*, 74:10407; incorporated herein by reference). One of ordinary skill in the art will readily recognize that experiments such as those described herein can be carried out to find out whether domain B HCV antibodies inhibit E2 interaction with other HCV receptor molecules.

[00258] Specific residues critical for CD81 binding have been localized to mainly two discontinuous sequences on E2 (Drummer *et al.*, 2006, *J. Virol.*, 80:7844; and Owsianka *et al.*, 2006, *J. Virol.*, 80:8695; both of which are incorporated herein by reference). They include a conserved motif located between HVR1 and HVR2 (amino acid 436-443) (Drummer *et al.*, 2006, *J. Virol.*, 80:7844; incorporated herein by reference) and specific residues conserved on all subtypes/subtypes, Trp420, Tyr527, Trp529, Gly530 and Asp535 (Owsianka *et al.*, 2006, *J. Virol.*, 80:8695; incorporated herein by reference). Alanine substitution studies on one of these two regions from 523-540 showed that two of the HCV antibodies representing the entire group revealed critical shared contact residues. HC-1 requires Trp529, Gly530 and Asp535, and HC-12 requires Gly530 and Asp535. These two patterns place HC-12 more similar to the CBH-5 epitope that also involves Gly530 and Asp535 but with at least one different contact point at Gly523 (Owsianka *et al.*, 2008, *J. Gen. Virol.*, 89:653-9; incorporated herein by reference). Indeed, competition studies also showed that the HC-11 epitope is spatially closer to CBH-5 than to either HC-1. Both HC-1 and HC-11 bind to alanine substitution at Gly523 while CBH-5 showed no binding. Recent studies showed that other broadly neutralizing human antibodies from combinatorial libraries isolated from an individual infected with subtype 2b also recognize epitopes containing Gly523, Trp529, Gly530, and Asp535 (Johansson *et al.*, 2007, *Proc. Natl. Acad. Sci., USA*, 104:16269; incorporated herein by reference). Why HC-1 and HC-12 have greater neutralizing potencies than CBH-5 with subtype 1a can be attributed in part by different antibody affinity to subtype 1a with HC-1, HC-12 and CBH-5 having nearly a two-log drop in K_d . This might reflect differences in their epitopes on contact residues outside of 523-540 that

could account for the differences in K_d values and neutralizing activities. Also, the location of critical contact residues of all domain B HCV antibodies at Gly530 and Asp535 on HCV E2 provides added proof of specific glycosylation sites on E2 modulating the neutralizing antibody response to HCV. In two independent studies, the glycan at Asn532 has been shown to decrease neutralizing activities of HCV polyclonal sera as well as HCV-specific neutralizing monoclonal antibodies (Falkowska *et al.*, 2007, *J. Virol.*, 81:8072; and Helle *et al.*, 2007, *J. Virol.*, 81:8101; both of which are incorporated herein by reference). When this residue is substituted with alanine, the mutant HCVpp showed greater sensitivity to be neutralized by these antibodies. The location of the glycan at Asn532 could reduce access of all domain B HCV antibodies to two shared contact residues at Gly530 and Asp535. Two other glycans as Asn417 and Asn645 have also been shown to reduce the neutralizing activity of a domain B HCV antibody, CBH-5 (Helle *et al.*, 2007, *J. Virol.*, 81:8101; incorporated herein by reference). Additional studies could provide evidence of other contact points of domain B HCV antibodies near these two glycans.

[00259] In summary, three sets of related neutralizing monoclonal antibodies from different laboratories have been isolated from individuals infected with HCV subtype 1a, 1b or 2b isolates (Hadlock *et al.*, 2000, *J. Virol.*, 74:10407; Johansson *et al.*, 2007, *Proc. Natl. Acad. Sci., USA*, 104:16269-74; and Keck *et al.*, 2007, *J. Virol.*, 81:1043; all of which are incorporated herein by reference). Cross-competition studies and epitope mapping to a region on HCV E2 involved in E2 binding to CD81 demonstrate that these antibodies are to overlapping epitopes with varying degrees of conservation among different HCV subtype and subtype isolates. Clearly, domain B is an immunodominant region on HCV E2 containing multiple overlapping epitopes. The fact that an HCVpp alanine substitution mutant at Asn532 having greater sensitivity to be neutralized by a panel of sera obtained from individuals infected with subtype 1a, 1b, 2b, 3, 4 and 5 showed that these sera contain antibodies directed at this region which further confirms that the epitopes in domain B are preferential targets of the immune response (Falkowska *et al.*, 2007, *J. Virol.*, 81:8072; incorporated herein by reference). Although the majority of domain B HCV antibodies are to conserved epitopes, some such as CBH-8C and CBH-11 do not bind to subtype 1a isolates, suggesting that an antibody response to some domain B epitopes could lead to escape virus mutants. An effective vaccine approach may involve eliciting an antibody response to those epitopes as represented by HCV antibodies while eliminating epitopes associated with escape mutants. This can be accomplished through point mutations or through deletions without

altering the native structure of the E2 glycoprotein as recently shown for the hypervariable regions (McCaffrey *et al.*, 2007, *J. Virol.*, 81:9584; incorporated herein by reference). Lastly, the biochemical and functional characterizations of the HCV antibodies, the earlier group of domain B HCV antibodies and the described recombinant human antibodies are consistent with the proposed model of antibodies to related epitopes within a domain having similar biophysical and functions properties.

Example 3: Antibody Production

[00260] The following Example provides a method that can be utilized to produce HCV monoclonal antibodies.

