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(54) Title: ANTI-HEPATITIS C ANTIBODIES AND ANTIGEN BINDING FRAGMENTS THEREOF

(57) Abstract: The invention provides an antibody or antigen binding fragment thereof capable of binding to the antigen binding pocket of the AP_{33} antibody, wherein said antibody or antigen binding fragment thereof comprises VL CDR1 (L1), VL CDR2 (L2), and VL CDR_3 (L_3) consisting of the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:23 respectively, and comprises VH CDR1 (H1), VH CDR2 (H2), and VH CDR_3 (H_3) consisting of the amino acid sequences of SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26 respectively. The invention also provides compositions, methods, nucleic acids and uses.

ANTI-HEPATITIS C ANTIBODIES AND ANTIGEN BINDING FRAGMENTS THEREOF

Field Of The Invention

The invention relates to antibodies or fragments thereof capable of generating an immune response against the Hepatitis C Virus (HCV) E2 protein.

Background To The Invention

There is an urgent need for a vaccine that will protect from infection with hepatitis C virus (HCV), which is a leading cause of liver cirrhosis and liver cancer. At present no such vaccine exists, and HCV infection is a major global public health problem. One of the obstacles to vaccine development is the high genetic diversity of the viral envelope glycoproteins.

HCV vaccine development has been thwarted by the high genetic diversity of the envelope glycoproteins and the presence of immunodominant, hypervariable regions within them. To elicit protective antibodies, the immune response needs to be focused on conserved, functionally important regions. The epitopes of broadly neutralizing antibodies (bnAbs) are therefore attractive leads for vaccine design.

One such bnAb is known antibody AP33, which binds to a conserved linear epitope (residues 412-423) on the HCV E2 envelope glycoprotein and potently neutralizes all genotypes of HCV.

The AP33 epitope, which spans residues 412 to 423 of HCV E2, is linear and highly conserved and encompasses a tryptophan residue that plays a critical role in CD81 recognition. The antibody has been shown to be capable of neutralising HCV across all the major genotypes. The rational development of immunogens that might mimic such epitopes and elicit AP33-like antibodies has been stymied by a range of factors in the art including the lack of detailed structural information available for the viral glycoproteins. Moreover, vaccination with peptides representing the epitope did not elicit antibodies that recognise E2.

It is a problem in the art to elicit antibodies that recognise E2.

The present invention seeks to overcome problem(s) associated with the prior art.

Summary Of The Invention

The generation of broadly neutralising antibodies for Hepatitis C virus (HCV) has been a problem in the art. Conventional approaches such as immunisation with peptides representing the key epitope of HCV E2 (residues 412 to 423 of E2) has failed to elicit antibodies that recognise E2.

The inventors rejected conventional approaches based on E2 peptide immunisations. The inventors instead pursued an anti-idiotypic approach. More specifically, the inventors have generated anti-idiotype antibodies against the established AP33 broadly neutralising antibody. Even this approach initially failed, until the inventors applied insights from a structural analysis of the epitope binding pocket of the AP33 antibody in order to design a radical selection technique allowing them to obtain the B2.1A anti-idiotypic antibody having remarkable properties.

The present invention is based upon the B2.1A antibody and its unique characteristics.

Thus, in one aspect the invention provides an antibody or antigen binding fragment thereof capable of binding to the antigen binding pocket of the AP33 antibody, wherein said antibody or antigen binding fragment thereof comprises VL CDR1 (L1), VL CDR2 (L2), and VL CDR3 (L3) consisting of the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:23 respectively, and comprises VH CDR1 (H1), VH CDR2 (H2), and VH CDR3 (H3) consisting of the amino acid sequences of SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26 respectively.

Suitably said antibody or antigen binding fragment thereof comprises VL amino acid sequence consisting of the amino acid sequence of SEQ ID NO:20.

Suitably said antibody or antigen binding fragment thereof comprises VH amino acid sequence consisting of the amino acid sequence of SEQ ID NO:22.

Suitably said antibody or antigen binding fragment thereof comprises VL amino acid sequence consisting of the amino acid sequence of SEQ ID NO:20 and said antibody or antigen binding fragment thereof comprises VH amino acid sequence consisting of the amino acid sequence of SEQ ID NO:22.

In another aspect, the invention relates to an antibody or antigen binding fragment thereof as described above, wherein the antigen binding fragment thereof is selected from the group consisting of a Fab fragment, a Fab' fragment, a F(ab')2 fragment, a scFv, a Fv, a rIgG, and a diabody.

Suitably said antigen binding fragment is a scFv and wherein said scFv comprises the amino acid sequence of SEQ ID NO:11 or SEQ ID NO:12 or SEQ ID NO:13.

In another aspect, the invention relates to a nucleic acid comprising a nucleotide sequence encoding the variable heavy chain domain and/or the variable light chain domain of the antibody or antigen binding fragment as described above.

Suitably the nucleic acid comprises one or more nucleotide sequences selected from the group consisting of SEQ ID NO:19 and SEQ ID NO:21.

In another aspect, the invention relates to a nucleic acid comprising a nucleotide sequence complementary to the nucleotide sequence as described above.

In another aspect, the invention relates to a vector comprising the nucleic acid as described above.

Suitably the vector further comprises an expression control sequence operatively linked to the nucleic acid encoding the variable heavy chain domain and/or the variable light chain domain.

In another aspect, the invention relates to a host cell containing the vector as described above.

Suitably the cell is a eukaryotic cell.

Suitably the eukaryotic cell is a Chinese Hamster Ovary (CHO) cell or a human embryonic kidney (HEK) cell.

In another aspect, the invention relates to a method of producing an antibody or antigen binding fragment thereof, comprising incubating a host cell as described above such that the encoded variable heavy chain domain and/or variable light chain domain is expressed by the cell; and recovering the expressed the antibody or antigen binding fragment thereof.

Suitably the method further comprises isolating and/or purifying the recovered antibody or antigen binding fragment thereof.

In another aspect, the invention relates to a composition comprising the antibody or antigen binding fragment thereof as described above and a pharmaceutically acceptable carrier or excipient.

In another aspect, the invention relates to a composition as described above further comprising a carrier protein, the carrier protein preferably selected from the group consisting of tetanus toxoid and CRM 197 mutant diphtheria toxin.
Suitably said composition further comprises an adjuvant.

In another aspect, the invention relates to a composition as described above formulated for use in humans.

In another aspect, the invention relates to an antibody or antigen binding fragment thereof capable of inducing in a mammal an immune response against the hepatitis C virus E2 protein, wherein said antibody or antigen binding fragment thereof is capable of binding to the antigen binding pocket of the monoclonal AP33 antibody.

In another aspect, the invention relates to an antibody or antigen binding fragment thereof capable of inducing in a mammal an immune response against the hepatitis C virus E2 protein, wherein said antibody or antigen binding fragment thereof comprises VL CDR1 (L1), VL CDR2 (L2), and VL CDR3 (L3) consisting of the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:23 respectively, and comprises VH CDR1 (H1), VH CDR2 (H2), and VH CDR3 (H3) consisting of the amino acid sequences of SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26 respectively.

In another aspect, the invention relates to an antibody or antigen binding fragment thereof capable of binding to the AP33 antibody wherein said antibody or antigen binding fragment thereof exhibits binding to AP33 antibody mutants FL32A, NL91A, WL96A, YH33A, YH50A, YH58A, IH95A and YH100A of less than 50% of its binding to the AP33 antibody.

In another aspect, the invention relates to an antibody that binds to an antibody or antigen binding fragment thereof as described above, which is not AP33 antibody or a fragment thereof.
Suitably said antibody is obtained by immunisation of a mammal with an antibody or antigen binding fragment thereof as described above.

In another aspect, the invention relates to a method of inducing in a mammal an immune response against the hepatitis C virus E2 protein, the method comprising administering to said mammal an antibody as described above, a nucleic acid as described above, a vector as described above, or a composition as described above.

In another aspect, the invention relates to an antibody as described above, a nucleic acid as described above, a vector as described above, or a composition as described above for inducing in a mammal an immune response against the hepatitis C virus E2 protein.

In one aspect, the invention relates to an antibody, a nucleic acid, a vector, or a composition as described above for use in manufacture of a composition for immunising against HCV.

In one aspect, the invention relates to an antibody, a nucleic acid, a vector, or a composition as described above for inducing in a mammal an immune response against the hepatitis C virus E2 protein.

Suitably said immune response induced is a humoral or antibody immune response.

Suitably said antibody induced binds HCV E2, suitably binding is at the 412-423 AP33 epitope. Suitably the antibodies induced bind HCV particles. Suitably the antibodies induced are neutralising antibodies.

Detailed Description Of The Invention

To obtain a molecule that correctly represents the 3-dimensional binding surface of the HCV E2 412-423 epitope, we pursued an anti-idiotype approach.

Mice were immunized with AP33 (Ab1) to generate a large number of anti-idiotypic (Ab2) monoclonal antibodies, all of which were able to potently inhibit AP33-E2 binding. The crystal structure of AP33 Fab complexed with its peptide epitope shows which amino acid residues comprise the antigen-binding pocket. By individually replacing these with alanine, we established exactly which residues are required for E2 binding. The AP33 mutants were then used to differentiate between the Ab2s. This screen identified one Ab2 with a binding profile very similar to that of E2.

When used as an immunogen in mice, this Ab2 induced Ab3 antibodies that recognize the same epitope and the same residues within it as AP33. The affinity of the Ab3 antibodies for E2 is similar to that of AP33, and they neutralize infectivity of cell-culture infectious HCV with an IC50 that is about twice that of AP33.

In one aspect the polypeptide of the invention comprises a B2.1A IgG molecule. A B2.1A IgG molecule is suitably an IgG molecule which comprises amino acid sequence of the CDRs of B2.1A e.g. the CDRs as shown in SEQ ID NO:s 1, 2, 23, 24, 25 and 26.

Suitably the polypeptide of the invention is a Fab fragment of the B2.1A IgG. The inventors have surprisingly discovered that the Fab fragment of the B2.1A antibody in fact performs better than the parent antibody itself. In addition, the Fab fragment is smaller and easier to handle. In addition, by removing sequences not required for antigen recognition the Fab fragment presents fewer irrelevant sequences to the immune system of the recipient, and therefore provides a more efficient antigen for immunisation.

Suitably the polypeptide of the invention may be a single chain variable fragment (scFv) derived from the B2.1A antibody sequence. This has the advantage of being of the smallest possible size whilst retaining the antigen binding activity. scFvs can also be cheap and efficient to produce by recombinant means.

The polypeptide or antibody or antigen binding fragment thereof of the invention may take any of the known forms. For example, the polypeptide may comprise an IgG. For example, the polypeptide may comprise a F(ab')2. For example, the polypeptide may comprise a Fab'. For example, the polypeptide may comprise a Fab. For example, the polypeptide may comprise a Fv. For example, the polypeptide may comprise a rIgG.

A person skilled in the art can make these or any other antibody variants according to their choice and/or the desired application. The production of each of these and any other antibody variants is enabled by the amino acid sequences of the variable regions of the B2.1A antibody provided herein, in particular the exact sequences of the CDRs. For example, in order to produce IgG, the variable region sequences such as the CDRs (i.e. nucleotide sequence encoding the CDRs or the larger variable regions) may be inserted into a standard heavy/light chain expression vector.

For example, B2.1A antibody chains may be produced using conventional antibody expression systems incorporating the CDRs of the B2.1A as disclosed herein. Suitably a conventional expression system such as the 'antibody generation' system which is commercially available from InvivoGen at 5, rue Jean Rodier, F-31400 Toulouse, France may be used.

This vector may then be transfected into any suitable host cell. Suitably the host cell is eukaryotic such as mammalian. For example, suitable host cells may include CHO cells, 293T cells, HEK cells or any other suitable cell line. Following transfection, the host cells are incubated to allow expression of the antibody chains. These are the collected, for example from the supernatant in which the cells are incubated.

Purification of the antibody chains from that supernatant may be carried out. Purification may be by any known means such as chromatography, for example affinity chromatography (e.g. Protein A, Protein G, Protein L, Peptide M etc) or any other suitable means known in the art.

Thus, when a full IgG is desired, then the expression vector is so chosen so as to express the chains for a full IgG. If it is desired to produce a Fab fragment from that IgG, then any standard method known in the art such as papain digestion, pepsin digestion or ficin digestion may be used to generate that Fab. Most suitably, papain digestion of IgG is used to generate Fab.

Generation of antibodies or antigen binding fragments thereof, for example via antibody fragmentation, is well known in the art using commercially available reagents such as from Pierce (Pierce Protein Biology Products also known as ThermoScientific (ThermoFisher Scientific) of 3747 N Meridian Rd, Rockford, IL 61101, USA.

Suitably the antibody or antigen binding fragment thereof of the invention may be administered in conjunction with, or formulated into a composition with, a carrier that is suitable for use in humans.

Suitably the antibody or antigen binding fragment thereof of the invention may be administered in conjunction with, or formulated into a composition with, an adjuvant that is suitable for use in humans

Alum is a most commonly used adjuvant in human vaccination. It is found in numerous vaccines, including diphtheria-tetanus-pertussis, human papillomavirus and hepatitis vaccines. Alum provokes a strong Th2 response. Suitably the adjuvant comprises Alum. Suitably alum means aluminium hydroxide, such as in the form of a wet gel suspension.
The adjuvant suitably induces both Th1 and Th2 responses.

Further guidance on adjuvants is provided by the European Medicines Agency's (EMEA) committee for medicinal products for human use. In particular, reference is made to their guideline on adjuvants in vaccines for human use document, which is incorporated herein by reference.

Suitably the antibody or antigen binding fragment thereof of the invention may be administered as, or provided as, a formulation that is suitable for use in humans.

Known carrier proteins include Keyhole Limpet Haemocyanin (KLH), self assembling carrier proteins such as Ferritin or luminaze synthase. There are numerous carrier proteins that are commonly used in compositions such as human vaccines: suitably the carrier protein is tetanus toxoid or CRM 197 mutant diphtheria toxin. As will be apparent to the skilled person, these are vaccines in their own right, against tetanus and diphtheria, respectively. They are also effective as immunogenic carrier proteins for molecules such as bacterial polysaccharides, which on their own are poorly immunogenic.

In principle, any protein molecule that is used in approved human vaccines could be a suitable carrier. The choice of carrier may be made by the skilled worker and confirmed either experimentally and/or through clinical trials.

The same principles apply to a suitable adjuvant. There is a limited number of adjuvants approved for human use, although there are a lot of candidate adjuvants and ongoing research into better human adjuvants, especially within the pharmaceutical industry. In principle, any adjuvant approved for use in human vaccines could be suitable. The choice of adjuvant may be made by the skilled worker and confirmed either experimentally and/or through clinical trials.

The same principles apply to a suitable vaccination regimen. Suitably a first administration of the of the antibody or fragment thereof (or nucleic acid or vector or composition) is provided. This may be referred to as a primary (or 'prime') injection. This is day 0. The immune response, for example as measured by antibody titer, can be maintained or enhanced ('boosted') in a mammal by providing one or more further or booster injections of the of the antibody or fragment thereof (or nucleic acid or vector or composition) at 2 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 1 year, or more after the primary injection. The primary and further or booster

injections need not be the same. Formulations may be different between injections such as carrier proteins may change, or nucleic acid may be alternated with peptide components as the operator chooses.

The same principles apply to a suitable formulation. In principle, any formulation suitable for use in human vaccines could be used. The choice of formulation may be made by the skilled worker and confirmed either experimentally and/or through clinical trials.

The composition may be a pharmaceutical composition.
The composition is suitably a composition suitable for generating an immune response to the antibody such as B2.1A antibody or fragment thereof as described herein.
Suitably said immune response induced is a humoral or antibody immune response.
Suitably said antibody induced binds HCV E2, suitably binding is at the 412-423 AP33 epitope. Suitably the antibodies induced bind HCV particles. Suitably the antibodies induced are neutralising antibodies.
Suitably the composition is a vaccine composition, suitably a vaccine composition for use in humans. Suitably the antibodies induced are protective against HCV infection.

Pharmaceutical compositions useful in the present invention may comprise an amount of the antibody or fragment thereof effective to induce an immune response in a subject and a pharmaceutically acceptable carrier, dilutent or excipient (including combinations thereof).
Pharmaceutical compositions may be for human or animal usage in human and veterinary medicine and will typically comprise any one or more of a pharmaceutically acceptable dilutent, carrier, or excipient. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985).
Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as olyvinylpyrrolidone; amino acids such as glycine,

glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

The choice of pharmaceutical carrier, excipient or dilutent may be selected with regard to the intended route of administration and standard pharmaceutical practice.

Pharmaceutical compositions may comprise as - or in addition to - the carrier, excipient or dilutent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s) or solubilizing agent(s).

Preservatives, stabilizers, dyes and even flavoring agents may be provided in pharmaceutical compositions. Examples of preservatives include sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used.

There may be different composition/formulation requirements dependent on the different delivery systems. By way of example, pharmaceutical compositions useful in the present invention may be formulated to be administered using a mini-pump or by a mucosal route, for example, as a nasal spray or aerosol for inhalation or ingestible solution, or parenterally in which the composition is formulated by an injectable form, for delivery, by, for example, an intravenous, intramuscular or subcutaneous route. Alternatively, the formulation may be designed to be administered by a number of routes. Most suitably the formulation is designed to be administered by injection via a route effective in inducing an immune response such as subcutaneously or intramuscularly.