Expression and purification of antibody from a plasmid vector

[00261] A gene encoding an entire antibody molecule can be amplified from its parent hybridoma by RT-PCT and cloned into a heterologous expression cassette capable of driving expression of the antibody in a constitutive or inducible manner in a eukaryotic cell line. For example, antibody genes can be expressed in an expression plasmid such as pcDNA3.1 Zeo, pIND(SP1), pREP8, (all from Invitrogen, Carlsbad, California), and/or other expression vectors. Alternatively or additionally, antibody genes can be expressed via viral or retroviral vectors such as MLV based vectors, vaccinia virus-based vectors, and/or Adenovirus-based vectors. Similarly, antibody genes can be expressed in insect virus vectors such as baculovirus vectors. Other vectors, such as the pCOMB series of vectors allow for expression of an antibody heavy and light chain pair or a single chain antibody on the surface of M13 phage or bacteria.

[00262] Once expressed, antibodies can be purified using, *e.g.*, protein-A or protein-G sepharose, and purified antibody can be chemically or enzymatically modified in any of the following ways: biotinylation (*e.g.*, biotin-C11-hydroxysuccinimide ester, *etc.*); coupling to beads of various formats (*e.g.*, sepharose, agarose, magnetic, polystyrene, through the use of cyanogen bromide, *etc.*); and/or bridging the antibody to a useful chemical moiety (*e.g.*, by modifying lysine residues, by modifying basic residues, and/or through the use of reagents specific for free sulfhydryl groups, *etc.*).

Equivalents and Scope

[00263] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention, described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the appended claims.

[00264] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the appended claims.

[00265] In the claims articles such as “a,” “an,” and “the” may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Thus, for example, reference to “an antibody” includes a plurality of such antibodies, and reference to “the cell” includes reference to one or more cells known to those skilled in the art, and so forth. Claims or descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process. Furthermore, it is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, *etc.*, from one or more of the listed claims is introduced into another claim. For example, any claim that is dependent on another claim can be modified to include one or more limitations found in any other claim that is dependent on the same base claim. Furthermore, where the claims recite a composition, it is to be understood that methods of using the composition for any of the purposes disclosed herein are included, and methods of making the composition according to any of the methods of making disclosed herein or other methods known in the art are included, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise.

[00266] Where elements are presented as lists, *e.g.*, in Markush group format, it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be

removed from the group. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements, features, *etc.*, certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements, features, *etc.* For purposes of simplicity those embodiments have not been specifically set forth *in haec verba* herein. It is noted that the term “comprising” is intended to be open and permits the inclusion of additional elements or steps.

[00267] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[00268] In addition, it is to be understood that any particular embodiment of the present invention that falls within the prior art may be explicitly excluded from any one or more of the claims. Since such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular embodiment of the compositions of the invention (*e.g.*, any HCV genotype/subtype, any HCV antibody, any epitope, any pharmaceutical composition, any method of administration, any therapeutic application, *etc.*) can be excluded from any one or more claims, for any reason, whether or not related to the existence of prior art.

[00269] The publications discussed above and throughout the text are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior disclosure.

Other Embodiments

[00270] Those of ordinary skill in the art will readily appreciate that the foregoing represents merely certain preferred embodiments of the invention. Various changes and modifications to the procedures and compositions described above can be made without departing from the spirit or scope of the present invention, as set forth in the following claims.

Claims

What is claimed is:

1. An antibody directed to a conformational epitope within amino acids 523 to 540 of hepatitis C virus (HCV) envelope glycoprotein 2 (E2).
2. An antibody, wherein the antibody is monoclonal antibody HC-1.
3. An antibody, wherein the antibody is monoclonal antibody HC-3.
4. An antibody, wherein the antibody is monoclonal antibody HC-11.
5. An antibody, wherein the antibody is monoclonal antibody CBH-23.
6. An antibody directed to the epitope recognized by HC-1.
7. An antibody directed to the epitope recognized by HC-3.
8. An antibody directed to the epitope recognized by HC-11.
9. An antibody directed to the epitope recognized by CBH-23.
10. An antibody, wherein the antibody competes with HC-1 for binding to its epitope.
11. An antibody, wherein the antibody competes with HC-3 for binding to its epitope.
12. An antibody, wherein the antibody competes with HC-11 for binding to its epitope.
13. An antibody, wherein the antibody competes with CBH-23 for binding to its epitope.
14. The antibody of any one of claims 1-13, wherein the antibody is a monoclonal antibody.
15. The antibody of any one of claims 1-13, wherein the antibody is a humanized antibody.
16. The antibody of any one of claims 1-13, wherein the antibody is a mammalian antibody.

17. A fragment of an antibody selected from the group consisting of HC-1, HC-3, HC-11, and CBH-23, wherein the fragment retains the antigen binding activity of the whole antibody.
18. A cell line expressing the antibody of any one of claims 1-17.
19. The cell line of claim 18, wherein the cell line is a B cell line.
20. The cell line of claim 18, wherein the cell line is a human cell line.
21. The cell line of claim 18, wherein the cell line is a mammalian cell line.
22. The cell line of claim 21, wherein the cell line is a eukaryotic cell line.
23. The cell line of claim 18, wherein the cell line is a hybridoma.
24. A pharmaceutical composition comprising:
 - an antibody selected from the group consisting of HC-1, HC-3, HC-11, and CBH-23; and;
 - a pharmaceutically acceptable excipient.
25. A pharmaceutical composition comprising:
 - an antibody that recognizes an epitope recognized by an antibody selected from the group consisting of HC-1, HC-3, HC-11, and CBH-23; and;
 - a pharmaceutically acceptable excipient.
26. A pharmaceutical composition comprising:
 - a fragment of an antibody selected from the group consisting of HC-1, HC-3, HC-11, and CBH-23, wherein the fragment retains the antigen binding activity of the whole antibody; and
 - a pharmaceutically acceptable excipient.
27. The pharmaceutical composition of any one of claims 24-26, further comprising at least one additional antiviral agent.