The formulations to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

The antibody or fragment thereof may even be prepared in situ in the subject being treated. In this respect, nucleotide sequences encoding said antibody or fragment thereof may be delivered by use of non-viral techniques (e.g., by use of liposomes) and/or viral techniques (e.g., by use of retroviral vectors) such that the said protein is expressed from said nucleotide sequence.

The pharmaceutical compositions may be used in any of the methods described herein.

The pharmaceutical composition may be used among those subjects (e.g., humans) susceptible to infection with HCV i.e. to prevent or reduce/decrease the onset of HCV infection, such as by inducing an immune reaction against HCV.

The pharmaceutical composition may be used among those subjects (e.g., humans) already infected with HCV i.e. to treat HCV infection. Such treatment may facilitate

clearance of the virus from those subjects who are acutely or chronically infected including infected patients undergoing liver transplantation.

Thus, in a further aspect the invention provides a method for the treatment and/or prevention of hepatitis C virus infection, comprising the use of the antibody or the antibody fragment or the pharmaceutical composition. Suitably, an effective amount of the antibody or fragment thereof or the pharmaceutical composition is administered to the subject to induce an immune response.

There is also provided an antibody of a fragment thereof or the pharmaceutical composition for use in the treatment and/or prevention of hepatitis C virus infection in a subject.

Preferably the administered antibody/fragments thereof are substantially purified (e.g., preferably at least 95% homogeneity, more preferably at least 97% homogeneity, and most preferably at least 98% homogeneity, as judged by SDS-PAGE).

The active immunisation methods of the invention should allow for the protection or treatment of individuals against infection with viruses of a range of HCV genotypes, more suitably any of genotypes 1-6 of HCV, except for very occasional mutant isolates which contain several amino acid differences to that of the consensus peptide epitope 412-423 of E2.

Construction and operation of standard antibody expression systems as outlined above is well within the ambit of the skilled reader. Such systems are widely commercially available and are used as a matter of routine in order to produce antibody molecules having the desired CDRs.

In one aspect the polypeptide of the invention is a polypeptide comprising at least the six CDRs of the B2.1A antibody.

Unless otherwise indicated, all discussion of nucleotide and/or amino acid numbering herein follows the usual conventions. Numbering for polypeptide or polynucleotide sequences follows the numbering of the wild type version or the version apparent from the context. Numbering for antibody polypeptides / residues / mutants etc follows the established Kabat numbering (Kabat EA, Wu TT, Perry HM, Gottesman KS, Foeller C. 1991. Sequences of proteins of immunological interest, 5th ed. U.S. Department of Health and Human Services/NIH, Bethesda, MD.).

The polypeptide of the invention may be fused to another polypeptide such as a carrier polypeptide, a scaffold polypeptide or any other polypeptide.

It is further surprising that the Fab fragment of B2.1A performed better than the scFv of B2.1A. It is further surprising that the Fab fragment of B2.1A performed so well, especially since Fab fragments lose their divalence, but that did not appear to adversely affect performance.

The inventors took an unusual approach in selection of B2.1A. Firstly, they tried the conventional approach of immunising with target antibody (AP33) and generating anti-AP33 anti-idiotypic sera. However, those sera repeatedly failed. In order to address this problem, the inventors studied the crystal structure of AP33 complexed with its target, the linear E2 peptide. Based on this crystal structure, the inventors generated alanine mutants at fifteen different carefully selected sites on the AP33 antibody. In this manner, the inventors generated a panel of fifteen mutated antibodies based closely on AP33, each bearing a separate single alanine mutation in the key antigen binding pocket. The inventors tested the binding of these AP33 mutants to the E2 polypeptide. The inventors found that a single mutation at each of these carefully chosen sites was enough to abrogate the binding of the AP33 mutants to the AP33 epitope on the E2 polypeptide. In a remarkable new approach, the inventors then took this panel of mutant antibodies and analysed their binding to a panel of candidate anti-idiotype antibodies generated by immunisation with AP33. The results from this analysis varied widely. All of the anti-idiotype antibodies studied inhibited E2 binding to AP33. However, the anti-idiotype antibodies varied widely in their binding to the panel of fifteen mutant AP33 antibodies. Through a careful analysis of the binding of the anti-idiotype candidate antibodies to the fifteen alanine mutant AP33 antibodies, the inventors were able to select the remarkable B2.1A anti-idiotype antibody. This was the only anti-idiotype antibody in the analysis which showed a binding which was negatively affected by each of the individual alanine mutated AP33 mutant antibodies. This striking result is illustrated in Table 1. The key mutated residues in the AP33 light and heavy chains are highlighted in the "E2" row of the table. These correspond to eight alanine substitutions that reduce binding to E2 by more than 90%. These residues were therefore considered crucial to the AP33 – E2 interaction. As can be seen in the row entitled "B2.1A", this anti-idiotypic antibody also showed a drastically reduced binding to each of the AP33 alanine mutants bearing substitutions at those crucial residues. In sharp contrast, all of the other candidate anti-idiotype antibodies shown in Table 1 maintained a high level of binding to at least one of those AP33-derived antibodies bearing alanine substitutions at crucial residues. For example, L1.1A shows 85% binding even to a N91A AP33 mutant antibody. Therefore, B2.1A was

unique amongst all of the candidate anti-idiotypic antibodies analysed in that it showed a pattern of depressed binding to all of the AP33 mutant antibodies bearing alanine substitutions at the crucial residues for the AP33 – E2 interaction. This was interpreted by the inventors as the strongest possible evidence that they had created an anti-idiotypic antibody whose 3-dimensional structure most closely mimicked the 3-dimensional structure of the crucial epitope on the E2 polypeptide itself.

For all of these reasons, it is clear that the B2.1A antibody has unique and valuable characteristics which could not be expected, and which are not shown by any other known antibody, nor any other candidate antibody studied by the inventors.

A more conventional approach might have been to use all of the candidate anti-idiotype antibodies to immunise. Resulting sera (anti-Ab2 or anti-(anti-idiotype) sera) which show antibodies recognising E2 would then be selected. However, when the inventors followed this approach they experienced problematic rates of failure. In fact, the inventors did this for 25 candidate anti-Ab2 sera. Although the anti-Ab2 sera showed inhibition of binding of AP33 to E2 (indicating that they contained anti-Ab2 antibodies), the anti-Ab2 sera did not bind E2, nor did they inhibit HCV in cell culture. The inventors therefore rethought their approach as described above.

For illustrative/comparative purposes, a selection of the failed sera results are presented in a comparative example (see below).

It should be noted that the B2.1A antibody was very challenging to produce. For example, as described above, the inventors initially tried to obtain this antibody using twenty five separate immune sera generated by immunisation with AP33 antibodies. As explained above, none of those yielded the successful anti-idiotypic antibody having the features of B2.1A. In addition, prior attempts to induce anti-HCV E2 412 to 423 antibodies by immunising with E2 peptides, such as peptides comprising the 412 to 423 E2 antigen were unsuccessful. In view of these robust attempts to generate a successful immunogenic anti-idiotype antibody, the expectation would have been that such an antibody could not be produced. However, even in the face of this stark scientific situation, the inventors were able to adapt and make progress over a long period of arduous research as described herein. The result was the B2.1A antibody which is both structurally novel in terms of its sequence, in particular the unique and novel sequences of the CDRs and/or of the VL and/or of the VH chains, and also provides striking and

unique characteristics which are beneficial and render it susceptible of industrial application/utility. These properties are discussed in more detail below.

More specifically, the fact that the inventors were able to produce an antibody capable of replicating the key binding characteristics between the broadly neutralising AP33 antibody and its target epitope of residues 412 to 423 of E2 is an unexpected and extremely valuable achievement.

With reference to Table 1 showing the binding properties of E2 and anti-idiotypic antibodies to wild-type and mutant AP33, by "high" binding is meant binding of the test polypeptide to AP33 mutants at scores of 50% or higher of the binding of E2 to wild-type AP33. In particular, the key mutants under consideration are F_L32A, N_L91A, W_L96A; Y_H33A, Y_H50A, Y_H58A, I_H95A and Y_H100A.

Antibodies

Antibodies are naturally occurring immunoglobulin molecules which have varying structures, all based upon the immunoglobulin fold. For example, IgG antibodies such as AP33 have two 'heavy' chains and two 'light' chains that are disulphide-bonded to form a functional antibody. Each heavy and light chain itself comprises a "constant" (C) and a "variable" (V) region. The V regions determine the antigen binding specificity of the antibody, whilst the C regions provide structural support and function in non-antigen-specific interactions with immune effectors. The antigen binding specificity of an antibody or antigen-binding fragment of an antibody is the ability of an antibody or fragment thereof to specifically bind to a particular antigen.

The antigen binding specificity of an antibody is determined by the structural characteristics of the V region. The variability is not evenly distributed across the 110-amino acid span of the variable domains. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-12 amino acids long. The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in

binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

In some embodiments, the hypervariable regions are the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region may comprise amino acid residues from a "complementarity determining region" or "CDR" (e.g., around about residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the VL, and around about 31-35B (H1), 50-65 (H2) and 95-102 (H3) in the VH (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) and/or those residues from a "hypervariable loop" (e.g. residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the VL, and 26-32 (H1), 52A-55 (H2) and 96-101 (H3) in the VH (Chothia and Lesk J. Mol. Biol. 196:901-917 (1987)).

Each V region typically comprises three complementarity determining regions ("CDRs", each of which contains a "hypervariable loop"), and four framework regions. An antibody binding site, the minimal structural unit required to bind with substantial affinity to a particular desired antigen, will therefore typically include the three CDRs, and at least three, preferably four, framework regions interspersed there between to hold and present the CDRs in the appropriate conformation. Classical four chain antibodies, such as AP33, have antigen binding sites which are defined by VH and VL domains in cooperation. Certain antibodies, such as camel and shark antibodies, lack light chains and rely on binding sites formed by heavy chains only. Single domain engineered immunoglobulins can be prepared in which the binding sites are formed by heavy chains or light chains alone, in absence of cooperation between VH and VL.

Throughout the present specification and claims, unless otherwise indicated, the numbering of the residues in the constant domains of an immunoglobulin heavy chain is that of the EU index as in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), expressly incorporated herein by reference. The "EU index as in Kabat" refers to the residue numbering of the human IgG1 EU antibody. The residues in the V region are numbered according to Kabat numbering unless sequential or other numbering system is specifically indicated.

The antibody or antibody fragment described herein may be isolated or purified to any degree. As used herein, “isolated” means that that antibody or antibody fragment has been removed from its natural environment. In some embodiments, contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic or immunisation uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In some

embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

"Purified" means that the antibody or antibody fragment has been increased in purity, such that it exists in a form that is more pure than it exists in its natural environment and/or when initially synthesized and/or amplified under laboratory conditions. Purity is a relative term and does not necessarily mean absolute purity.

AP33 Antibody

AP33 is a mouse monoclonal antibody (MAb) that can strongly inhibit the interaction between E2 (in various forms, including soluble E2, E1E2, and virus-like particles) and CD81 (Clayton RF, et al. 2002. Analysis of antigenicity and topology of E2 glycoprotein present on recombinant hepatitis C virus-like particles. J. Virol. 76:7672–7682, Owsianka A, Clayton RF, Loomis-Price LD, McKeating JA, Patel AH. 2001. Functional analysis of hepatitis C virus E2 glycoproteins and viruslike particles reveals structural dissimilarities between different forms of E2. J. Gen. Virol. 82:1877–1883, Owsianka A, et al. 2005. Monoclonal antibody AP33 defines a broadlyneutralizing epitope on the hepatitis C virus E2 envelope glycoprotein. J. Gen. Virol. 79:11095–11104).

The AP33 epitope, which spans residues 412 to 423 of HCV E2, is linear and highly conserved and encompasses a tryptophan residue that plays a critical role in CD81 recognition. Indeed, the antibody has been shown to be capable of potently neutralizing infection across all the major genotypes.

Any known AP33 antibody may be used in the methods and techniques described herein. AP33 has been humanised, for example as in WO2009/081285. Suitably references herein to 'AP33 antibody' refer to the wild type mouse monoclonal AP33 antibody. Most suitably 'AP33 antibody' means an antibody or antigen binding fragment thereof comprising the AP33 CDRs, more suitably comprising the AP33 VL and/or VH sequences as described below.

AP33 (WT) vh and vL coding sequences

AP33 WT V_H seq

The sequence is arranged Leader-vH.
The Leader sequence is boxed.

ATG GTG TTA AGT CTT CTG TAC CTG TTG ACA GCC CTT CCG GGT ATC CTG TCA GAG GTG
CAG CTT CAG GAG TCA GGA CCT AGC CTC GTG AAA CCT TCT CAG ACT CTG TCC CTC ACC
TGT TCT GTC ACT GGC GAC TCC ATC ACC AGT GGT TAC TGG AAC TGG ATC CGG AAA TTC
CCA GGG AAT AAA CTT GAG TAC ATG GGA TAC ATA AGT TAC AGT GGT AGC ACT TAC TAC
AAT CTA TCT CTC AGA AGT CGC ATC TCC ATC ACT CGA GAC ACA TCC AAG AAT CAG TAC
TAC CTG CAG TTG AAT TCT GTG ACT ACT GAG GAC ACA GCC ACA TAT TAC TGT GCG CTC
ATT ACT ACG ACT ACC TAT GCT ATG GAC TAC TGG GGT CAA GGA ACC TCA GTC ACC GTC
TCC (SEQ ID NO: 14)

The amino acid sequence is disclosed by virtue of the above coding sequence which may be translated into the amino acid sequence using the universal genetic code.

AP33 WT V_L seq

The sequence is arranged Leader-vL
The Leader sequence is boxed.

ATG GAG ACA GAC ACA CTC CTG CTA TGG GTG CTG CTG CTC TGG GTT CCA GGT
TCC ACA GGT AAC ATT GTG CTG ACC CAA TCT CCA GTT TCT TTG GCT GTG TCT
CTG GGG CAG AGG GCC ACC ATT TCC TGC AGA GCC AGT GAA AGT GTT GAT GGT
TAT GGC AAT AGT TTT CTG CAC TGG TTC CAG CAG AAA CCA GGA CAG CCA CCC
AAA CTC CTC ATC TAT CTT GCA TCC AAC CTA AAC TCT GGG GTC CCT GCC AGG
TTC AGT GGC AGT GGG TCT AGG ACA GAC TTC ACC CTC ACC ATT GAT CCT GTG
GAG GCT GAT GAT GCT GCA ACC TAT TAC TGT CAG CAA AAT AAT GTG GAC CCG
TGG ACG TTC GGT GGA GGC ACC AAG CTG GAA ATC AAA (SEQ ID NO: 15)

The amino acid sequence is disclosed by virtue of the above coding sequence which may be translated into the amino acid sequence using the universal genetic code.

The sequences of the CDRs of AP33 are suitably as disclosed by the above nucleotide coding sequence of the VL and VH regions of AP33 antibody since it is a routine matter for the skilled worker to identify the CDRs given the VL/VH sequences.

The 'antigen binding pocket of the monoclonal AP33 antibody' is defined as known in the art, for example in Potter et al 2012 (J. Virol. vol 86 No 23 pages 12923-12932 "Toward a Hepatitis C Virus Vaccine: the Structural Basis of HepatitisC Virus Neutralization by AP33, a Broadly Neutralizing Antibody") which is incorporated herein by reference for the specific disclosure of the antigen binding pocket, with particular reference to Figure 3 of Potter et al 2012.
In case any further guidance is required, reference is made to the examples section herein.

Suitably the 'antigen binding pocket of the monoclonal AP33 antibody' is that part of AP33 which comprises the AP33 residues shown in Table 1.
Most suitably the 'antigen binding pocket of the monoclonal AP33 antibody' is that part of AP33 which comprises the AP33 residues highlighted in Table 1 (double underlined and bold in the head of the table in the 'WT AP33' row).

HCV E2 Protein

The HCV E2 protein is known in the art. For ease of reference representative HCV E2 sequences (both amino acid and nucleotide sequences) are provided in FIGURE 5.

The sequences presented are translation of: HCV strain H77c
The sequence shown is from HCV nucleotides 1 to 2600 encoding viral proteins core, E1 and E2 as annotated. The E2 sequence from amino acid residue 384-746 is underlined.

B2.1A Antibody

The sequence of B2.1A light and heavy chain variable regions is shown below.

Following convention, the vL CDRs are described as CDR1, CDR2 and CDR3. Alternatively, the light chain CDRs may be referred to as L1, L2, L3 and heavy chain CDRs as H1, H2, H3.

The CDRs are shown in boxed type on the amino acid sequence (the three sections of boxed type are CDR1, 2 and 3, respectively). These are also shown separately for ease of reference. Where there is underlining in a particular sequence, any non-underlined sequences are nucleotides/residues at the beginning of the sequence that did not appear in our sequencing, i.e. they were missing from the sequence because they were too close to the primer. They are therefore taken from the germline sequence that matches the rest of the obtained sequence.

In the preferred sequences:

CDRs defined by Kabat analysis are in **bold**

<u>CDRs defined by Chothia analysis are underlined</u>

Preferred CDRs based on crystal structure are boxed. In all instances, unless otherwise apparent from the context, reference to the CDRs of the B2.1A antibody (or derivative thereof) refers to the preferred CDRs as boxed above.

In the heavy chain preferred sequences, the *T* in italics was originally sequenced as A but corrected to T. Corresponding codon is *ACT*.

B2.1A Light chain		
	Example Sequence	**Preferred Sequence**
B2.1A Light chain nucleotide sequence	TGTGATGACCCAGTCTCCAA AATTCATGTCCACATCAGTAG GAGACAGGGTCAGCATCACC TGCAAGGCCAGTCAGAATGT TCGTACTGCTGTAGCCTGGT ATCAACAGAAACCAGGGCAG TCTCCTAAAGCACTGATTTAC TTGGCATCCAGCCGGCACAC TGGAGTCCCTGATCGCTTCA CAGGCAGTGGATCTGGGACA GATTTCACTCTCACCATTAGC AATGTGCAATCTGAAGACCT GGCAGATTATTTCTGTCTGCA ACATTGGAATTATCCGTACAC GTTCGGAGGGGGGACCAAGC TGGAAATAAAACGGGCTGAT GCTGCACCAACTG (SEQ ID NO:9)	GATATAGTGATGACCCAGTCT CCAAAATTCATGTCCACATCAG TAGGAGACAGGGTCAGCATCA CCTGCAAGGCCAGTCAGAATG TTCGTACTGCTGTAGCCTGGT ATCAACAGAAACCAGGGCAGT CTCCTAAAGCACTGATTTACTT GGCATCCAGCCGGCACACTGG AGTCCCTGATCGCTTCACAGG CAGTGGATCTGGGACAGATTT CACTCTCACCATTAGCAATGTG CAATCTGAAGACCTGGCAGAT TATTTCTGTCTGCAACATTGGA ATTATCCGTACACGTTCGGAG GGGGGACCAAGCTGGAAATAA AACGG (SEQ ID NO: 19)
B2.1A Light chain amino acid sequence	DIVMTQSPKFMSTSVGDRVSI TCKASQNVRTAVAWYQQKP GQSPKALIYLASSRHTGVPDR FTGSGSGTDFTLTISNVQSED LADYFCLQHWNYPYTFGGGT KLEIKRA (SEQ ID NO: 7)	DIVMTQSPKFMSTSVGDRVSIT C**KASQNVRTAVA**WYQQKPG QSPKALIY**LASSRHT**GVPDRF TGSGSGTDFTLTISNVQSEDLA DYFC**LQHWNYPYT**FGGGTK LEIKR (SEQ ID NO: 20)
VL CDR1 (L1)	KASQNVRTAVA (SEQ ID NO: 1)	KASQNVRTAVA (SEQ ID NO: 1)
VL CDR2 (L2)	LASSRHT (SEQ ID NO: 2)	LASSRHT (SEQ ID NO: 2)
VL CDR3 (L3)	LQHWNYPY (SEQ ID NO: 3)	LQHWNYPYT (SEQ ID NO: 23)

B2.1A Heavy chain		
	Example Sequence	**Preferred Sequence**
B2.1A Heavy chain nucleotide sequence	CTTCCGGAATTNCAGGTNCA GCTGCAGGAGTCTGGGGCTG AGCTGGTGAAGCCTGGGGCT TCAGTGAAGCTGTCCTGCAA GGCTTCTGGCTACACCTTCAC CAACTACTGGATGCACTGGG TTAAGCAGAGGCCTGGACAA GGCCTTGAGTGGATTGGAGA GATTAATCCTAGCGACGGTC ATACTAACTACAATGAGAAG TTCAAGAGCAAGGCCACACT GACTGTAGACAAATCCTCCA GCACAGCCTACATGCAACTC AGCAGCCTGACATCTGAGGA CTCTGCGGTCTATTACTGTGC AAGACCTTGGGCGTTTGGTA ACTACGGGGCCTGGTTTGCT TACTGGGGCCAAGGGACTCT GGTCACTGTCTCTGCAGCCA AAACGACACCCCCATCT (SEQ ID NO:10)	CAGGTTCAGCTGCAGGAGTC TGGG*A CT*GAGCTGGTGAAGC CTGGGGCTTCAGTGAAGCTG TCCTGCAAGGCTTCTGGCTA CACCTTCACCAACTACTGGAT GCACTGGGTTAAGCAGAGGC CTGGACAAGGCCTTGAGTGG ATTGGAGAGATTAATCCTAG CGACGGTCATACTAACTACA ATGAGAAGTTCAAGAGCAAG GCCACACTGACTGTAGACAA ATCCTCCAGCACAGCCTACAT GCAACTCAGCAGCCTGACAT CTGAGGACTCTGCGGTCTAT TACTGTGCAAGACCTTGGGC GTTTGGTAACTACGGGGCCT GGTTTGCTTACTGGGGCCAA GGGACTCTGGTCACTGTCTC TGCA (SEQ ID NO: 21)
B2.1A Heavy chain amino acid sequence	QVQLQESGAELVKPGASVKLS CKASGYTFTNYWMHWVKQR PGQGLEWIGEINPSDGHTNY NEKFKSKATLTVDKSSSTAY MQLSSLTSEDSAVYYCARPW AFGNYGAWFAYWGQGTLVT VSA (SEQ ID NO: 8)	QVQLQESG*T*ELVKPGASVKLS CKASGYTFT**NYW**MH WVKQ RPGQGLEWIG**EI**NPSDGH**T NYNEKFKS**KATLTVDKSSST AYMQLSSLTSEDSAVYYCAR**P WAFGNYGAWFAY**WGQGT LVTVSA (SEQ ID NO: 22)
VH CDR1 (H1)	NYWMH (SEQ ID NO: 4)	GYTFTNYW (SEQ ID NO: 24)
VH CDR2 (H2)	EINPSDGHTNYNEKFKS (SEQ ID NO: 5)	NPSDGH (SEQ ID NO: 25)
VH CDR3 (H3)	PWAFGNYGAWFA (SEQ ID NO: 6)	PWAFGNYGAWFAY (SEQ ID NO: 26)

Regarding the Preferred Sequences compared to the Example Sequences, there are some minor differences: (1) There are three extra codons at the beginning of the LC sequence, which code for DIV; (2) extra nucleotides at the 3' end of the LC sequence that do not code for the aa sequence of the LC variable region have been deleted; (3) Extra nucleotides at the 5' end of the HC sequence that do not code for the aa sequence of the HC variable region have been deleted; (4) The nucleotide given as N within the coding sequence of the HC is actually a T, i.e. the first two codons are CAG GTT (coding for aa's QV); (5) The ninth aa of the HC is T, not A. The corresponding codon is ACT, not GCT.

Regarding the preferred CDR sequences, as the skilled worker will appreciate, there are various models for assigning/identifying the CDR sequences in antibody VL/VH chains. The most popular/widely accepted versions are the Chothia and Kabat models, although others also exist such as the ABM and CONTACT models. The 'Example Sequence' CDR sequences were determined using the Kabat model as is conventional in the art. Therefore, whilst the Kabat determined CDRs represent a robust determination, they are in fact only modelled/predicted CDRs. The absolute/correct CDR sequences are those which are experimentally determined. The inventors have carried out this labour intensive analysis by creating a crystal structure. The experimentally determined CDRs are the 'Preferred Sequences'.

Expression of Recombinant Antibodies

Also provided are isolated nucleic acids encoding the antibodies and fragments thereof described herein such as the B2.1A antibodies, vectors and host cells comprising the nucleic acid, and recombinant techniques for the production of the antibody. The antibodies described herein may be produced by recombinant expression.

Nucleic acids encoding light and heavy chain variable regions as described herein are optionally linked to constant regions, and inserted into an expression vector(s). The light and heavy chains can be cloned in the same or different expression vectors. The DNA segments encoding immunoglobulin chains are operably linked to control sequences in the expression vector(s) that ensure the expression of immunoglobulin polypeptides. Expression control sequences include, but are not limited to, promoters (*e.g.*, naturally-associated or heterologous promoters), signal sequences, enhancer elements, and transcription termination sequences.

Suitably, the expression control sequences are eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells (*e.g.*, COS cells – such as COS 7 cells – or CHO cells). Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the

nucleotide sequences, and the collection and purification of the cross-reacting antibodies.

These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA.

Selection Gene Component- Commonly, expression vectors contain selection markers (*e.g.*, ampicillin-resistance, hygromycin-resistance, tetracycline resistance, kanamycin resistance or neomycin resistance) to permit detection of those cells transformed with the desired DNA sequences (*see, e.g.*, Itakura et al., US 4,704,362). In some embodiments, selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, *e.g.*, the gene encoding D-alanine racemase for Bacilli.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the nucleic acid encoding antibodies or fragments thereof described herein such as the B2.1A antibodies, such as DHFR, thymidine kinase, metallothionein-I and -III, preferably primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, etc.

For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity (*e.g.*, ATCC CRL-9096).

Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding an antibody described herein, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, *e.g.*, kanamycin, neomycin, or G418. *See* U.S. Pat. No. 4,965,199.

A suitable selection gene for use in yeast is the trp1 gene present in the yeast plasmid YRp7 (Stinchcomb et al., *Nature*, 282:39 (1979)). The trp1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1. Jones, *Genetics*, 85:12 (1977). The presence of the trp1 lesion in the yeast host cell genome then provides an effective environment for

detecting transformation by growth in the absence of tryptophan. Similarly, Leu2-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the Leu2 gene.

In addition, vectors derived from the 1.6 μm circular plasmid pKD1 can be used for transformation of Kluyveromyces yeasts. Alternatively, an expression system for large-scale production of recombinant calf chymosin was reported for *K. lactis*. Van den Berg, *Bio/Technology*, 8:135 (1990). Stable multi-copy expression vectors for secretion of mature recombinant human serum albumin by industrial strains of *Kluyveromyces* have also been disclosed. Fleer et al., *Bio/Technology*, 9:968-975 (1991).

Signal Sequence Component- The antibodies described herein such as the B2.1A antibodies may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which is preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. The heterologous signal sequence selected preferably is one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by the host cell. A signal sequence can be substituted with a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, 1 pp, or heat-stable enterotoxin II leaders. For yeast secretion the native signal sequence may be substituted by, *e.g.*, the yeast invertase leader, α factor leader (including *Saccharomyces* and *Kluyveromyces* α-factor leaders), or acid phosphatase leader, the *C. albicans* glucoamylase leader, or the signal described in WO 90/13646. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available.

The DNA for such precursor region is ligated in reading frame to DNA encoding the antibodies described herein such as the B2.1A antibodies.

Origin of Replication-Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Promoter Component- Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the nucleic acid encoding an antibody described herein such as a B2.1A antibody. Promoters suitable for use with prokaryotic hosts include the phoA promoter, β-lactamase and lactose promoter systems, alkaline phosphatase promoter, a tryptophan (trp) promoter system, and hybrid promoters such as the tac promoter. However, other known bacterial promoters are suitable. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the antibody.

Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CNCAAT region where N may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into eukaryotic expression vectors.

Examples of suitable promoter sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase or other glycolytic enzymes, such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657. Yeast enhancers also are advantageously used with yeast promoters.

The transcription of an antibody described herein such as the B2.1A antibody from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as *Adenovirus* 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, *e.g.*, the actin promoter or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Pat. No. 4,419,446. A modification of this system is described in U.S. Pat. No. 4,601,978. *See also* Reyes et al., Nature 297:598-601 (1982) on expression of human .beta.-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus. Alternatively, the Rous Sarcoma Virus long terminal repeat can be used as the promoter.

Enhancer Element Component- Transcription of a DNA encoding the antibody described herein such as the B2.1A antibody by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, .alpha.-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. *See also* Yaniv, *Nature* 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the HCV binding antibody-encoding sequence, but is preferably located at a site 5' from the promoter.

Transcription Termination Component- Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. One useful transcription termination component is the bovine growth hormone polyadenylation region. *See* WO94/11026 and the expression vector disclosed therein.

The vectors containing the polynucleotide sequences (*e.*g., the variable heavy and/or variable light chain encoding sequences and optional expression control sequences) can be transferred into a host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment, electroporation, lipofection, biolistics or viral-based transfection may be used for other cellular hosts. (*See generally* Sambrook et al., *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Press, 2nd ed., 1989). Other methods used to transform mammalian cells include the use of polybrene, protoplast fusion, liposomes, electroporation, and

microinjection (*see generally*, Sambrook et al., *supra*). For production of transgenic animals, transgenes can be microinjected into fertilized oocytes, or can be incorporated into the genome of embryonic stem cells, and the nuclei of such cells transferred into enucleated oocytes.

When heavy and light chains are cloned on separate expression vectors, the vectors are co-transfected to obtain expression and assembly of intact immunoglobulins. Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, HPLC purification, gel electrophoresis and the like (*see generally* Scopes, Protein Purification (Springer-Verlag, N.Y., (1982)). Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity is most preferred, for pharmaceutical uses.

Constructs

The invention further provides a nucleic acid construct comprising a polynucleotide as described herein.

Typically the construct will be an expression vector allowing expression, in a suitable host, of the polypeptide(s) encoded by the polynucleotide. The construct may comprise, for example, one or more of the following: a promoter active in the host; one or more regulatory sequences, such as enhancers; an origin of replication; and a marker, preferably a selectable marker. The host may be a eukaryotic or prokaryotic host, although eukaryotic (and especially mammalian) hosts may be preferred. The selection of suitable promoters will obviously depend to some extent on the host cell used, but may include promoters from human viruses such as HSV, SV40, RSV and the like. Numerous promoters are known to those skilled in the art.

The construct may comprise a polynucleotide which encodes a polypeptide comprising three light chain hypervariable loops or three heavy chain hypervariable loops. Alternatively the polynucleotide may encode a polypeptide comprising three heavy chain hypervariable loops and three light chain hypervariable loops joined by a suitably flexible linker of appropriate length. Another possibility is that a single construct may comprise a polynucleotide encoding two separate polypeptides – one comprising the light chain loops and one comprising the heavy chain loops. The separate polypeptides may be independently expressed or may form part of a single common operon.

The construct may comprise one or more regulatory features, such as an enhancer, an origin of replication, and one or more markers (selectable or otherwise). The construct may take the form of a plasmid, a yeast artificial chromosome, a yeast mini-

chromosome, or be integrated into all or part of the genome of a virus, especially an attenuated virus or similar which is non-pathogenic for humans.

The construct may be conveniently formulated for safe administration to a mammalian, preferably human, subject. Typically, they will be provided in a plurality of aliquots, each aliquot containing sufficient construct for effective immunization of at least one normal adult human subject.

The construct may be provided in liquid or solid form, preferably as a freeze-dried powder which, typically, is rehydrated with a sterile aqueous liquid prior to use.

The construct may be formulated with an adjuvant or other component which has the effect of increasing the immune response of the subject (*e.g.*, as measured by specific antibody titer) in response to administration of the construct.

Vectors

The term "vector" includes expression vectors and transformation vectors and shuttle vectors.

The term "expression vector" means a construct capable of *in vivo* or *in vitro* expression.

The term "transformation vector" means a construct capable of being transferred from one entity to another entity - which may be of the species or may be of a different species. If the construct is capable of being transferred from one species to another - such as from an *Escherichia coli* plasmid to a bacterium, such as of the genus *Bacillus*, then the transformation vector is sometimes called a “shuttle vector”. It may even be a construct capable of being transferred from an *E. coli* plasmid to an *Agrobacterium* to a plant.

Vectors may be transformed into a suitable host cell as described below to provide for expression of a polypeptide encompassed in the present invention. Thus, in a further aspect the invention provides a process for preparing polypeptides for use in the present invention which comprises cultivating a host cell transformed or transfected with an expression vector as described above under conditions to provide for expression by the vector of a coding sequence encoding the polypeptides, and recovering the expressed polypeptides.

The vectors may be for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of the said polynucleotide and optionally a regulator of the promoter.

Vectors may contain one or more selectable marker genes which are well known in the art.

There are many known heavy and light chain expression vectors commercially available. The skilled operator may choose vectors expressing the same constant region

subtype as the original antibody. The sequence of the heavy and light chain variable regions is then easily placed into the vector accordingly.

Suitably InvivoGen (of 5, rue Jean Rodier, F-31400 Toulouse, France) vectors may be used for heterologous expression of antibodies or antigen binding fragments of the invention. For example, B2.1A may be expressed using pFUSE2ss-CLIg-mk for the K light chain and pFUSEss-CHIg-mG1 for the IgG1 heavy chain variable region. Similarly, there is a wide range of known vectors commercially available for scFV expression. To make the B2.1A scFv's, suitably vector(s) such as pDisplay or derivatives thereof may be used.

Host Cells

The invention further provides a host cell – such as a host cell *in vitro* - comprising the polynucleotide or construct described herein. The host cell may be a bacterium, a yeast or other fungal cell, insect cell, a plant cell, or a mammalian cell, for example.