28. The pharmaceutical composition of claim 27, wherein the at least one additional antiviral agent is selected from the group consisting of interferons, anti-HCV monoclonal antibodies, anti-HCV polyclonal antibodies, RNA polymerase inhibitors, ribavirin, protease inhibitors, IRES inhibitors, helicase inhibitors, antisense compounds, short interfering RNAs, short hairpin RNAs, micro RNAs, RNA aptamers, and ribozymes.
29. The pharmaceutical composition of claim 27, wherein the at least one additional antiviral agent is interferon.
30. The pharmaceutical composition of claim 27, wherein the at least one additional antiviral agent is ribavirin.
31. The pharmaceutical composition of claim 27, wherein the at least one additional antiviral agent is a combination of interferon and ribavirin.
32. The pharmaceutical composition of claim 27, wherein the at least one additional antiviral agent is an anti-HCV monoclonal antibody.
33. The pharmaceutical composition of claim 32, wherein the anti-HCV monoclonal antibody recognizes envelope glycoprotein 2 (E2).
34. The pharmaceutical composition of claim 32, wherein the antibodies are directed to proteins of identical HCV genotypes.
35. The pharmaceutical composition of claim 32, wherein the antibodies are directed to proteins of two or more HCV genotypes.
36. A method of treating a patient infected with HCV comprising steps of:
 - providing a patient infected with HCV or susceptible to HCV infection; and
 - administering to the patient the antibody or pharmaceutical composition of any one of claims 1-35.
37. A method of treating a patient exposed to HCV comprising steps of:
 - providing a patient infected with HCV or susceptible to HCV infection; and

administering to the patient the antibody or pharmaceutical composition of any one of claims 1-35.

38. The method of claim 36 or 37, wherein a therapeutically effective amount is an amount sufficient to treat HCV infection in the subject.
39. The method of claim 36 or 37, wherein a therapeutically effective amount is an amount sufficient to reduce the recurrence of HCV infection in the subject.
40. The method of claim 36 or 37, wherein a therapeutically effective amount is an amount sufficient to prevent or delay onset of HCV infection in the subject
41. The method of claim 36 or 37, wherein the subject is a baby born to an HCV infected mother.
42. The method of claim 36 or 37, wherein the subject is a liver transplant recipient.
43. The method of claim 36 or 37, wherein the subject is an individual who is susceptible to HCV infection.
44. The method of claim 36 or 37, wherein the subject is an individual who is suffering from HCV infection.
45. A method, comprising administering to a patient in need thereof an effective amount of a pharmaceutical composition, wherein the pharmaceutical composition comprises a pharmaceutically acceptable excipient and a human monoclonal antibody selected from the group consisting of:
 - (a) the monoclonal antibody HC-1 which is secreted by the hybridoma cell line deposited in the ATCC under Accession Number PTA-9416;
 - (b) the monoclonal antibody HC-3 which is secreted by the hybridoma cell line deposited in the ATCC under Accession Number PTA-9417;
 - (c) the monoclonal antibody HC-11 which is secreted by the hybridoma cell line deposited in the ATCC under Accession Number PTA-9418;
 - (d) the monoclonal antibody CBH-23 which is secreted by the hybridoma cell line deposited in the ATCC under Accession Number PTA-9419; and

- (e) fragments of the antibodies of (a)-(d) which retain the antigen binding characteristics of the whole antibody.
46. A kit comprising:
at least one HCV antibody selected from the group consisting of HC-1, HC-3, HC-11, and CBH-23;
a syringe, needle, or applicator for administration of the at least one HCV antibody to a subject; and
instructions for use.
47. A kit comprising:
a lyophilized powder comprising at least one HCV antibody selected from the group consisting of HC-1, HC-3, HC-11, and CBH-23;
a diluent, wherein the diluent is used to reconstitute the powder.
48. A small molecule, wherein the small molecule binds to the same epitope as HC-1.
49. A small molecule, wherein the small molecule binds to the same epitope as HC-3.
50. A small molecule, wherein the small molecule binds to the same epitope as HC-11.
51. A small molecule, wherein the small molecule binds to the same epitope as CBH-23.
52. A small molecule, wherein the small molecule competes with HC-1 for binding to its epitope.
53. A small molecule, wherein the small molecule competes with HC-3 for binding to its epitope.
54. A small molecule, wherein the small molecule competes with HC-11 for binding to its epitope.
55. A small molecule, wherein the small molecule competes with CBH-23 for binding to its epitope.

HMAbs	HCVpp					
	1a	1b	2A	2B	3A	4
HC-1	+	+	+	+	+	+
HC-3	+	+	+	+	+	+
HC-11	+	+	+	+	+	+
CBH-23	+	+	+	+	+	+
RO4	--	--	--	--	--	--

a: Indirect immunofluorescent assay against E1E2 transfected 293T cells

FIG. 1

Heavy Chain

	<u>CDR1</u>	<u>CDR2</u>	<u>CDR3</u>
HC-1	GGTYNSEV	FIPMFGTA	AKVLQVGGNLVVRPL
HC-3	GFSLSTTGVG	IYWDDDK	ALNSYRSGTILYRELELRGLFYI
HC-11	GATFSSFI	IIPMFGTA	AMEVPGFCRGGSCSGYMDV
CBH-23	GGTFSSYA	IVPMFGTE	ARHENIYGTPFDY

Light Chain

	<u>CDR1</u>	<u>CDR2</u>	<u>CDR3</u>
HC-1	QTISSTH	GVS	HQYGNSPQT
HC-3	QSISSW	ESS	QQYESSSWT
HC-11	HSVSSSN	GAS	QQYGSSPIT
CBH-23	HSITRY	AAS	QQSYSTLLT