The invention also provides a transgenic multicellular host organism which has been genetically manipulated so as to produce a polypeptide in accordance with the invention. The organism may be, for example, a transgenic mammalian organism (*e.g.*, a transgenic goat or mouse line).

E. coli is one prokaryotic host that may be of use. Other microbial hosts include bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella*, *Serratia*, and various *Pseudomonas* species. In these prokaryotic hosts, one can make expression vectors, which will typically contain expression control sequences compatible with the host cell (*e.g.*, an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation.

Other microbes, such as yeast, may be used for expression. *Saccharomyces* is a preferred yeast host, with suitable vectors having expression control sequences (*e.g.*, promoters), an origin of replication, termination sequences and the like as desired. Typical promoters include 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase, isocytochrome C, and enzymes responsible for maltose and galactose utilization.

In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the antibodies or fragments thereof as described herein and in some instances are preferred (*See* Winnacker, *From Genes to Clones*, VCH Publishers,

N.Y., N.Y. (1987). For some embodiments, eukaryotic cells (*e.g.*, COS7 cells) may be preferred, because a number of suitable host cell lines capable of secreting heterologous proteins (*e.g.*, intact immunoglobulins) have been developed in the art, and include CHO cell lines, various Cos cell lines, HeLa cells, preferably, myeloma cell lines, or transformed B-cells or hybridomas.

In some embodiments, the host cell is a vertebrate host cell. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR(CHO, Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)) or CHO-DP-12 line; mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Alternatively, antibody-coding sequences can be incorporated into transgenes for introduction into the genome of a transgenic animal and subsequent expression in the milk of the transgenic animal (*see, e.g.*, Deboer et al., U.S. Pat. No. 5,741,957, Rosen, U.S. Pat. No. 5,304,489, and Meade et al., U.S. Pat. No. 5,849,992). Suitable transgenes include coding sequences for light and/or heavy chains in operable linkage with a promoter and enhancer from a mammary gland specific gene, such as casein or beta lactoglobulin.

Alternatively, the antibodies described herein can be produced in transgenic plants (*e.g.*, tobacco, maize, soybean and alfalfa). Improved `plantibody` vectors (Hendy et al. (1999) *J. Immunol. Methods* 231:137-146) and purification strategies coupled with an increase in transformable crop species render such methods a practical and efficient means of producing recombinant immunoglobulins not only for human and animal therapy, but for industrial applications as well (*e.g.*, catalytic antibodies). Moreover, plant produced antibodies have been shown to be safe and effective and avoid the use of animal-derived materials. Further, the differences in glycosylation patterns of plant and mammalian cell-produced antibodies have little or no effect on antigen binding or specificity. In addition, no evidence of toxicity or HAMA has been observed in patients receiving topical oral application of a plant-derived secretory dimeric IgA antibody (*see* Larrick *et al.* (1998) *Res. Immunol.* 149:603-608).

Full length antibody, antibody fragments, and antibody fusion proteins can be produced in bacteria, in particular when glycosylation and Fc effector function are not needed, such as when the therapeutic antibody is conjugated to a cytotoxic agent (*e.g.*, a toxin) and the immunoconjugate by itself shows effectiveness in tumor cell destruction. Full length antibodies have greater half life in circulation. Production in E. coli is faster and more cost efficient. For expression of antibody fragments and polypeptides in bacteria, *see, e.g.*, U.S. Pat. No. 5,648,237 (Carter et. al.), U.S. Pat. No. 5,789,199 (Joly et al.), and U.S. Pat. No. 5,840,523 (Simmons et al.) which describes translation initiation region (TIR) and signal sequences for optimizing expression and secretion, these patents incorporated herein by reference. After expression, the antibody is isolated from the E. coli cell paste in a soluble fraction and can be purified through, e.g., a protein A or G column depending on the isotype. Final purification can be carried out similar to the process for purifying antibody expressed *e.g.*, in CHO cells. Suitable host cells for the expression of glycosylated antibodies such as a glycosylated B2.1A antibody are derived from multicellular organisms. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified. A variety of viral strains for transfection are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Purification of Antibody

When using recombinant techniques, the antibody can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, are removed, for example, by centrifugation or ultrafiltration. Carter et al., *Bio/Technology* 10: 163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of *E. coli*. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human γ1, γ2, or γ4 heavy chains (Lindmark et al., *J. Immunol. Meth.* 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human γ3 (Guss et al., *EMBO J.* 5:15671575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a C.sub.H3 domain, the Bakerbond ABX™ resin (J. T. Baker, Phillipsburg, N.J.) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (*e.g.*, from about 0-0.25M salt).

Antibody Fragments

F(ab')2 (110,000 daltons) fragments contain two antigen-binding regions joined at the hinge through disulfides. This fragment is void of most, but not all, of the Fc region.

Fab' (55,000 daltons) fragments can be formed by the reduction of F(ab')2 fragments. The Fab' fragment contains a free sulfhydryl group that may be alkylated or utilised in conjugation with an enzyme, toxin or other protein of interest. Fab' is derived from F(ab')2; therefore, it may contain a small portion of Fc.

Fab (50,000 daltons) is a monovalent fragment that can be produced from IgG and/or IgM, consisting of the VH, CH1 and VL, CL regions, linked by an intramolecular disulfide bond.

Fv (25,000 daltons) is the smallest fragment produced from IgG and/or IgM that contains a complete antigen-binding site. Fv fragments have the same binding properties and similar three-dimensional binding characteristics as Fab. The VH and VL chains of the Fv fragments are held together by non-covalent interactions. These chains tend to dissociate upon dilution, so methods have been developed to cross-link the chains through glutaraldehyde, intermolecular disulfides or a peptide linker. scFv are single chain Fvs and can be conveniently made recombinantly.

"rIgG" Fragments or "rIgG" refers to reduced IgG (75,000 daltons) or half-IgG. It is the product of selectively reducing just the hinge-region disulfide bonds. Although several disulfide bonds occur in IgG, those in the hinge-region are most accessible and easiest to reduce, especially with mild reducing agents like 2-mercaptoethylamine (2-MEA). Half-IgG are frequently prepared for the purpose of targeting the exposing hinge-region sulfhydryl groups that can be targeted for conjugation, either antibody immobilization or enzyme labeling.

Techniques for producing these different fragments are well known in the art. Examples of production and reagents needed are provided below such as in the examples section in case any further guidance is needed.

Immunisation and Challenge Studies

The immunocompetent Cre-lox mouse model developed by Marcus Dorner is the most appropriate model for testing HCV vaccines (Dorner et al 2011; Dorner et al 2013). Commercially available transgenic mice, strain FVB.129S6(B6)-Gt(ROSA)26Sor$^{tm1(Luc)Kael}$/J, contain a LoxP-flanked STOP cassette restricting firefly luciferase expression. Expression of cyclization recombination (CRE) recombinase catalyses recombination between the two loxP sites, which removes the STOP cassette and activates the luciferase reporter gene, leading to intracellular luciferase expression. The mice are made permissive for HCV entry by infection with adenoviruses encoding essential cell surface receptors (human CD81, occludin, claudin 1 and SR-BI), and then infected with recombinant bicistronic HCVcc expressing cyclization recombination (CRE) recombinase. Upon entry into mouse hepatocytes, the recombinant viral genome is translated and the CRE protein is expressed. The CRE recombinase excises the STOP cassette and activates the luciferase reporter, leading to expression of luciferase. Subsequent injection of luciferin results in bioluminescence that can be

measured using a using a whole body bioluminescence imager. The HCV-CRE virus does not undergo a full infectious cycle because mouse cells do not support HCV replication and assembly. Therefore infection does not proceed beyond the entry step. Given that they have a normal immune system, the Rosa26-Fluc mice can be immunised with a potential vaccine, for example B2.1A Fab-KLH. The mice are given a primary vaccination followed by several booster vaccinations. A test bleed is taken after each booster, to monitor the induction of anti-E2 antibodies. If the vaccination induces anti-E2 antibodies, vaccinated and unvaccinated mice are made permissive for HCV infection by administering adenovirus vectors as described above, and then challenged with HCV-CRE. Entry of HCV into hepatocytes is detected as bioluminescence, as described herein. An inverse correlation between HCV entry and anti-E2 titre indicates that the vaccine protects against HCV challenge. Further details are provided in the Examples section.

Properties of B2.1A

B2.1A is an anti-idiotype antibody.

B2.1A binds AP33 monoclonal antibody.

B2.1A inhibits the binding of AP33 monoclonal antibody to the E2 412 to 423 epitope.

B2.1A does not bind (or has low binding i.e. binding less than 50% compared to binding to wild-type AP33) to mutant AP33 antibodies such as F_L32A, N_L91A, W_L96A; Y_H33A, Y_H50A, Y_H58A, I_H95A and/or Y_H100A.

Advantages

It is an advantage of the invention the B2.1A antibodies and derivatives are provided in spite of various failures during the intensive research carried out.

It is an advantage of the invention that a complex structure based selection procedure was devised in order to obtain the antibodies of the invention.

It is an advantage of the invention that a structurally novel antibody/antibody derivatives is/are provided in the form of B2.1A and the CDRs of B2.1A.

It is an advantage of the invention that the antibody and antibody derivatives described herein have not been able to be obtained by conventional routes.

It is an advantage of the antibody and antibody derivatives described herein that they exhibit a pattern of binding to AP33 mutants which closely emulates the binding pattern of E2 to those AP33 mutants.

It is an advantage that the invention provides an anti-idiotypic antibody useful for inducing an immune response capable of recognising E2 polypeptide.

It is an advantage of the invention that it enables the induction of an immune response targeting HCV E2 polypeptide.

It is an advantage of the invention that it enables the induction of an immune response against HCV E2 polypeptide.

It is an advantage of the invention that the paratope presented by B2.1A and its derivatives is an excellent mimic of the 3-dimensional structure of a key broadly neutralising epitope of the HCV E2 polypeptide.

It is an advantage of the invention that the paratope presented by B2.1A and its derivatives induce antibodies that recognise the same epitope as AP33. It is a further advantage that the induced antibodies recognise the same residues within that epitope it as AP33 recognises.

It is an advantage of the invention that the affinity of the Ab3 antibodies (i.e. the antibodies induced by the B2.1A antibodies or derivatives of the invention) for E2 is similar to that of AP33. It is a further advantage that the induced antibodies neutralize infectivity of cell-culture infectious HCV. They neutralise infectivity with an IC50 that is about twice that of AP33.

It is an advantage of the invention that fewer animals were required in order to obtain the B2.1A antibody.

It is a feature of the approach that the structural data available for the AP33 – E2 interaction were extensively used in the rational design of the selection scheme.

The invention is now further described by way of numbered paragraphs:

paragraph 1. An antibody or antigen binding fragment thereof capable of binding to the antigen binding pocket of the AP33 antibody, wherein said antibody or antigen binding fragment thereof comprises VL CDR1 (L1), VL CDR2 (L2), and VL CDR3 (L3) consisting of the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3 respectively, and comprises VH CDR1 (H1), VH CDR2 (H2), and VH CDR3 (H3) consisting of the amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6 respectively.

paragraph 2. An antibody according to paragraph 1 wherein said antibody or antigen binding fragment thereof comprises VL amino acid sequence consisting of the amino acid sequence of SEQ ID NO:7.

paragraph 3. An antibody according to paragraph 1 wherein said antibody or antigen binding fragment thereof comprises VH amino acid sequence consisting of the amino acid sequence of SEQ ID NO:8.

paragraph 4. An antibody according to paragraph 1 wherein said antibody or antigen binding fragment thereof comprises VL amino acid sequence consisting of the amino acid sequence of SEQ ID NO:7 and wherein said antibody or antigen binding fragment thereof comprises VH amino acid sequence consisting of the amino acid sequence of SEQ ID NO:8.

paragraph 5. An antibody or antigen binding fragment thereof according to any preceding paragraph, wherein the antigen binding fragment thereof is selected from the group consisting of a Fab fragment, a Fab' fragment, a F(ab')2 fragment, a scFv, a Fv, a rIgG, and a diabody.

paragraph 6. An antibody or antigen binding fragment thereof according to paragraph 5 wherein said antigen binding fragment is a scFv and wherein said scFv comprises the amino acid sequence of SEQ ID NO:11 or SEQ ID NO:12 or SEQ ID NO:13.

paragraph 7. A nucleic acid comprising a nucleotide sequence encoding the variable heavy chain domain and/or the variable light chain domain of the antibody or antigen binding fragment according to any preceding paragraph.

paragraph 8. The nucleic acid of paragraph 7, wherein the nucleic acid comprises one or more nucleotide sequences selected from the group consisting of SEQ ID NO:9 and SEQ ID NO:10.

paragraph 9. A nucleic acid comprising a nucleotide sequence complementary to the nucleotide sequence of paragraph 7 or paragraph 8.

paragraph 10. A vector comprising the nucleic acid of paragraph 7 or paragraph 8.

paragraph 11. The vector of paragraph 10, wherein the vector further comprises an expression control sequence operatively linked to the nucleic acid encoding the variable heavy chain domain and/or the variable light chain domain.

paragraph 12. A host cell containing the vector of paragraph 10 or paragraph 11.

paragraph 13. The host cell of paragraph 12, wherein the cell is a eukaryotic cell.

paragraph 14. The host cell of paragraph 13, wherein the eukaryotic cell is a Chinese Hamster Ovary (CHO) cell or a human embryonic kidney (HEK) cell.

paragraph 15. A method of producing an antibody or antigen binding fragment thereof, comprising incubating a host cell according to any of paragraphs 12 to 14 such that the encoded variable heavy chain domain and/or variable light chain domain is expressed by the cell; and recovering the expressed the antibody or antigen binding fragment thereof.

paragraph 16. The method of paragraph 15, which further comprises isolating and/or purifying the recovered antibody or antigen binding fragment thereof.

paragraph 17. A composition comprising the antibody or antigen binding fragment thereof according to any of paragraphs 1 to 6 and a pharmaceutically acceptable carrier or excipient.

paragraph 18. A composition according to paragraph 17 further comprising a carrier protein, the carrier protein preferably selected from the group consisting of tetanus toxoid and CRM 197 mutant diphtheria toxin.

paragraph 19. A composition according to paragraph 17 or paragraph 18 further comprising an adjuvant.

paragraph 20. A composition according to any of paragraphs 17 to 19 formulated for use in humans.

paragraph 21. An antibody or antigen binding fragment thereof capable of inducing in a mammal an immune response against the hepatitis C virus E2 protein, wherein said antibody or antigen binding fragment thereof is capable of binding to the antigen binding pocket of the monoclonal AP33 antibody.

paragraph 22. An antibody or antigen binding fragment thereof capable of inducing in a mammal an immune response against the hepatitis C virus E2 protein, wherein said antibody or antigen binding fragment thereof comprises VL CDR1 (L1), VL CDR2 (L2), and VL CDR3 (L3) consisting of the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3 respectively, and comprises VH CDR1 (H1), VH CDR2 (H2), and VH CDR3 (H3) consisting of the amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6 respectively.

paragraph 23. An antibody or antigen binding fragment thereof capable of binding to the AP33 antibody wherein said antibody or antigen binding fragment thereof exhibits binding to AP33 antibody mutants FL32A, NL91A, WL96A, YH33A, YH50A, YH58A, IH95A and YH100A of less than 50% of its binding to the AP33 antibody.

paragraph 24. An antibody that binds to an antibody or antigen binding fragment thereof according to any of paragraphs 1 to 6, which is not AP33 antibody or a fragment thereof.

paragraph 25. An antibody according to paragraph 24 which is obtained by immunisation of a mammal with an antibody or antigen binding fragment thereof according to any of paragraphs 1 to 6.

paragraph 26. A method of inducing in a mammal an immune response against the hepatitis C virus E2 protein, the method comprising administering to said mammal an antibody according to any of paragraphs 1 to 6 or 21 to 25, a nucleic acid according to any of paragraphs 7 to 9, a vector according to paragraph 10 or paragraph 11, or a composition according to any of paragraphs 17 to 20.

paragraph 27. An antibody according to any of paragraphs 1 to 6 or 21 to 25, a nucleic acid according to any of paragraphs 7 to 9, a vector according to paragraph 10 or paragraph 11, or a composition according to any of paragraphs 17 to 20 for inducing in a mammal an immune response against the hepatitis C virus E2 protein.

Further Aspects and Applications

In a broad aspect, the invention relates to an antibody or antigen binding fragment thereof capable of binding to the monoclonal AP33 antibody.

In a broad aspect, the invention relates to an antibody or antigen binding fragment thereof as described above which comprises at least one of the V_L CDR1 (L1), V_L CDR2 (L2), V_L CDR3 (L3), V_H CDR1 (H1), V_H CDR2 (H2), and V_H CDR3 (H3) consisting of the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26 respectively. Suitably said antibody or antigen binding fragment thereof comprises at least two of said sequences, suitably said antibody or antigen binding fragment thereof comprises at least three of said sequences, suitably said antibody or antigen binding fragment thereof comprises at least four of said sequences, suitably said antibody or antigen binding fragment thereof comprises at least five of said sequences, suitably said antibody or antigen binding fragment thereof comprises all six of said sequences. When said antibody or antigen binding fragment thereof comprises at least three of said sequences, suitably it comprises each of the three V_L sequences or the each of the three V_H sequences.

In one aspect, the invention relates to a method of identifying a polypeptide having, or comprising a region or domain having, essentially the same three dimensional structure as the AP33 epitope residues 412 to 423 HCV E2, the method comprising

(i) assaying the binding of said polypeptide to the AP33 antibody, and

(ii) assaying the binding of said polypeptide to AP33 antibody mutants F_L32A, N_L91A, W_L96A, Y_H33A, Y_H50A, Y_H58A, I_H95A and Y_H100A,

wherein if the polypeptide exhibits binding to AP33 antibody mutants F_L32A, N_L91A, W_L96A, Y_H33A, Y_H50A, Y_H58A, I_H95A and Y_H100A of less than 50% of its binding to the AP33 antibody, said polypeptide is identified as having essentially the same three dimensional structure as the AP33 epitope residues 412 to 423 HCV E2.

Suitably the polypeptide is an antibody or antigen binding fragment thereof.

Suitably the polypeptide is an antibody or antigen binding fragment thereof generated by immunisation of a mammal with AP33 antibody.

Suitably binding is assayed by ELISA.

Suitably binding to AP33 antibody mutants is less than 60% of binding to the AP33 antibody.

In one aspect, the invention relates to a method of producing an antibody or antigen binding fragment thereof having, or comprising a region or domain having, essentially the same three dimensional structure as the AP33 epitope residues 412 to 423 HCV E2, said method comprising identifying a polypeptide having, or comprising a region or domain having, essentially the same three dimensional structure as the AP33 epitope residues 412 to 423 HCV E2 as described above, and expressing said antibody or antigen binding fragment thereof *in vitro*, and optionally purifying same.

In one aspect, the invention relates to a kit comprising the antibody or antigen binding fragment thereof as described above and instructions for administering said antibody or antigen binding fragment thereof.

In one aspect, the invention relates to a method for treating or preventing a hepatitis C virus infection in a human, comprising administering an effective amount of the antibody or antigen binding fragment thereof as described above. Suitably the antigen binding fragment thereof is selected from the group consisting of a Fab fragment, a Fab' fragment, a F(ab')2 fragment, a scFv, a Fv, a rIgG and a diabody.

Suitably the hepatitis C virus infection is an acute hepatitis C virus infection.

Suitably the hepatitis C virus infection is a chronic hepatitis C virus infection.

Suitably treating the hepatitis C virus infection comprises reducing viral load.

Suitably treating or preventing the hepatitis C virus infection comprises inducing an immune response against the hepatitis C virus, suitably against the E2 protein of the hepatitis C virus, most suitably against the AP33 epitope 412 to 423 of the E2 protein of the hepatitis C virus.

In some embodiments, suitably the method for treating or preventing a hepatitis C virus infection comprises administering a second therapeutic agent.

Further particular and preferred aspects are set out in the accompanying independent and dependent claims. Features of the dependent claims may be combined with features of the independent claims as appropriate, and in combinations other than those explicitly set out in the claims.

Where an apparatus feature is described as being operable to provide a function, it will be appreciated that this includes an apparatus feature which provides that function or which is adapted or configured to provide that function.

Brief Description Of The Drawings

Embodiments of the present invention will now be described further, with reference to the accompanying drawings, in which:

Figure 1 shows a graph.

Figure 2 shows a graph.

Figure 3 shows a bar chart.

Figure 4 shows a diagram.

Figure 5 shows HCV E2 sequence.

Figure 6 shows examples of antibodies and antigen binding fragments of the invention.

Figure 7 shows The molecular surface of the AP33 binding pocket.

Figure 8 shows bar charts.

Figure 9 shows graphs.

Figure 10 shows bar charts.

Figure 11 shows graphs.

Figure 12 shows a graph.

Figure 13 shows a ribbon diagram.

Figure 14 shows that B2.1A docks into the AP33 antigen-binding site. Ribbon and surface representation of AP33 Fab (Ab1; heavy chain: orange, light chain: yellow) in complex with (a) B2.1A scFv (Ab2; heavy chain: purple, light chain: pink), and (b) a peptide corresponding to the HCV E2 epitope (Ag; teal; pdb accession code 4gag).

Figure 15 shows Antigen mimicry by B2.1A. Structural alignment of the Ab_1-Ab_2 complex (AP33 heavy chain: orange, light chain: yellow; B2.1A heavy chain: purple, light chain: pink) with the Ab_1-Ag complex (AP33 heavy chain: blue, light chain: teal; peptide: magenta; pdb accession code 4gag). Hydrogen bonds in the Ab_1-Ab_2 complex are shown as black dashed lines and those in the Ab_1-Ag complex as grey dashed lines. Water molecules are shown as red spheres. The panels show the mimicry by B2.1A of E2 residues W420 (a); G418 (b); N415 (c) and L413 (d) and their interactions with AP33.

Figure 16 shows site-directed mutagenesis of B2.1A. Purified MBP-B2.1A scFv proteins carrying the indicated mutations were captured on immobilised AP33 and detected by anti-MBP-HRP conjugate in an ELISA assay. (a) WT protein and mutants F98W and N100G, which retained binding; (b) WT protein and mutants that did not bind. Sigmoidal curves were fitted to the absorbance data.

Figure 17 shows anti-E2 titre of Rosa26-Fluc mice. Three mice (A, B, C) were given a primary vaccination with B2.1A Fab coupled to KLH, followed by five booster

vaccinations. Test bleeds were taken 7–10 days after each booster, with an additional bleed 15 days after the last booster The sera were tested for E2 reactivity by ELISA. The values shown are the mean of two independent titrations

Figure 18 shows Ab3 antibodies in vaccinated Rosa26-Fluc mice are specific for the AP33 epitope. Pooled high-titre sera from Rosa26 Fluc mice B & C were pre-incubated with a range of peptide concentrations and then transferred to E2-coated microtitre plates. Two peptides were used, one corresponding to the WT AP33 epitope (aa residues 412 – 424 of E2) and the other containing a W420R substitution. Monoclonal antibodies AP33 and ALP98 served as positive and negative controls, respectively. Bound antibodies were detected with anti-mouse-HRP conjugate followed by TMB substrate.

Examples

Example 1: Creation of anti-idiotypic antibody

Antibodies to AP33 were generated using a standard immunisation protocol for antibody production with AP33 as the antigen. Anti-idiotypic (Ab2) antibodies were identified by their ability to inhibit AP33-E2 interaction in a competition ELISA. Nine fusions yielded 122 hybridomas secreting the Ab2 antibodies shown in Table A below:

Table A: A summary of the Ab2 data obtained from Dec 2008 to October 2011

	Vaccination[a]		Binding to AP33[b]			Sequenced[c]
Ab2	Tested	Outcome	IgG	LC	HC hybrid	V_L & V_H
A1	Yes	Negative	Positive	Negative	Negative	
A1.5	Yes	Negative	Positive	Negative	Negative	Yes
A5	Yes	Negative	Positive	Negative	Negative	
A164	Yes	Negative	Positive	Negative	Negative	
A164.3	Yes	Negative	Positive	Negative	Negative	
A8A	No		Positive	Negative	Negative	Yes
A8B	No		Positive	Negative	Negative	Yes
A8C	No		Positive	Negative	Negative	Yes
A8D	No		Positive	Negative	Negative	Yes
A8E	No		Positive	Negative	Negative	Yes
A8F	No		Positive	Negative	Negative	Yes
A8G	No		Positive	Negative	Negative	Yes
A8H	No		Positive	Negative	Negative	Yes
A8I	No		Positive	Negative	Negative	Yes
A8L	No		Positive	Negative	Negative	Yes
A8M	No		Positive	Negative	Negative	Yes
A11A	No		Positive	Negative	Negative	Yes
A11B	Yes	Negative	Positive	Negative	Negative	Yes

A11C	No		Positive	Negative	Negative	
A12.1	No		Positive	Negative	Negative	Yes
A12.3	No		Positive	Negative	Negative	
A12.5	No		Positive	Negative	Negative	Yes
A14.2	No		Positive	Negative	Negative	
A14.4	No		Positive	Negative	Negative	
A14.5	No		Positive	Negative	Negative	
A16A	Yes	Negative	Positive	Negative	Negative	Yes
A17.5	No		Positive	Negative	Negative	
A22A	No		Positive	Negative	Negative	Yes
A22B	No		Positive	Negative	Negative	
A22C	No		Positive	Negative	Negative	
A22D	No		Positive	Negative	Negative	Yes
A22E	No		Positive	Negative	Negative	
A22F	No		Positive	Negative	Negative	
A22G	No		Positive	Negative	Negative	
A22H	No		Positive	Negative	Negative	
A25D	Yes	Negative	Positive	Negative	Negative	Yes
A25H	No		Positive	Negative	Negative	Yes
A31A	No		Positive	Negative	Negative	Yes
A31B	No		Positive	Negative	Negative	Yes
A31C	No		Positive	Negative	Negative	Yes
A31D	Yes	Negative	Positive	Negative	Negative	Yes
A31E	No		Positive	Negative	Negative	
A31F	No		Positive	Negative	Negative	
A31G	No		Positive	Negative	Negative	
A34A	No		Positive	Negative	Negative	Yes
A34B	No		Positive	Negative	Negative	
A34C	No		Positive	Negative	Negative	Yes
A46A	No		Positive	Negative	Negative	
A46B	Yes	Negative	Positive	Negative	Negative	Yes
A46C	No		Positive	Negative	Negative	Yes
A46D	No		Positive	Negative	Negative	
A49A	No		Positive	Negative	Negative	
A49B	No		Positive	Negative	Negative	
A49C	No		Positive	Negative	Negative	
A49D	No		Positive	Negative	Negative	
A49E	No		Positive	Negative	Negative	
A49F	No		Positive	Negative	Negative	Yes
A52B	No		Positive	Negative	Negative	
A52C	No		Positive	Negative	Negative	Yes
A52D	No		Positive	Negative	Negative	
A52E	No		Positive	Negative	Negative	
A52F	No		Positive	Negative	Negative	
A52G	No		Positive	Negative	Negative	
A52H	No		Positive	Negative	Negative	Yes
A52I	No		Positive	Negative	Negative	
A52O	No		Positive	Negative	Negative	
A52P	No		Positive	Negative	Negative	
A53B	No		Positive	Negative	Negative	
A53C	No		Positive	Negative	Negative	

A53D	Yes	Negative	Positive	Negative	Negative	
A53E	No		Positive	Negative	Negative	
A53I	No		Positive	Negative	Negative	
A53J	No		Positive	Negative	Negative	
A53K	No		Positive	Negative	Negative	
A53M	No		Positive	Negative	Negative	Yes
A53N	No		Positive	Negative	Negative	
A53O	No		Positive	Negative	Negative	Yes
A53P	No		Positive	Negative	Negative	
A57B	No		Positive	Negative	Negative	
A57C	No		Positive	Negative	Negative	
A57D	No		Positive	Negative	Negative	Yes
A57F	No		Positive	Negative	Negative	
A57G	No		Positive	Negative	Negative	Yes
A57H	No		Positive	Negative	Negative	
A57J	No		Positive	Negative	Negative	
A570	No		Positive	Negative	Negative	
A71.2	No		Positive	Negative	Negative	
A71.5	No		Positive	Negative	Negative	
A71.9	No		Positive	Negative	Negative	
B2.1A	No		Positive	Negative	Negative	Yes
B2.1B	No		Positive	Negative	Negative	
B4.1A	No		Positive	Negative	Negative	
B4.1D	Yes	Negative	Positive	Negative	Negative	Yes
B4.1E	Yes	Negative	Positive	Negative	Negative	Yes
B4.1F	Yes	Negative	Positive	Negative	Negative	Yes
B4.1G	No		Positive	Negative	Negative	Yes
K201	Yes	Negative	Positive	Negative	Negative	Yes
K271	No		Positive	Negative	Negative	
K391	Yes	Negative	Positive	Negative	Negative	Yes
2K19	Yes	Negative	Positive	Negative	Negative	Yes
2K49	Yes	Negative	Positive	Negative	Negative	
2K55	Yes	Negative	Positive	Negative	Negative	Yes
2K56	Yes	Negative	Positive	Negative	Negative	Yes
2K160	Yes	Negative	Positive	Negative	Negative	Yes
L1.1A	No		Positive	Negative	Negative	Yes
L1.1D	Yes	Negative	Positive	Negative	Negative	
L1.2A	Yes	Negative	Positive	Negative	Negative	Yes
L1.2B	No		Positive	Negative	Negative	
L1.2C	No		Positive	Negative	Negative	Yes
L1.2D	No		Positive	Negative	Negative	Yes
L1.2E	No		Positive	Negative	Negative	Yes
L1.2F	No		Positive	Negative	Negative	Yes
L1.2H	No		Positive	Negative	Negative	
L1.2I	No		Positive	Negative	Negative	Yes
L1.2K	No		Positive	Negative	Negative	Yes
L1.2L	No		Positive	Negative	Negative	
L1.2M	No		Positive	Negative	Negative	
L1.2N	No		Positive	Negative	Negative	
L1.2O	No		Positive	Negative	Negative	
L1.2P	No		Positive	Negative	Negative	Yes

P1.52	Yes	Negative	Positive	Negative	Negative	Yes
P1.T	Yes	Negative	Positive	Negative	Negative	Yes

[a] Balb/c mice were vaccinated with purified antibody coupled to KLH and the immune sera were tested for reactivity with E2. A negative result denotes lack of reactivity. An example of negative ELISA data is shown separately
[b] Binding of the Ab2s to (a) AP33 whole IgG, (b) AP33 light-chain alone and (c) a hybrid comprising AP33 heavy-chain and an irrelevant κ-light-chain. None of the Ab2s bound AP33 LC or HC hybrid.
[c] Sequencing of Ab2 variable regions.

Over the course of 18 months, twenty-five Ab2s were picked at random and used to vaccinate mice (Table A), in order to identify one or more internal-image antibodies (Ab2β) that would be capable of eliciting an immune response to HCV E2. The immune sera were tested by ELISA for:

1. Blocking of AP33-Ab2 interaction.
2. Binding to E2.
3. Inhibition of HCV infection in cell culture

RESULTS: The immune sera strongly inhibited binding of AP33 to Ab2, indicating that they contained anti-Ab2 antibodies. However, the anti-Ab2 antibodies did not bind to E2, nor did they inhibit HCV in cell culture. This was a significant problem. See Figs 2 and 3 for an example of these negative results.

Fig. 1 shows inhibition of AP33 binding to A164 by immune sera
Six Balb/c mice were vaccinated with A164 conjugated to KLH. Primary vaccination was followed by 4 boosters at 14-day intervals, and a final bleed taken 5 days after the last booster.
Serial dilutions of pre-immune and immune sera were co-incubated with biotinylated AP33 (b-AP33) on A164-coated microtitre plates. Binding of b-AP33 was detected with streptavidin-HRP and TMB. A decreased signal indicates blocking of b-AP33-A164 interaction by competing serum antibodies. The graph represents the response of two mice (No 1 & 2) within the group. All other animals showed the same response.
RESULT: The immune sera contain A164-specific antibodies that block AP33 binding to E2, whereas the pre-immune bleeds have no effect on the interaction.

Fig. 2 shows binding of serum antibodies to E2 - *example of negative result*
Six Balb/c mice were vaccinated with A164 conjugated to KLH. Primary vaccination was followed by 4 boosters at 14-day intervals, and a final bleed taken 5 days after the last booster.
Serial dilutions of immune sera were incubated on E2-coated microtitre plates. Binding of serum antibodies was detected with anti-mouse-HRP and TMB. An increased signal indicates the presence of E2-specific antibodies. AP33 served as a positive control. . The graph represents the response of two mice (No 1 & 2) within the group. All other animals showed the same response.
RESULT: The immune sera from mice immunized with A164 do not contain antibodies that recognize E2.

Fig. 3 shows virus neutralization by immune sera - *example of negative result*

Wild type JFH1 virus (WT) and two E2 mutant viruses, G451R and W420Y were incubated with sera (1/100 dilution) obtained from mice immunized with the Ab2 P1T (TB = terminal bleed). Serum was taken from the same mice prior to immunization and served as controls (PB = pre-bleed). After 1 hour at 37°C, the virus/serum mixture was used to infect Huh7-J20 cells. The Huh7-J20 cell-line is engineered to release secreted alkaline phosphatase (SEAP) reporter into the medium following HCV infection, thus enabling a rapid and sensitive quantification of virus infectivity. At 3 hours post-infection, the inoculum was replaced with fresh medium and incubated for 72 hours. The virus infectivity levels were determined by measurement of SEAP released into the medium. The percent infectivity was calculated by quantifying viral infectivity in the presence of mouse immune serum (TB) relative to its respective control non-immune serum (PB). Error bars indicate standard deviation from the mean. A33 is included as a control. The G451R virus is more sensitive than WT to neutralization by AP33. The W420Y virus is resistant to neutralization by AP33.
RESULT: Infectivity of WT and G451R virus is significantly reduced by pre-incubation with AP33, but not by any of the mouse sera, indicating that the immune sera from mice immunized with P1T do not contain neutralizing antibodies.