FIG. 2

	IgG1	IgG2	IgG3	IgG4
HC-1	--	+++	--	--
HC-3	+++	--	--	--
HC-11	+++	--	--	--
CBH-23	+++	--	--	--

FIG. 3

HMAb	HCVpp ^a						HCVcc ^b			
	1a	1b	2a	2b	3a	4	5	6	1a	2a
HC-1	86 ^c	78	38	41	39	29	58	48	95	100
HC-3	70	18	NA ^d	NA	NA	NA	NA	NA	NA	100
HC-11	90	87	58	52	37	52	11	45	90	100
CBH-23	82	40	42	25	21	20	7	-10	NA	85
RO4	5	5	0	4	2	0	0	0	0	0

^a: HCVpp is HCV pseudoparticles
^b: HCVcc is cell cultured infectious HCV virions
^c: Number is percent neutralization
^d: Not available

FIG. 4

5/11

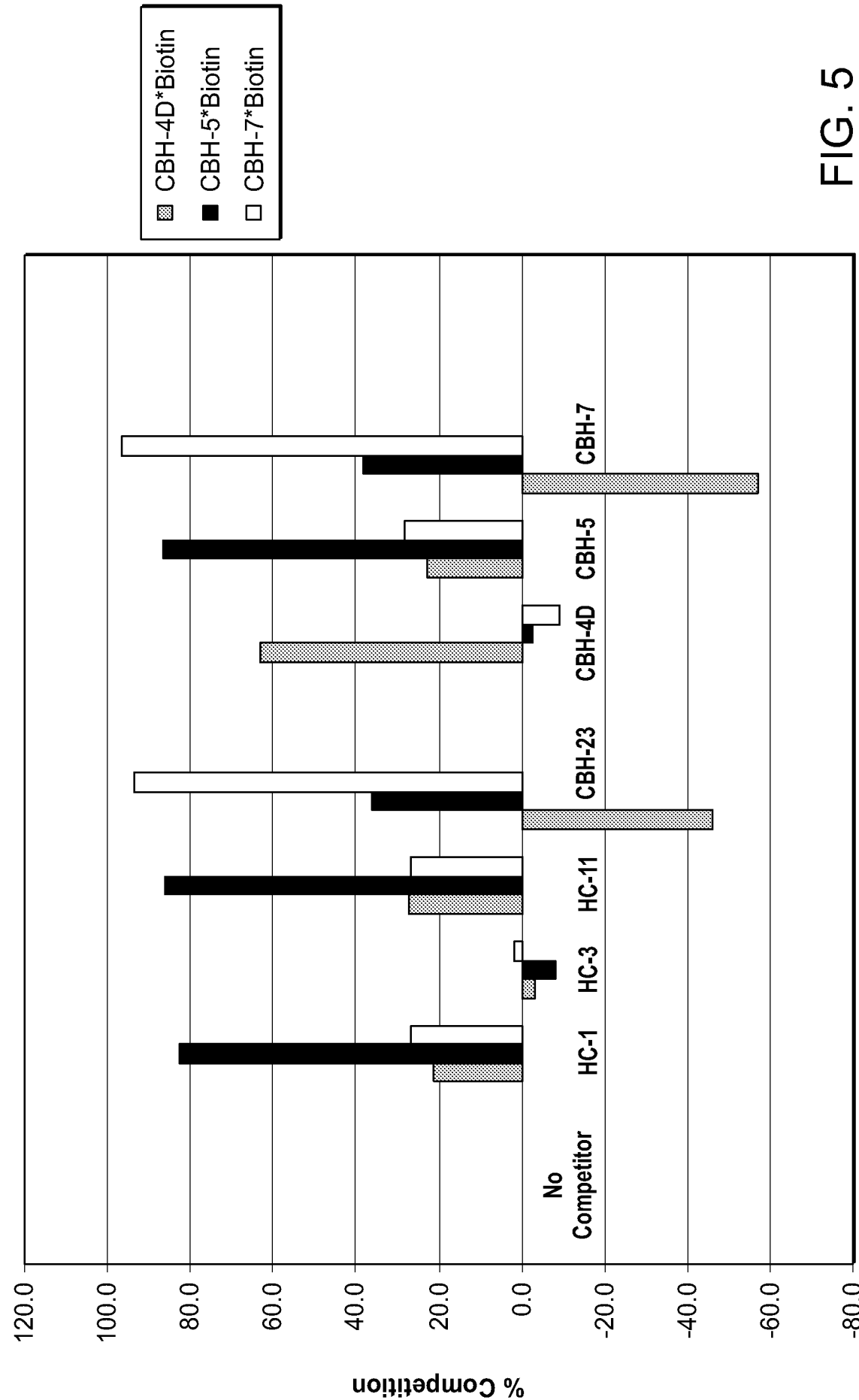


FIG. 5

Antibody at 20 µg/ml

6/11

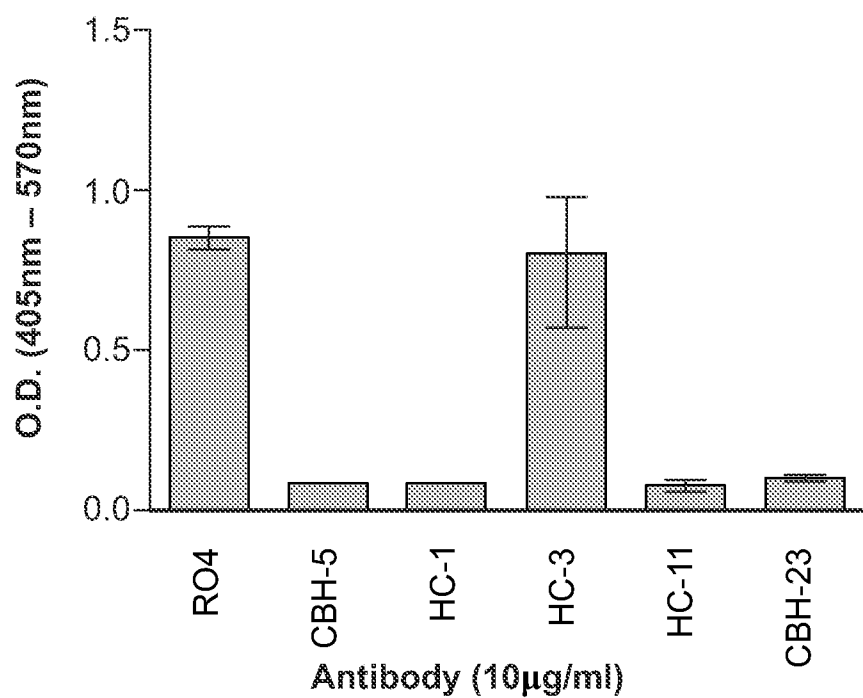


FIG. 6

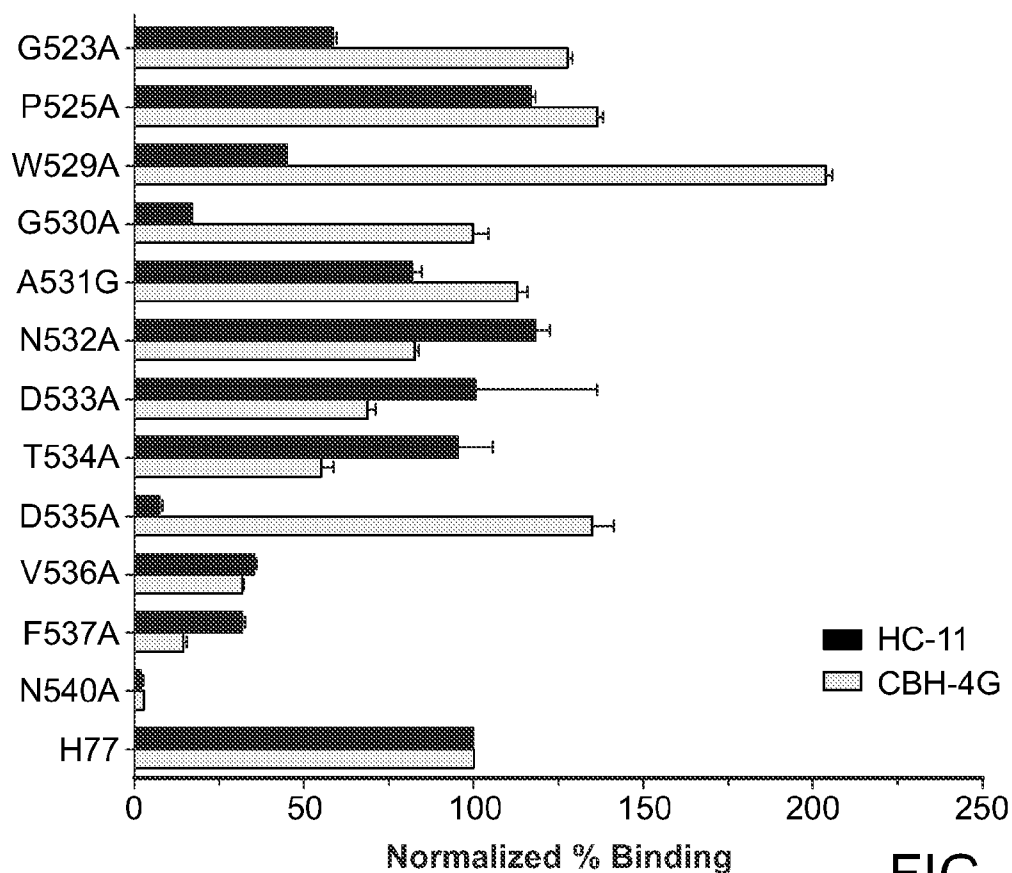
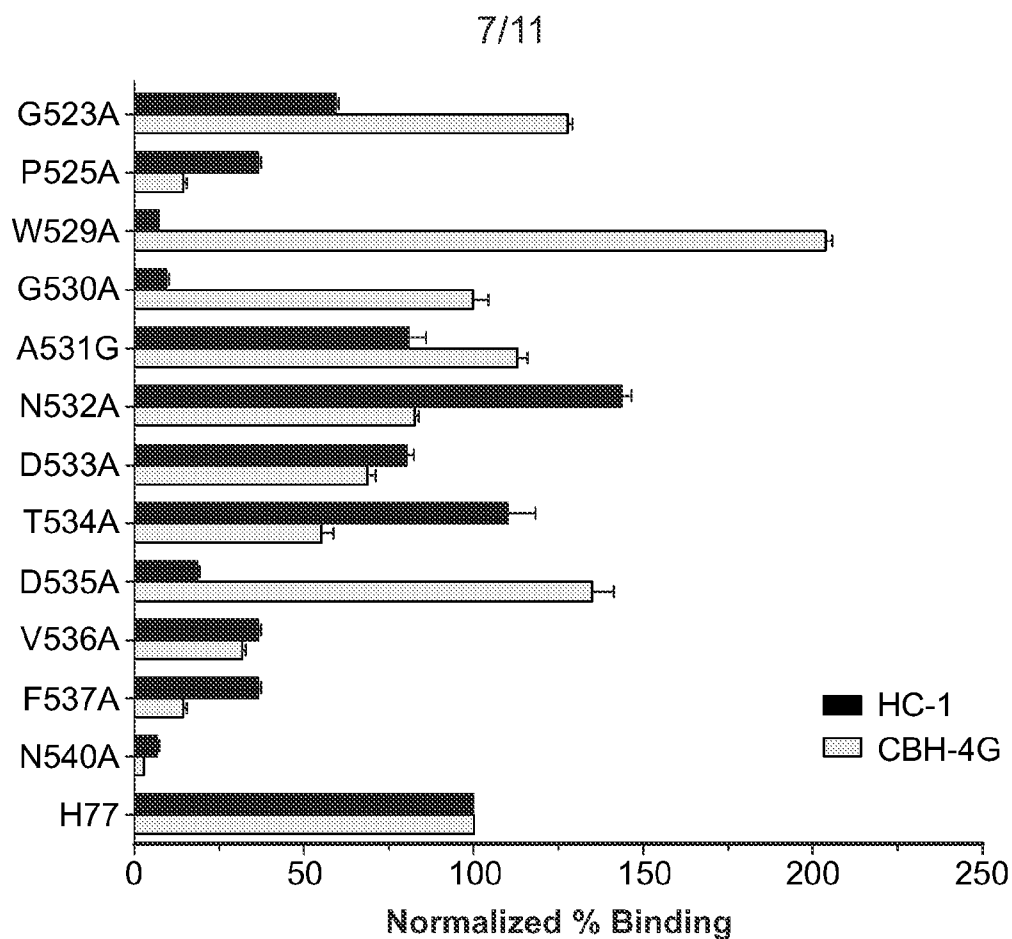
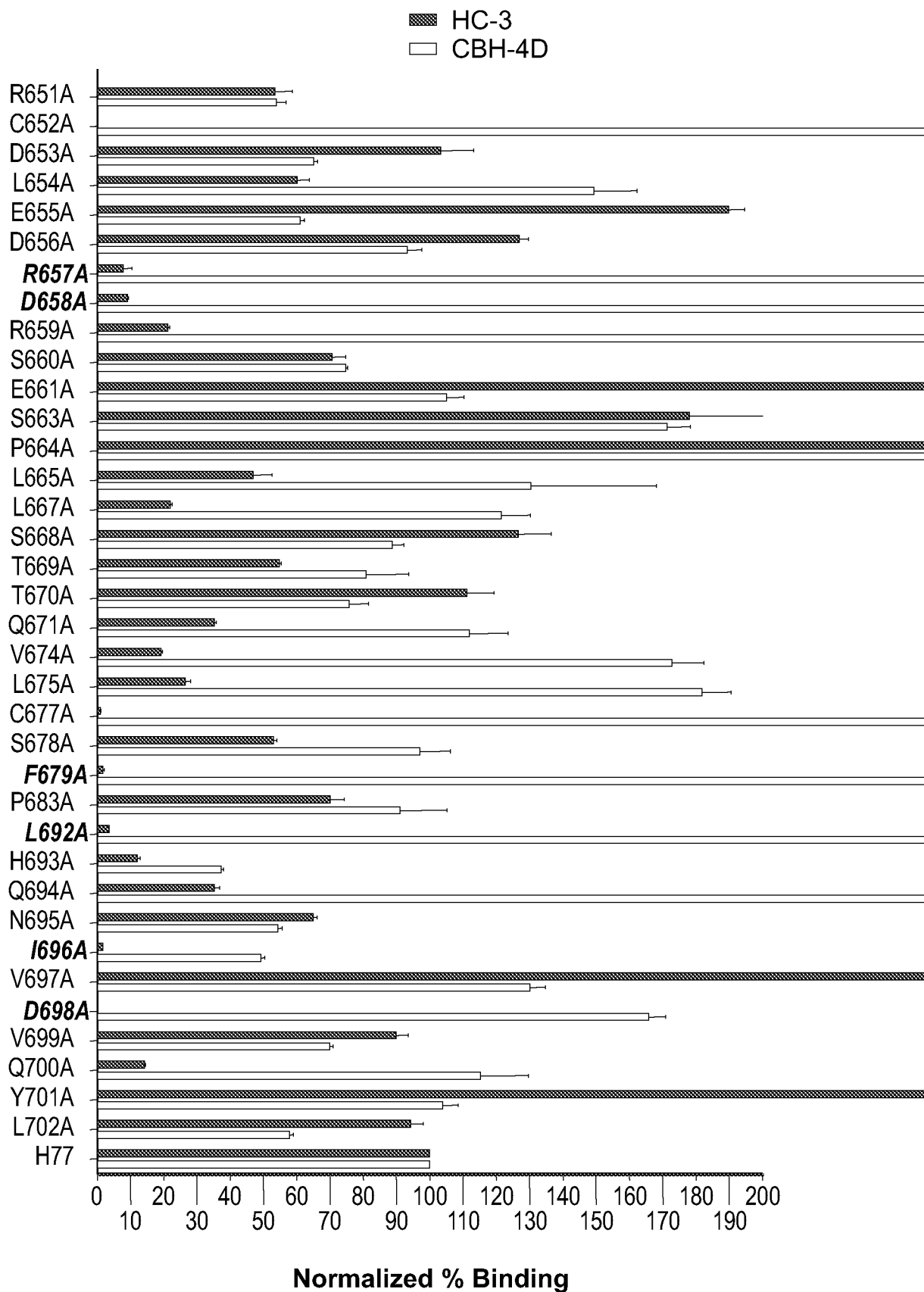