Obtaining B2.1A Antibody

These results presented a significant challenge: how to identify the Ab2βs?

- By immunisation to produce Ab3
- By testing for binding to AP33 light chain and heavy chain

This is illustrated in Figure 4.
Result: all 120 Ab2s behave like Ab2β

We realised that there were problems in screening 122 antibodies by vaccination, for example time constraints and/or the number of animals that would be required, so we did the following:

1. We compared the binding of the Ab2s to (a) AP33 whole IgG, (b) AP33 light-chain alone and (c) a hybrid comprising AP33 heavy-chain and an irrelevant κ-light-chain. This approach is illustrated in Fig.4., and is based on the expectation that an Ab2β would bind to the entire antigen-binding pocket present in (a) but it would not bind to (b) or (c), whereas an Ab2 that did not represent an internal-image of the antigen-binding pocket would bind to either (b) or (c) In fact, all the Ab2s behaved as Ab2βs and bound only to (a), so this assay failed to differentiate between them.

2. We sequenced the variable regions of all the Ab2s, to remove any duplicates. This reduced the panel to 18 unique antibodies.

Our crystal structure of AP33 Fab complexed with a peptide corresponding to its epitope allowed us to identify the amino acid residues that make up the antigen-binding pocket of AP33. Using a panel of mutant AP33 antibodies in which these residues were individually replaced by alanine, we established which amino acid residues are involved in E2 binding and which are not (Potter et al. 2012 and Table 1 below).

The same panel of mutant AP33 antibodies was used to differentiate between the Ab2s. This approach proved to be a real breakthrough, because it revealed striking differences between the Ab2s. Some were unaffected by the mutations, whereas

others shared binding characteristics with E2. The binding profile of B2.1A most closely resembled that of E2 (Table 1).

Table 1: Binding of Ab2s to wild-type and mutant AP33, data obtained Nov 2011 - Jan 2012

	Relative strength of binding (%)															Score[d] = binding reduced by	
	WT AP33	AP33 light chain mutants[a]							AP33 heavy chain mutants[a]								
		Y_L28A	N_L30A	F_L32A	N_L91A	N_L92A	V_L93A	D_L94A	W_L96A	Y_H33A	Y_H50A	Y_H53A	Y_H58A	I_H95A	T_H97A	Y_H100A	>50%	>80%
E2[b]	100	111.9	88.8	**-3.5**	**6.6**	59.8	79	74.4	**-0.4**	**2.5**	**-1**	100.4	**0.7**	**9.9**	100.6	**4.9**	8	8
Anti-Id[c]																		
B2.1A	100	82	73	**21**	**36**	36	49	41	**1**	**10**	**4**	68	**5**	**29**	89	**8**	8	5
L1.1A	100	5	88	**3**	85	83	88	86	**1**	**26**	**9**	85	**9**	**26**	84	**13**	7	5
P1.T	100	20	88	54	**18**	48	72	50	**4**	67	**15**	90	**13**	**15**	86	**11**	6	6
L1.2A	100	76	90	52	**31**	63	83	88	**1**	**30**	**24**	88	**9**	**41**	86	54	6	2
A16A	100	93	92	64	57	66	82	70	**7**	**15**	**35**	46	**40**	63	75	**10**	5	3
K391	100	112	74	**45**	66	82	77	82	**14**	91	**37**	82	**41**	**35**	98	54	5	1
A53M	100	80	85	55	**21**	86	77	89	**11**	94	88	104	59	54	98	**17**	3	2
2K55	100	87	69	**23**	81	83	84	88	**1**	50	**27**	77	61	60	92	89	3	1
2K49	100	21	93	**43**	94	105	94	97	70	**29**	90	88	91	84	93	**25**	3	0
B4.1F	100	79	95	**49**	75	78	100	92	**10**	76	79	94	62	74	97	57	2	1
K201	100	79	90	68	89	115	96	90	**3**	55	**34**	79	67	57	104	82	2	1
2K56	100	89	84	61	88	90	86	94	**1**	57	**39**	74	67	66	90	83	2	1
A5	100	90	98	53	98	105	98	100	**4**	81	54	89	71	74	98	88	1	1
2K19	100	96	88	75	90	94	88	97	**2**	72	54	79	73	75	96	88	1	1
A1.5	100	82	98	94	94	100	96	98	55	93	86	69	85	65	96	78	0	0
A164	100	60	100	66	83	106	98	106	70	73	68	65	93	72	95	59	0	0
2K160	100	78	100	82	86	82	90	104	96	90	91	80	94	91	97	75	0	0
P1.52	100	96	103	79	95	105	106	107	64	84	79	102	71	89	94	80	0	0

[a] The amino acid residues that comprise the antigen-binding pocket were identified from the crystal structure of AP33 Fab complexed with a peptide corresponding to its epitope. Mutant AP33 antibodies were made in which these residues were individually replaced by alanine. The mutants were named according to the identity and position of the wild type (WT) amino acid, eg Y_L28A has tyrosine at position 28 in the light chain changed to alanine.

[b] The reactivity of HCV E2 with each mutant was determined by ELISA and expressed as a percentage of reactivity with WT AP33.

[c] The reactivity of each anti-idiotype (anti-Id) with each mutant was determined by ELISA and expressed as a percentage of reactivity with WT AP33.

[d] The score is the number of mutants to which binding was reduced by >50% and >80% relative to WT AP33. Values contributing to the score are highlighted.

RESULT: AP33 binding to E2 was reduced by >90% by mutation of light chain residues F32, N91 and W96, and of heavy chain residues Y33, Y50, Y58, I95 and Y100 (values highlighted in bold, double underlined). The same eight mutations reduced AP33 binding to anti-Id B2.1A (top line, values highlighted in bold and boxed), whereas binding to other anti-Ids was affected by fewer, or none of the mutations, which shows that B2.1A most closely resembles E2. Binding to some anti-Ids was reduced by mutations that did not affect E2 binding (eg Y_L28A), therefore these reduced values are not highlighted or included in the score.

Example 2.1: Selection of an anti-idiotypic antibody that represents an internal image of the AP33 paratope

Figure 7 shows the molecular surface of the AP33 binding pocket. The positions of eight alanine substitutions that reduced binding by >90% are colored purple, while those that had little or no effect on E2 binding are colored cyan. The epitope peptide is shown as sticks with yellow carbon atoms.

Figure 7 also shows a schematic diagram to illustrate the principles of the anti-idiotype network theory. Exposure to antigen induces the production of antibodies, termed Ab1. The specificity of an Ab1 antibody is determined by the sequence and structure of its hypervariable regions, and this unique antigen-binding site is also recognised as a set of idiotypic epitopes, or idiotopes, by the immune system. Anti-idiotypic (anti-Id) antibodies generated against the Ab1 are termed Ab2, and a subset of these, termed Ab2β, fit into the antigen-binding site (paratope) of the Ab1 precisely enough to be an "internal image" of it, and, by the same token, an effective mimic of the original antigen. An Ab2β antibody can therefore be used as a surrogate antigen to elicit anti-anti-Id antibodies (Ab3), which have the same binding properties as the Ab1.

Balb/c mice were vaccinated with AP33 to generate a large number of hybridomas. These were screened for the production of Ab2 antibodies that were able to block the AP33-E2 interaction by binding to the hypervariable region of AP33.

To identify, from this panel of various anti-idiotypes, the desired Ab2β that represents an "internal image" of the AP33 paratope, we used a panel of AP33 antibody mutants, in which each residue within the antigen-binding pocket was individually mutated to alanine. Eight residues in the centre of the pocket were essential for E2 recognition, and the same eight residues were also required for binding of one of the Ab2s, designated B2.1A. This indicates that the molecular surface of B2.1A closely resembles that of the AP33 epitope on E2.

Example 2.2: Vaccination with B2.1A elicits Ab3 antibodies that recognise HCV E2

Balb/c mice were vaccinated with B2.1A conjugated to KLH. A different adjuvant was used for each group of four mice: (A) Complete Freunds/Incomplete Freunds (CFA/IFA); (B) Alum; (C) Alum & lipopolysaccharide (LPS); (D) Quil-A. The immune and pre-immune sera were tested by ELISA for

1. Blocking of AP33-B2.1A interaction: Sera at 1:300 dilution were co-incubated with biotinylated AP33 (b-AP33) on B2.1A-coated microtitre plates. Decreased binding of b-AP33 to B2.1A indicates blocking of the interaction by competing serum antibodies.
2. Binding to E2: Sera at 1:300 dilution were incubated on E2-coated microtitre plates. Binding of serum antibodies indicates the presence of E2-specific Ab3 antibodies.

RESULT

All the immune sera strongly inhibited binding of b-AP33 to B2.1A, indicating that they contain B2.1A-specific antibodies. However, not all of them contain E2-specific antibodies. Immune sera A2 and D3 show the strongest E2 reactivity, with an anti-E2 titre of over 300. As expected, the pre-immune sera are uniformly negative. These results show that B2.1A is able to elicit an E2-specific response.

See Figure 8.

Example 2.3: Vaccination with B2.1A elicits Ab3 antibodies that bind to the same epitope as AP33

A) Peptide inhibition

Immune sera A2 and D3 and anti-E2 monoclonal antibodies (MAbs) AP33 and ALP98 were pre-incubated with peptide, transferred to E2-coated microtitre plates and bound antibodies were detected with anti-mouse-HRP.

RESULT

The binding to E2 of Ab1 (AP33) and of Ab3 in the immune sera is specifically inhibited by a peptide that corresponds to the AP33 epitope. There is no inhibition by a peptide in which W420, an essential contact residue for AP33, has been replaced by R, nor by an unrelated control sequence. As expected, ALP98, which binds to a different linear epitope on E2, is not inhibited.

See Figure 9.

B) Alanine scanning across AP33 the epitope

ELISA was used to test the reactivity of Ab3 antibodies in immune sera A2 and D3 with a panel of E2 mutants, in which each residue across the AP33 epitope was individually replaced by alanine. MAbs AP33 and ALP98 served as positive and negative controls, respectively.

RESULT

The binding of AP33 to E2 was reduced by alanine substitution of L413, N415, G418 or W420. This agrees with our previous data[2] and with the crystal structure of the AP33-peptide complex, in which these four residues are buried at the molecular interface[1]. The binding profile of the Ab3 antibodies was very similar to that of AP33: Their binding to E2 was reduced or abrogated by the same four mutations, and also by alanine substitution of I414. As expected, the binding of ALP98 was not affected by any of the substitutions.

This is compelling evidence that vaccination with B2.1A elicits AP33-like antibodies.

See Figure 10

Example 2.4. The titre of E2-specific Ab3 antibodies in immune sera

Figure 11A shows Serial dilutions of purified total IgG from immune sera A2 and D3, from non-immune mouse serum (NIM) and from a mouse vaccinated with anti-Id A164 were tested for E2 binding by ELISA. MAbs AP33 and ALP98 served as positive controls.

Figure 11B shows E2-specific Ab3 antibodies from immune sera A2 and D3 were affinity-purified on immobilized E2. Serial dilutions of the purified Ab3 antibodies and of AP33 were tested for E2 binding by ELISA.

RESULT

The anti-E2 titre of total IgG from sera A2 and D3 was about 1000-fold lower than that of AP33, whereas the anti-E2 titre of the E2-specific affinity-purified IgG was only 2- to 3-fold lower than that of AP33. Taken together, these data indicate that the proportion of E2-specific antibody to total IgG in the immune sera is in the range of 1/500 – 1/2000.

Example 2.5. Vaccination with B2.1A elicits Ab3 antibodies that neutralize virus

HCVcc were pre-incubated for 1 h with serial dilutions of E2-specific IgG affinity-purified from the serum of a mouse vaccinated with B2.1A. The virus-IgG mix was used to infect Huh7-J20 reporter cells[3]. Virus growth was measured by the level of secreted alkaline phosphatase (SEAP) reporter present in the cell culture medium after 3 days. MAb AP33 and IgG purified from a mouse vaccinated with another anti-Id served as positive and negative controls, respectively.

RESULT

The Ab3 antibodies elicited by B2.1A neutralize virus infectivity very effectively, with an IC_{50} that is about twice that of AP33.

Summary

We have used a broadly neutralizing antibody, AP33, as a template to reverse engineer an immunogen that induces similar antibodies upon vaccination. This has been achieved by isolating an anti-idiotypic antibody that represents the internal image of the AP33 binding pocket and thus mimics the protective epitope. We demonstrate, for the first time in the HCV vaccine field, the success of such a focused, structure-based approach.

References to Example 2

1. Potter, J.A. *et. al* (2012). Towards a hepatitis C virus vaccine: the structural basis of hepatitis C virus neutralization by AP33, a broadly neutralizing antibody. *J. Virol.* 86, 12923-12932.
2. Tarr, A. W. *et. al* (2006). Characterization of the hepatitis C virus E2 epitope defined by the broadly neutralizing monoclonal antibody AP33. *Hepatology* 43, 592-601.
3. Iro, M. *et. al* (2009). A reporter cell line for rapid and sensitive evaluation of hepatitis C virus infectivity and replication. *Antivir. Res.* 83, 148-155.

Example 3: scFv's

scFv's were produced from B2.1A. scFv amino acid sequences for eukaryotic such as mammalian expression and for prokaryotic such as bacterial expression are shown below.

Mammalian expression construct.

A mammalian expression construct containing the B2.1A scFv sequence was generated. This sequence was expressed in CHO cells and purified. The purified product was shown to interact with AP33 in ELISA. The B2.1A scFv protein sequence is shown below.

METDTLLLWVLLLWVPGSTGDANSQVQLQESGTELVKPGASVKLSCKASGYTFTNYWMHWVKQRPGQG
LEWIGEINPSDGHTNYNEKFKSKATLTVDKSSSTAYMQLSSLTSEDSAVYYCARPWAFGNYGAWFAYWGQ
GTLVTVSAGGSGGSGGGGSGGGGSGGGASDIVMTQSPKFMSTSVGDRVSITCKASQNVRTAVAWYQQ
KPGQSPKALIYLASSRHTGVPDRFTGSGSGTDFTLTISNVQSEDLADYFCLQHWNYPYTFGGGTKLEIKSR
HHHHHH (SEQ ID NO: 11)

Text in underlined in dots = IgK leader sequence allowing secretion of the scFv into cell culture medium

Boxed Text = vH sequence; CDRs underlined; preferred CDRs shaded

Text in bold = vL sequence; CDRs underlined; preferred CDRs shaded

Text underlined in dashes = linker sequence

Text double underlined = 6-his tag for affinity purification of scFv

Bacterial expression construct.

The above B2.1A scFv mammalian expression construct was used as a template to provide the scFv-encoding sequence and this was sub-cloned in-frame to the maltose-binding protein (MBP) into the bacterial expression vector pMBP. The MBP-B2.1A scFv amino acid sequence is shown below. The scFv was expressed in bacteria and purified following cleavage of the MBP domain and tested in mouse immunization experiments. The bacterial scFv was effective in eliciting AP33-like antibodies, but less effective than the mammalian scFv.

The MBP-B2.1A scFv fusion protein sequence is shown below:

MKYYHHHHHHDYDHMKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGD
GPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKT
WEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIK
NKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAAS
PNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAFWY
AVRTAVINAASGRQTVDEALKDAQTNSSSNNNNNNNNNNLGENLYFQGA/MAQVQLQESGTELVKPGA
SVKLSCKASGYTFTNYWMHWVKQRPGQGLEWIGEINPSDGHTNYNEKFKSKATLTVDKSSSTAYMQLSSL

TSEDSAVYYCARPWAFGNYGAWFAYWGQGTLVTVSAGGSGGSGGGGSGGGGSGGGASDIVMTQSPKF MSTSVGDRVSITCKASQNVRTAVAWYQQKPGQSPKALIYLASSRHTGVPDRFTGSGSGTDFTLTISNVQ SEDLADYFCLQHWNYPYTFGGGTKLE (SEQ ID NO: 12)

Cleaved sequence:

MAQVQLQESGTELVKPGASVKLSCKASGYTFTNYWMHWVKQRPGQGLEWIGEINPSDGHTNYNEKFKS KATLTVDKSSSTAYMQLSSLTSEDSAVYYCARPWAFGNYGAWFAYWGQGTLVTVSAGGSGGSGGGGSG GGGSGGGASDIVMTQSPKFMSTSVGDRVSITCKASQNVRTAVAWYQQKPGQSPKALIYLASSRHTGVP DRFTGSGSGTDFTLTISNVQSEDLADYFCLQHWNYPYTFGGGTKLE (SEQ ID NO: 13)

Text in underlined in dots = MBP sequence

/ = proteolytic cleavage site to remove MBP from the MBP-scFv fusion protein

boxed text = vH sequence; CDRs underlined; preferred CDRs shaded

Text in bold = vL sequence; CDRs underlined; preferred CDRs shaded

Text underlined in dashes = linker sequence

Nucleic Acid Constructs

In the exemplary sequences presented below, the coding sequence may be separately taken and placed into the vector of choice if the skilled worker desires.

pDisMod2-B2.1A-scFv - Example Sequence

A modified pDisplay vector carrying the B2.1A scFv sequence (the coding sequence is highlighted)

scFv coding sequence key as follows:

Leader sequence-vH-linker-vL-**6his tag**-STOP

```
       1 GCGCGCGTTG ACATTGATTA TTGACTAGTT ATTAATAGTA ATCAATTACG GGGTCATTAG
      61 TTCATAGCCC ATATATGGAG TTCCGCGTTA CATAACTTAC GGTAAATGGC CCGCCTGGCT
     121 GACCGCCCAA CGACCCCCGC CCATTGACGT CAATAATGAC GTATGTTCCC ATAGTAACGC
     181 CAATAGGGAC TTTCCATTGA CGTCAATGGG TGGACTATTT ACGGTAAACT GCCCACTTGG
     241 CAGTACATCA AGTGTATCAT ATGCCAAGTA CGCCCCCTAT TGACGTCAAT GACGGTAAAT
     301 GGCCCGCCTG GCATTATGCC CAGTACATGA CCTTATGGGA CTTTCCTACT TGGCAGTACA
     361 TCTACGTATT AGTCATCGCT ATTACCATGG TGATGCGGTT TTGGCAGTAC ATCAATGGGC
     421 GTGGATAGCG GTTTGACTCA CGGGGATTTC CAAGTCTCCA CCCCATTGAC GTCAATGGGA
     481 GTTTGTTTTG GCACCAAAAT CAACGGGACT TTCCAAAATG TCGTAACAAC TCCGCCCCAT
     541 TGACGCAAAT GGGCGGTAGG CGTGTACGGT GGGAGGTCTA TATAAGCAGA GCTCTCTGGC
     601 TAACTAGAGA ACCCACTGCT TACTGGCTTA TCGAAATTAA TACGACTCAC TATAGGGAGA
     661 CCCAAGCTTG GTACCGAGCT CGGATCTACT AGTAACGGCC GCCAGTGTGC TGGATTTCGG
     721 CTTGGGGATA TCCACCATGG AGACAGACAC ACTCCTGCTA TGGGTACTGC TGCTCTGGGT
     781 TCCAGGTTCC ACTGGTGACG CGAATTCGCA GGTTCAGCTG CAGGAGTCTT GGGCTGAGCT
     841 GGTGAAGCCT GGGGCTTCAG TGAAGCTGTC CTGCAAGGCT TCTGGCTACA CCTTCACCAA
     901 CTACTGGATG CACTGGGTTA AGCAGAGGCC TGGACAAGGC CTTGAGTGGA TTGGAGAGAT
     961 TAATCCTAGC GACGGTCATA CTAACTACAA TGAGAAGTTC AAGAGCAAGG CCACACTGAC
    1021 TGTAGACAAA TCCTCCAGCA CAGCCTACAT GCAACTCAGC AGCCTGACAT CTGAGGACTC
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1081 TGCGGTCTAT TACTGTGCAA GACCTTGGGC GTTTGGTAAC TACGGGGCCT GGTTTGCTTA
1141 CTGGGGCCAA GGGACTCTGG TCACTGTCTC TGCCGGGGGA TCCGGTGGAT CAGGAGGTGG
1201 CGGATCTGGT GGAGGCGGTT CAGGAGGAGG TGCTAGCGAT ATAGTGATGA CCCAGTCTCC
1261 AAAATTCATG TCCACATCAG TAGGAGACAG GGTCAGCATC ACCTGCAAGG CCAGTCAGAA
1321 TGTTCGTACT GCTGTAGCCT GGTATCAACA GAAACCAGGG CAGTCTCCTA AAGCACTGAT
1381 TTACTTGGCA TCCAGCCGGC ACACTGGAGT CCCTGATCGC TTCACAGGCA GTGGATCTGG
1441 GACAGATTTC ACTCTCACCA TTAGCAATGT GCAATCTGAA GACCTGGCAG ATTATTTCTG
1501 TCTGCAACAT TGGAATTATC CGTACACGTT CGGAGGGGGG ACCAAGTCTA GACATCACCA
1561 TCACCATCAC TAGGCTTCCG CTCGAGATCA GCCTCGACTG TGCCTTCTAG TTGCCAGCCA
1621 TCTGTTGTTT GCCCCTCCCC CGTGCCTTCC TTGACCCTGG AAGGTGCCAC TCCCACTGTC
1681 CTTTCCTAAT AAAATGAGGA AATTGCATCG CATTGTCTGA GTAGGTGTCA TTCTATTCTG
1741 GGGGGTGGGG TGGGGCAGGA CAGCAAGGGG GAGGATTGGG AAGACAATAG CAGGCATGCT
1801 GGGGATGCGG TGGGCTCTAT GGCTTCTGAG GCGGAAAGAA CCAGTGGCGG TAATACGGTT
1861 ATCCACAGAA TCAGGGGATA ACGCAGGAAA GAACATGTGA GCAAAAGGCC AGCAAAAGGC
1921 CAGGAACCGT AAAAAGGCCG CGTTGCTGGC GTTTTTCCAT AGGCTCCGCC CCCCTGACGA
1981 GCATCACAAA AATCGACGCT CAAGTCAGAG GTGGCGAAAC CCGACAGGAC TATAAAGATA
2041 CCAGGCGTTT CCCCCTGGAA GCTCCCTCGT GCGCTCTCCT GTTCCGACCC TGCCGCTTAC
2101 CGGATACCTG TCCGCCTTTC TCCCTTCGGG AAGCGTGGCG CTTTCTCATA GCTCACGCTG
2161 TAGGTATCTC AGTTCGGTGT AGGTCGTTCG CTCCAAGCTG GGCTGTGTGC ACGAACCCCC
2221 CGTTCAGCCC GACCGCTGCG CCTTATCCGG TAACTATCGT CTTGAGTCCA ACCCGGTAAG
2281 ACACGACTTA TCGCCACTGG CAGCAGCCAC TGGTAACAGG ATTAGCAGAG CGAGGTATGT
2341 AGGCGGTGCT ACAGAGTTCT TGAAGTGGTG GCCTAACTAC GGCTACACTA GAAGGACAGT
2401 ATTTGGTATC TGCGCTCTGC TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTCTTG
2461 ATCCGGCAAA CAAACCACCG CTGGTAGCGG TGGTTTTTTT GTTTGCAAGC AGCAGATTAC
2521 GCGCAGAAAA AAAGGATCTC AAGAAGATCC TTTGATCTTT TCTACGGGGT CTGACGCTCA
2581 GTGGAACGAA AACTCACGTT AAGGGATTTT GGTCATGAGA TTATCAAAAA GGATCTTCAC
2641 CTAGATCCTT TTAAATTAAA AATGAAGTTT TAAATCAATC TAAAGTATAT ATGAGTAACC
2701 TGAGGCTATG GCAGGGCCTG CCGCCCCGAC GTTGGCTGCG AGCCCTGGGC CTTCACCCGA
2761 ACTTGGGGGG TGGGGTGGGG AAAAGGAAGA AACGCGGGCG TATTGGCCCC AATGGGGTCT
2821 CGGTGGGGTA TCGACAGAGT GCCAGCCCTG GGACCGAACC CCGCGTTTAT GAACAAACGA
2881 CCCAACACCG TGCGTTTTAT TCTGTCTTTT TATTGCCGTC ATAGCGCGGG TTCCTTCCGG
2941 TATTGTCTCC TTCCGTGTTT CAGTTAGCCT CCCCCTAGGG TGGGCGAAGA ACTCCAGCAT
3001 GAGATCCCCG CGCTGGAGGA TCATCCAGCC GGCGTCCCGG AAAACGATTC CGAAGCCCAA
3061 CCTTTCATAG AAGGCGGCGG TGGAATCGAA ATCTCGTGAT GGCAGGTTGG GCGTCGCTTG
3121 GTCGGTCATT TCGAACCCCA GAGTCCCGCT CAGAAGAACT CGTCAAGAAG GCGATAGAAG
3181 GCGATGCGCT GCGAATCGGG AGCGGCGATA CCGTAAAGCA CGAGGAAGCG GTCAGCCCAT
3241 TCGCCGCCAA GCTCTTCAGC AATATCACGG GTAGCCAACG CTATGTCCTG ATAGCGGTCC
3301 GCCACACCCA GCCGGCCACA GTCGATGAAT CCAGAAAAGC GGCCATTTTC CACCATGATA
3361 TTCGGCAAGC AGGCATCGCC ATGGGTCACG ACGAGATCCT CGCCGTCGGG CATGCTCGCC
3421 TTGAGCCTGG CGAACAGTTC GGCTGGCGCG AGCCCCTGAT GCTCTTGATC ATCCTGATCG
3481 ACAAGACCGG CTTCCATCCG AGTACGTGCT CGCTCGATGC GATGTTTCGC TTGGTGGTCG
3541 AATGGGCAGG TAGCCGGATC AAGCGTATGC AGCCGCCGCA TTGCATCAGC CATGATGGAT
3601 ACTTTCTCGG CAGGAGCAAG GTGAGATGAC AGGAGATCCT GCCCCGGCAC TTCGCCCAAT
3661 AGCAGCCAGT CCCTTCCCGC TTCAGTGACA ACGTCGAGCA CAGCTGCGCA AGGAACGCCC
3721 GTCGTGGCCA GCCACGATAG CCGCGCTGCC TCGTCTTGCA GTTCATTCAG GGCACCGGAC
3781 AGGTCGGTCT TGACAAAAAG AACCGGGCGC CCCTGCGCTG ACAGCCGGAA CACGGCGGCA
3841 TCAGAGCAGC CGATTGTCTG TTGTGCCCAG TCATAGCCGA ATAGCCTCTC CACCCAAGCG
3901 GCCGGAGAAC CTGCGTGCAA TCCATCTTGT TCAATCATGC GAAACGATCC TCATCCTGTC
3961 TCTTGATCGA TCTTTGCAAA AGCCTAGGCC TCCAAAAAAG CCTCCTCACT ACTTCTGGAA
4021 TAGCTCAGAG GCCGAGGAGG CGGCCTCGGC CTCTGCATAA ATAAAAAAAA TTAGTCAGCC
4081 ATGGGGCGGA GAATGGGCGG AACTGGGCGG AGTTAGGGGC GGGATGGGCG GAGTTAGGGG
4141 CGGGACTATG GTTGCTGACT AATTGAGATG CATGCTTTGC ATACTTCTGC CTGCTGGGGA
4201 GCCTGGGGAC TTTCCACACC TGGTTGCTGA CTAATTGAGA TGCATGCTTT GCATACTTCT
4261 GCCTGCTGGG GAGCCTGGGG ACTTTCCACA CCCTAACTGA CACACATTCC ACAGCTGGTT
4321 CTTTCCGCCT CAGGACTCTT CCTTTTTCAA TAAATCAATC TAAAGTATAT ATGAGTAAAC
4381 TTGGTCTGAC AGTTACCAAT GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT
4441 TCGTTCATCC ATAGTTGCCT GACTCCCCGT CGTGTAGATA ACTACGATAC GGGAGGGCTT
4501 ACCATCTGGC CCCAGTGCTG CAATGATACC GCGAGACCCA CGCTCACCGG CTCCAGATTT
4561 ATCAGCAATA AACCAGCCAG CCGGAAGGGC CGAGCGCAGA AGTGGTCCTG CAACTTTATC
4621 CGCCTCCATC CAGTCTATTA ATTGTTGCCG GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA
4681 TAGTTTGCGC AACGTTGTTG CCATTGCTAC AGGCATCGTG GTGTCACGCT CGTCGTTTGG
4741 TATGGCTTCA TTCAGCTCCG GTTCCCAACG ATCAAGGCGA GTTACATGAT CCCCCATGTT
4801 GTGCAAAAAA GCGGTTAGCT CCTTCGGTCC TCCGATCGTT GTCAGAAGTA AGTTGGCCGC
4861 AGTGTTATCA CTCATGGTTA TGGCAGCACT GCATAATTCT CTTACTGTCA TGCCATCCGT
4921 AAGATGCTTT TCTGTGACTG GTGAGTACTC AACCAAGTCA TTCTGAGAAT AGTGTATGCG
4981 GCGACCGAGT TGCTCTTGCC CGGCGTCAAT ACGGGATAAT ACCGCGCCAC ATAGCAGAAC
5041 TTTAAAAGTG CTCATCATTG GAAAACGTTC TTCGGGGCGA AAACTCTCAA GGATCTTACC
5101 GCTGTTGAGA TCCAGTTCGA TGTAACCCAC TCGTGCACCC AACTGATCTT CAGCATCTTT
5161 TACTTTCACC AGCGTTTCTG GGTGAGCAAA AACAGGAAGG CAAAATGCCG CAAAAAAGGG
5221 AATAAGGGCG ACACGGAAAT GTTGAATACT CATACTCTTC CTTTTTCAAT ATTATTGAAG
5281 CATTTATCAG GGTTATTGTC TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA
5341 ACAAATAGGG GTTCCGCGCA CATTTCCCCG AAAAGTGCCA CCTGACGCGC CCTGTAGCGG
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5401 CGCATTAAGC GCGGCGGGTG TGGTGGTTAC GCGCAGCGTG ACCGCTACAC TTGCCAGCGC
5461 CCTAGCGCCC GCTCCTTTCG CTTTCTTCCC TTCCTTTCTC GCCACGTTCG CCGGCTTTCC
5521 CCGTCAAGCT CTAAATCGGG GGCTCCCTTT AGGGTTCCGA TTTAGTGCTT TACGGCACCT
5581 CGACCCCAAA AAACTTGATT AGGGTGATGG TTCACGTAGT GGGCCATCGC CCTGATAGAC
5641 GGTTTTTCGC CCTTTGACGT TGGAGTCCAC GTTCTTTAAT AGTGGACTCT TGTTCCAAAC
5701 TGGAACAACA CTCAACCCTA TCTCGGTCTA TTCTTTTGAT TTATAAGGGA TTTTGCCGAT
5761 TTCGGCCTAT TGGTTAAAAA ATGAGCTGAT TTAACAAAAA TTTAACGCGA ATTTTAACAA
5821 AATATTAACG CTTACAATTT AC (SEQ ID NO: 16)
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pDisMod2-B2.1A-scFv - Preferred Sequence

A modified pDisplay vector carrying the B2.1A scFv sequence (the coding sequence is highlighted). There are TWO changes relative to Example Sequence (SEQ ID NO: 16) above - these are in line 781 and are marked in **bold**.

scFv coding sequence key as follows:

Leader sequence-vH-linker-vL-**6his tag**-STOP

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   1 GCGCGCGTTG ACATTGATTA TTGACTAGTT ATTAATAGTA ATCAATTACG GGGTCATTAG
  61 TTCATAGCCC ATATATGGAG TTCCGCGTTA CATAACTTAC GGTAAATGGC CCGCCTGGCT
 121 GACCGCCCAA CGACCCCCGC CCATTGACGT CAATAATGAC GTATGTTCCC ATAGTAACGC
 181 CAATAGGGAC TTTCCATTGA CGTCAATGGG TGGACTATTT ACGGTAAACT GCCCACTTGG
 241 CAGTACATCA AGTGTATCAT ATGCCAAGTA CGCCCCCTAT TGACGTCAAT GACGGTAAAT
 301 GGCCCGCCTG GCATTATGCC CAGTACATGA CCTTATGGGA CTTTCCTACT TGGCAGTACA
 361 TCTACGTATT AGTCATCGCT ATTACCATGG TGATGCGGTT TTGGCAGTAC ATCAATGGGC
 421 GTGGATAGCG GTTTGACTCA CGGGGATTTC CAAGTCTCCA CCCCATTGAC GTCAATGGGA
 481 GTTTGTTTTG GCACCAAAAT CAACGGGACT TTCCAAAATG TCGTAACAAC TCCGCCCCAT
 541 TGACGCAAAT GGGCGGTAGG CGTGTACGGT GGGAGGTCTA TATAAGCAGA GCTCTCTGGC
 601 TAACTAGAGA ACCCACTGCT TACTGGCTTA TCGAAATTAA TACGACTCAC TATAGGGAGA
 661 CCCAAGCTTG GTACCGAGCT CGGATCTACT AGTAACGGCC GCCAGTGTGC TGGATTTCGG
 721 CTTGGGGATA TCCACCATGG AGACAGACAC ACTCCTGCTA TGGGTACTGC TGCTCTGGGT

 781 TCCAGGTTCC ACTGGTGACG CGAATTCGCA GGTTCAGCTG CAGGAGTCTG GGACTGAGCT

 841 GGTGAAGCCT GGGGCTTCAG TGAAGCTGTC CTGCAAGGCT TCTGGCTACA CCTTCACCAA

 901 CTACTGGATG CACTGGGTTA AGCAGAGGCC TGGACAAGGC CTTGAGTGGA TTGGAGAGAT

 961 TAATCCTAGC GACGGTCATA CTAACTACAA TGAGAAGTTC AAGAGCAAGG CCACACTGAC

1021 TGTAGACAAA TCCTCCAGCA CAGCCTACAT GCAACTCAGC AGCCTGACAT CTGAGGACTC

1081 TGCGGTCTAT TACTGTGCAA GACCTTGGGC GTTTGGTAAC TACGGGGCCT GGTTTGCTTA

1141 CTGGGGCCAA GGGACTCTGG TCACTGTCTC TGCCGGGGGA TCCGGTGGAT CAGGAGGTGG

1201 CGGATCTGGT GGAGGCGGTT CAGGAGGAGG TGCTAGCGAT ATAGTGATGA CCCAGTCTCC

1261 AAAATTCATG TCCACATCAG TAGGAGACAG GGTCAGCATC ACCTGCAAGG CCAGTCAGAA

1321 TGTTCGTACT GCTGTAGCCT GGTATCAACA GAAACCAGGG CAGTCTCCTA AAGCACTGAT

1381 TTACTTGGCA TCCAGCCGGC ACACTGGAGT CCCTGATCGC TTCACAGGCA GTGGATCTGG

1441 GACAGATTTC ACTCTCACCA TTAGCAATGT GCAATCTGAA GACCTGGCAG ATTATTTCTG

1501 TCTGCAACAT TGGAATTATC CGTACACGTT CGGAGGGGGG ACCAAGTCTA GACATCACCA

1561 TCACCATCAC TAGGCTTCCG CTCGAGATCA GCCTCGACTG TGCCTTCTAG TTGCCAGCCA

1621 TCTGTTGTTT GCCCCTCCCC CGTGCCTTCC TTGACCCTGG AAGGTGCCAC TCCCACTGTC
1681 CTTTCCTAAT AAAATGAGGA AATTGCATCG CATTGTCTGA GTAGGTGTCA TTCTATTCTG
1741 GGGGGTGGGG TGGGGCAGGA CAGCAAGGGG GAGGATTGGG AAGACAATAG CAGGCATGCT
1801 GGGGATGCGG TGGGCTCTAT GGCTTCTGAG GCGGAAAGAA CCAGTGGCGG TAATACGGTT
1861 ATCCACAGAA TCAGGGGATA ACGCAGGAAA GAACATGTGA GCAAAAGGCC AGCAAAAGGC
1921 CAGGAACCGT AAAAAGGCCG CGTTGCTGGC GTTTTTCCAT AGGCTCCGCC CCCCTGACGA
1981 GCATCACAAA AATCGACGCT CAAGTCAGAG GTGGCGAAAC CCGACAGGAC TATAAAGATA
2041 CCAGGCGTTT CCCCCTGGAA GCTCCCTCGT GCGCTCTCCT GTTCCGACCC TGCCGCTTAC
2101 CGGATACCTG TCCGCCTTTC TCCCTTCGGG AAGCGTGGCG CTTTCTCATA GCTCACGCTG
2161 TAGGTATCTC AGTTCGGTGT AGGTCGTTCG CTCCAAGCTG GGCTGTGTGC ACGAACCCCC
2221 CGTTCAGCCC GACCGCTGCG CCTTATCCGG TAACTATCGT CTTGAGTCCA ACCCGGTAAG
2281 ACACGACTTA TCGCCACTGG CAGCAGCCAC TGGTAACAGG ATTAGCAGAG CGAGGTATGT
2341 AGGCGGTGCT ACAGAGTTCT TGAAGTGGTG GCCTAACTAC GGCTACACTA GAAGGACAGT
2401 ATTTGGTATC TGCGCTCTGC TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTCTTG
2461 ATCCGGCAAA CAAACCACCG CTGGTAGCGG TGGTTTTTTT GTTTGCAAGC AGCAGATTAC
2521 GCGCAGAAAA AAAGGATCTC AAGAAGATCC TTTGATCTTT TCTACGGGGT CTGACGCTCA
2581 GTGGAACGAA AACTCACGTT AAGGGATTTT GGTCATGAGA TTATCAAAAA GGATCTTCAC
2641 CTAGATCCTT TTAAATTAAA AATGAAGTTT TAAATCAATC TAAAGTATAT ATGAGTAACC
2701 TGAGGCTATG GCAGGGCCTG CCGCCCCGAC GTTGGCTGCG AGCCCTGGGC CTTCACCCGA
2761 ACTTGGGGGG TGGGGTGGGG AAAAGGAAGA AACGCGGGCG TATTGGCCCC AATGGGGTCT
2821 CGGTGGGGTA TCGACAGAGT GCCAGCCCTG GGACCGAACC CCGCGTTTAT GAACAAACGA
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2881 CCCAACACCG TGCGTTTTAT TCTGTCTTTT TATTGCCGTC ATAGCGCGGG TTCCTTCCGG
2941 TATTGTCTCC TTCCGTGTTT CAGTTAGCCT CCCCCTAGGG TGGGCGAAGA ACTCCAGCAT
3001 GAGATCCCCG CGCTGGAGGA TCATCCAGCC GGCGTCCCGG AAAACGATTC CGAAGCCCAA
3061 CCTTTCATAG AAGGCGGCGG TGGAATCGAA ATCTCGTGAT GGCAGGTTGG GCGTCGCTTG
3121 GTCGGTCATT TCGAACCCCA GAGTCCCGCT CAGAAGAACT CGTCAAGAAG GCGATAGAAG
3181 GCGATGCGCT GCGAATCGGG AGCGGCGATA CCGTAAAGCA CGAGGAAGCG GTCAGCCCAT
3241 TCGCCGCCAA GCTCTTCAGC AATATCACGG GTAGCCAACG CTATGTCCTG ATAGCGGTCC
3301 GCCACACCCA GCCGGCCACA GTCGATGAAT CCAGAAAAGC GGCCATTTTC CACCATGATA
3361 TTCGGCAAGC AGGCATCGCC ATGGGTCACG ACGAGATCCT CGCCGTCGGG CATGCTCGCC
3421 TTGAGCCTGG CGAACAGTTC GGCTGGCGCG AGCCCCTGAT GCTCTTGATC ATCCTGATCG
3481 ACAAGACCGG CTTCCATCCG AGTACGTGCT CGCTCGATGC GATGTTTCGC TTGGTGGTCG
3541 AATGGGCAGG TAGCCGGATC AAGCGTATGC AGCCGCCGCA TTGCATCAGC CATGATGGAT
3601 ACTTTCTCGG CAGGAGCAAG GTGAGATGAC AGGAGATCCT GCCCCGGCAC TTCGCCCAAT
3661 AGCAGCCAGT CCCTTCCCGC TTCAGTGACA ACGTCGAGCA CAGCTGCGCA AGGAACGCCC
3721 GTCGTGGCCA GCCACGATAG CCGCGCTGCC TCGTCTTGCA GTTCATTCAG GGCACCGGAC
3781 AGGTCGGTCT TGACAAAAAG AACCGGGCGC CCCTGCGCTG ACAGCCGGAA CACGGCGGCA
3841 TCAGAGCAGC CGATTGTCTG TTGTGCCCAG TCATAGCCGA ATAGCCTCTC CACCCAAGCG
3901 GCCGGAGAAC CTGCGTGCAA TCCATCTTGT TCAATCATGC GAAACGATCC TCATCCTGTC
3961 TCTTGATCGA TCTTTGCAAA AGCCTAGGCC TCCAAAAAAG CCTCCTCACT ACTTCTGGAA
4021 TAGCTCAGAG GCCGAGGAGG CGGCCTCGGC CTCTGCATAA ATAAAAAAAA TTAGTCAGCC
4081 ATGGGGCGGA GAATGGGCGG AACTGGGCGG AGTTAGGGGC GGGATGGGCG GAGTTAGGGG
4141 CGGGACTATG GTTGCTGACT AATTGAGATG CATGCTTTGC ATACTTCTGC CTGCTGGGGA
4201 GCCTGGGGAC TTTCCACACC TGGTTGCTGA CTAATTGAGA TGCATGCTTT GCATACTTCT
4261 GCCTGCTGGG GAGCCTGGGG ACTTTCCACA CCCTAACTGA CACACATTCC ACAGCTGGTT
4321 CTTTCCGCCT CAGGACTCTT CCTTTTTCAA TAAATCAATC TAAAGTATAT ATGAGTAAAC
4381 TTGGTCTGAC AGTTACCAAT GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT
4441 TCGTTCATCC ATAGTTGCCT GACTCCCCGT CGTGTAGATA ACTACGATAC GGGAGGGCTT
4501 ACCATCTGGC CCCAGTGCTG CAATGATACC GCGAGACCCA CGCTCACCGG CTCCAGATTT
4561 ATCAGCAATA AACCAGCCAG CCGGAAGGGC CGAGCGCAGA AGTGGTCCTG CAACTTTATC
4621 CGCCTCCATC CAGTCTATTA ATTGTTGCCG GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA
4681 TAGTTTGCGC AACGTTGTTG CCATTGCTAC AGGCATCGTG GTGTCACGCT CGTCGTTTGG
4741 TATGGCTTCA TTCAGCTCCG GTTCCCAACG ATCAAGGCGA GTTACATGAT CCCCCATGTT
4801 GTGCAAAAAA GCGGTTAGCT CCTTCGGTCC TCCGATCGTT GTCAGAAGTA AGTTGGCCGC
4861 AGTGTTATCA CTCATGGTTA TGGCAGCACT GCATAATTCT CTTACTGTCA TGCCATCCGT
4921 AAGATGCTTT TCTGTGACTG GTGAGTACTC AACCAAGTCA TTCTGAGAAT AGTGTATGCG
4981 GCGACCGAGT TGCTCTTGCC CGGCGTCAAT ACGGGATAAT ACCGCGCCAC ATAGCAGAAC
5041 TTTAAAAGTG CTCATCATTG GAAAACGTTC TTCGGGGCGA AAACTCTCAA GGATCTTACC
5101 GCTGTTGAGA TCCAGTTCGA TGTAACCCAC TCGTGCACCC AACTGATCTT CAGCATCTTT
5161 TACTTTCACC AGCGTTTCTG GGTGAGCAAA AACAGGAAGG CAAAATGCCG CAAAAAAGGG
5221 AATAAGGGCG ACACGGAAAT GTTGAATACT CATACTCTTC CTTTTTCAAT ATTATTGAAG
5281 CATTTATCAG GGTTATTGTC TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA
5341 ACAAATAGGG GTTCCGCGCA CATTTCCCCG AAAAGTGCCA CCTGACGCGC CCTGTAGCGG
5401 CGCATTAAGC GCGGCGGGTG TGGTGGTTAC GCGCAGCGTG ACCGCTACAC TTGCCAGCGC
5461 CCTAGCGCCC GCTCCTTTCG CTTTCTTCCC TTCCTTTCTC GCCACGTTCG CCGGCTTTCC
5521 CCGTCAAGCT CTAAATCGGG GGCTCCCTTT AGGGTTCCGA TTTAGTGCTT TACGGCACCT
5581 CGACCCCAAA AAACTTGATT AGGGTGATGG TTCACGTAGT GGGCCATCGC CCTGATAGAC
5641 GGTTTTTCGC CCTTTGACGT TGGAGTCCAC GTTCTTTAAT AGTGGACTCT TGTTCCAAAC
5701 TGGAACAACA CTCAACCCTA TCTCGGTCTA TTCTTTTGAT TTATAAGGGA TTTTGCCGAT
5761 TTCGGCCTAT TGGTTAAAAA ATGAGCTGAT TTAACAAAAA TTTAACGCGA ATTTTAACAA
5821 AATATTAACG CTTACAATTT AC (SEQ ID NO: 27)
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Example 4: Production of B2.1A Antibody

B2.1A Antibody Chains are produced using conventional antibody expression systems incorporating the CDRs of the B2.1A as disclosed herein.

In this example the conventional expression system used is the 'antibody generation' system which is commercially available from InvivoGen at 5, rue Jean Rodier, F-31400 Toulouse, France.

pFUSEss-CHIg-mG1-B2.1a-vH - Example Sequence

B2.1A vH sequence cloned into pFUSEss-CHIg-Mg1 to generate a full heavy chain.

Coding sequences highlighted:

Leader sequence (vector-derived)-B2.1A vH-constant heavy (vector-derived)-STOP

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   1 GGATCTGCGA TCGCTCCGGT GCCCGTCAGT GGGCAGAGCG CACATCGCCC ACAGTCCCCG
  61 AGAAGTTGGG GGGAGGGGTC GGCAATTGAA CGGGTGCCTA GAGAAGGTGG CGCGGGGTAA
 121 ACTGGGAAAG TGATGTCGTG TACTGGCTCC GCCTTTTTCC CGAGGGTGGG GGAGAACCGT
 181 ATATAAGTGC AGTAGTCGCC GTGAACGTTC TTTTTCGCAA CGGGTTTGCC GCCAGAACAC
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241 AGCTGAAGCT TCGAGGGGCT CGCATCTCTC CTTCACGCGC CCGCCGCCCT ACCTGAGGCC
301 GCCATCCACG CCGGTTGAGT CGCGTTCTGC CGCCTCCCGC CTGTGGTGCC TCCTGAACTG
361 CGTCCGCCGT CTAGGTAAGT TTAAAGCTCA GGTCGAGACC GGGCCTTTGT CCGGCGCTCC
421 CTTGGAGCCT ACCTAGACTC AGCCGGCTCT CCACGCTTTG CCTGACCCTG CTTGCTCAAC
481 TCTACGTCTT TGTTTCGTTT TCTGTTCTGC GCCGTTACAG ATCCAAGCTG TGACCGGCGC
541 CTACCTGAGA TCACCGGCGA AGGAGGGCCA CCATGTACAG GATGCAACTC CTGTCTTGCA
601 TTGCACTAAG TCTTGCACTT GTCACGAATT CGCAGGTTCA GCTGCAGGAG TCTGGGGCTG
661 AGCTGGTGAA GCCTGGGGCT TCAGTGAAGC TGTCCTGCAA GGCTTCTGGC TACACCTTCA
721 CCAACTACTG GATGCACTGG GTTAAGCAGA GGCCTGGACA AGGCCTTGAG TGGATTGGAG
781 AGATTAATCC TAGCGACGGT CATACTAACT ACAATGAGAA GTTCAAGAGC AAGGCCACAC
841 TGACTGTAGA CAAATCCTCC AGCACAGCCT ACATGCAACT CAGCAGCCTG ACATCTGAGG
901 ACTCTGCGGT CTATTACTGT GCAAGACCTT GGGCGTTTGG TAACTACGGG GCCTGGTTTG
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1021 TCTATCCACT GGCCCCTGGA TCTGCTGCCC AAACTAACTC CATGGTGACC CTGGGATGCC
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3421 CCAAAGCAAG GGGAAGTCAC GCGCCTGTAG CGCCAGCGTG TTGTGAAATG GGGGCTTGGG
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4681 CAATAAAATA TCTTTATTTT CATTACATCT GTGTGTTGGT TTTTTGTGTG AATCGTAACT
4741 AACATACGCT CTCCATCAAA ACAAAACGAA ACAAAACAAA CTAGCAAAAT AGGCTGTCCC
4801 CAGTGCAAGT GCAGGTGCCA GAACATTTCT CTATCGAA (SEQ ID NO: 17)
```