FIG. 7

8/11

FIG. 8



9/11

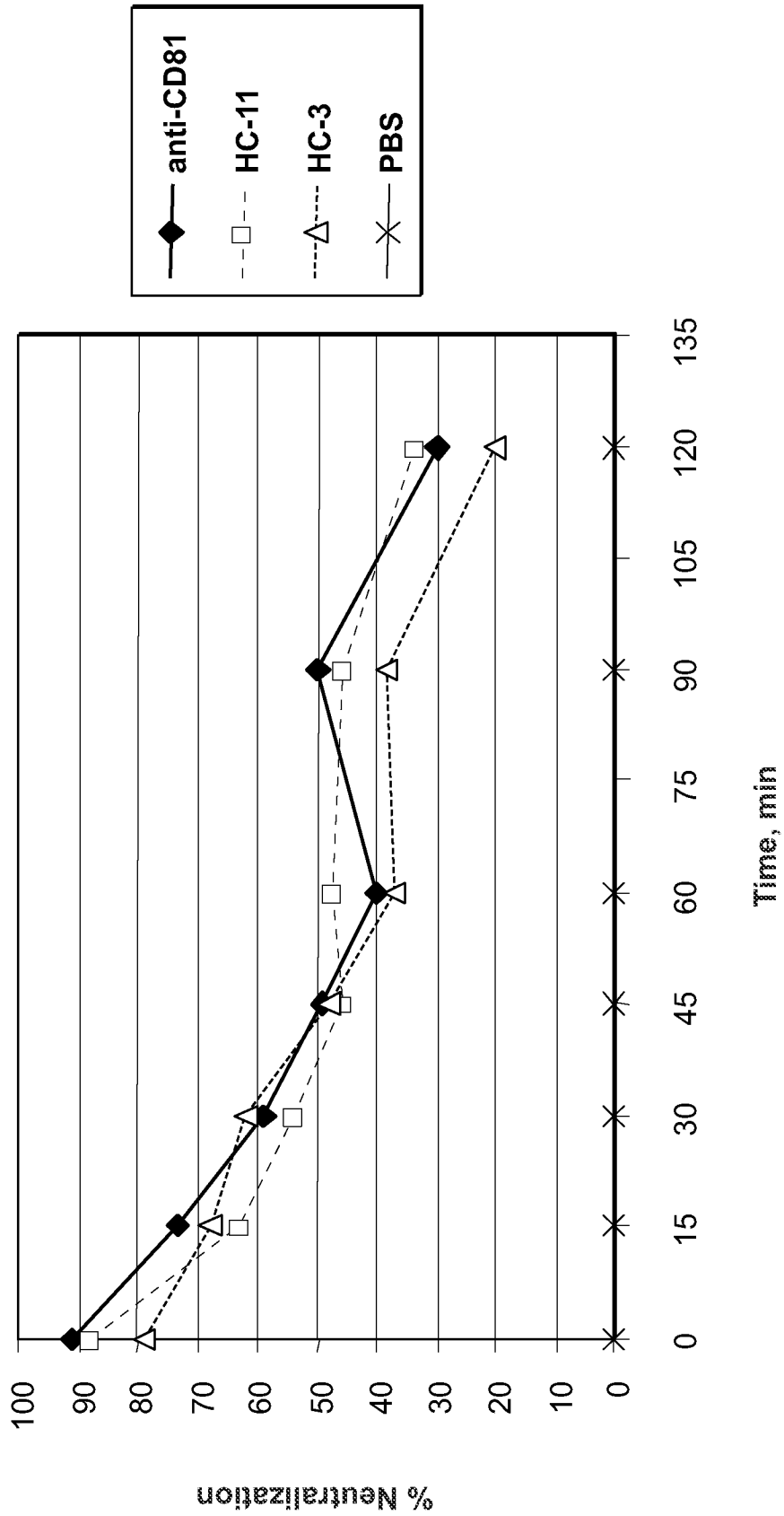


FIG. 9

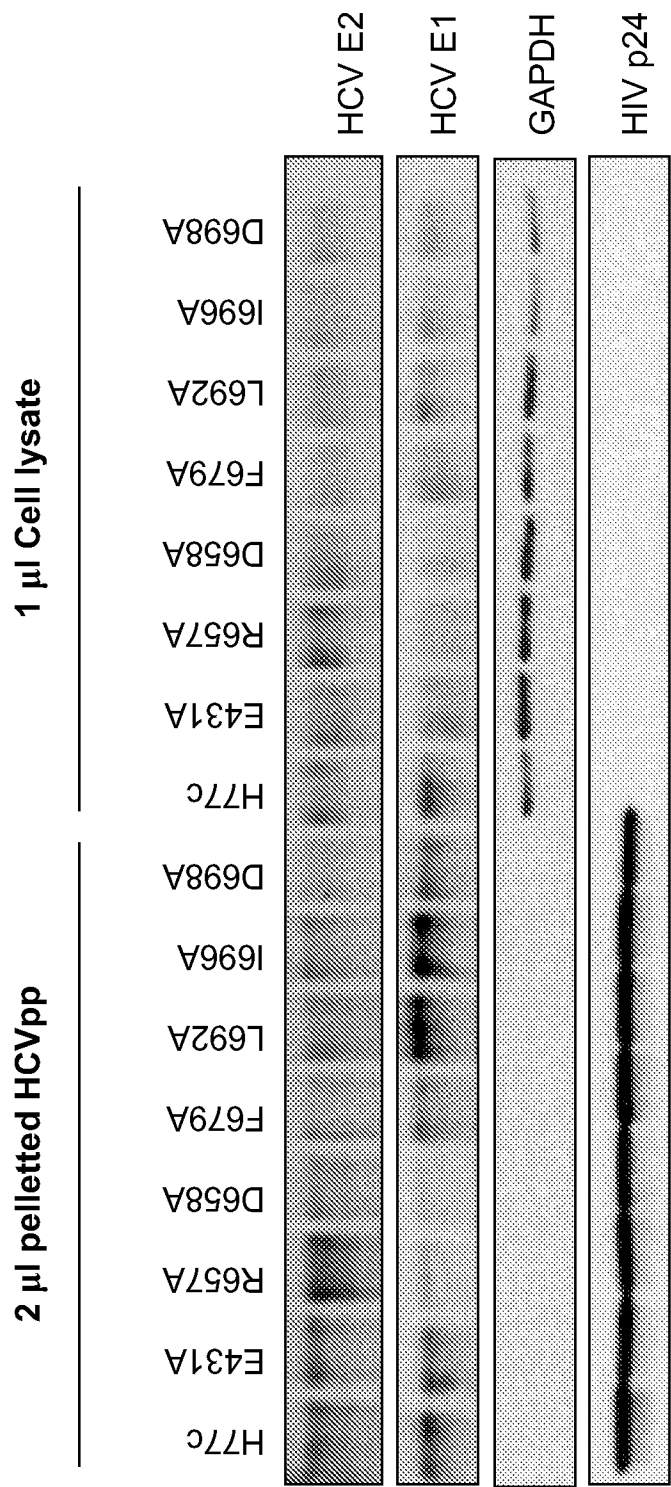


FIG. 10

11/11

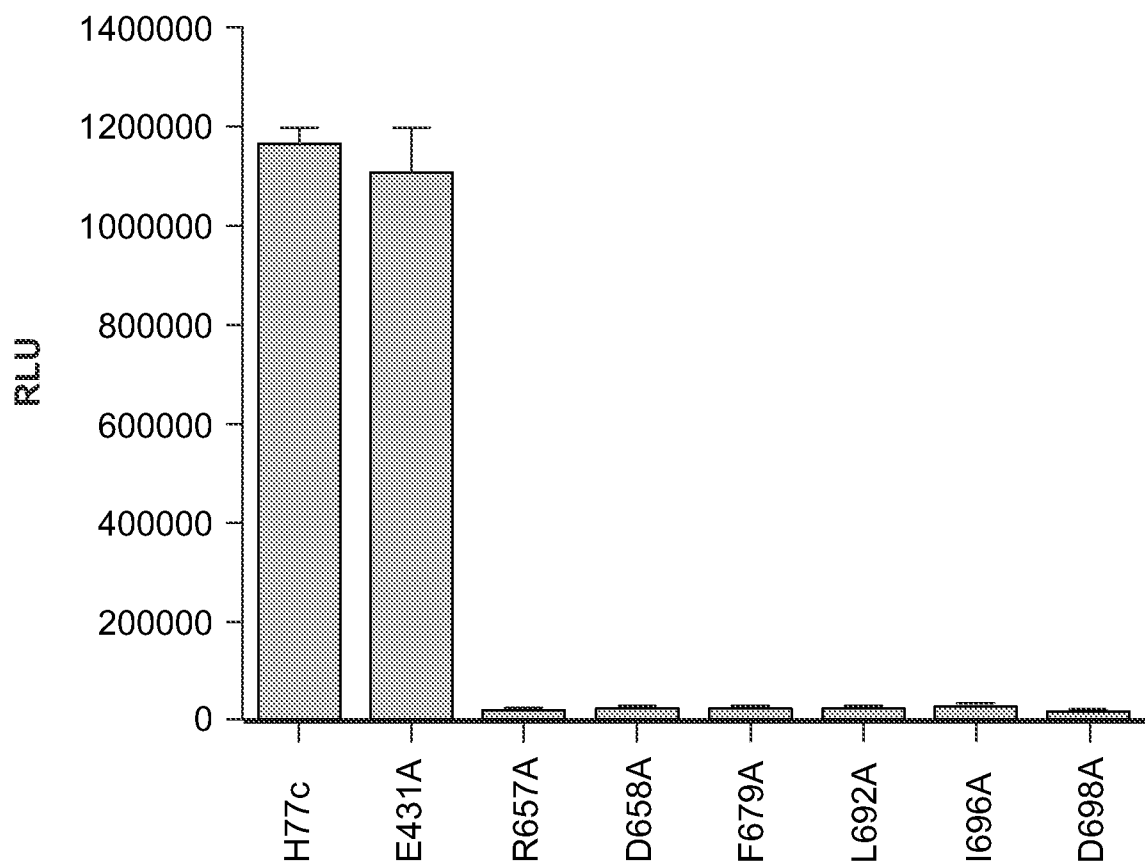


FIG. 11

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 08/78884

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 16/10, A61K 39/395 (2009.01)

USPC - 424/124.1, 424/149.1, 424/161.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

USPC -- 424/124.1, 424/149.1, 424/161.1, 530/388.3, 530/388.15

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubWEST(USPT,PGPB,EPAB,JPAB); Dialog Classic Files ? 654, 652, 351, 349, 315, 6, 35, 65, 155; Google Scholar; Entrez Pubmed; USPTO Web Page; Search terms -- monoclonal antibody, HC-1, HC-3, HC-11, CBH-23, epitope, HCV envelope glycoprotein E2, kit, excipient, diluents, kit, ribavirin, interferon, humanized, human

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - A	US 2006/0104980 A1 (FOUNG et al.) 18 May 2006 (18.05.2006) para [0004], [0015]-[0020], [0029] [0053], [0054], [0085], [0086], [0089], [0157]	1, 6-16 and 48-55 ----- 2-5, 17, 24-35, 45-47
A	US 2006/0257852 A1 (RAPPUOLI et al.) 16 November 2006 (16.11.2006) para [0003], [0017], [0028], [1452], [1467]	27-31, 47, 48
A	KECK et al., Analysis of a highly flexible conformational immunogenic domain A in hepatitis C virus E2, J. Virology, November 2005, Vol. 79, No. 21, pages 13199-13208	1-17, 24-35, 45-55

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

04 January 2009 (04.01.2009)

Date of mailing of the international search report

30 JAN 2009

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

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PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 08/78884

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 18-23 and 36-44
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.