pFUSEss-CHIg-mG1-B2.1a-vH – Preferred Sequence

B2.1A vH sequence cloned into pFUSEss-CHIg-Mg1 to generate a full heavy chain. There is one change relative to Example Sequence (SEQ ID NO: 17) above - this is in line 601 and is marked in **bold**.

Coding sequences highlighted:

Leader sequence (vector-derived)-B2.1A vH-constant heavy (vector-derived)-**STOP**

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   1 GGATCTGCGA TCGCTCCGGT GCCCGTCAGT GGGCAGAGCG CACATCGCCC ACAGTCCCCG
  61 AGAAGTTGGG GGGAGGGGTC GGCAATTGAA CGGGTGCCTA GAGAAGGTGG CGCGGGGTAA
 121 ACTGGGAAAG TGATGTCGTG TACTGGCTCC GCCTTTTTCC CGAGGGTGGG GGAGAACCGT
 181 ATATAAGTGC AGTAGTCGCC GTGAACGTTC TTTTTCGCAA CGGGTTTGCC GCCAGAACAC
 241 AGCTGAAGCT TCGAGGGGCT CGCATCTCTC CTTCACGCGC CCGCCGCCCT ACCTGAGGCC
 301 GCCATCCACG CCGGTTGAGT CGCGTTCTGC CGCCTCCCGC CTGTGGTGCC TCCTGAACTG
 361 CGTCCGCCGT CTAGGTAAGT TTAAAGCTCA GGTCGAGACC GGGCCTTTGT CCGGCGCTCC
 421 CTTGGAGCCT ACCTAGACTC AGCCGGCTCT CCACGCTTTG CCTGACCCTG CTTGCTCAAC
 481 TCTACGTCTT TGTTTCGTTT TCTGTTCTGC GCCGTTACAG ATCCAAGCTG TGACCGGCGC
 541 CTACCTGAGA TCACCGGCGA AGGAGGGCCA CCATGTACAG GATGCAACTC CTGTCTTGCA

 601 TTGCACTAAG TCTTGCACTT GTCACGAATT CGCAGGTTCA GCTGCAGGAG TCTGGGACTG

 661 AGCTGGTGAA GCCTGGGGCT TCAGTGAAGC TGTCCTGCAA GGCTTCTGGC TACACCTTCA

 721 CCAACTACTG GATGCACTGG GTTAAGCAGA GGCCTGGACA AGGCCTTGAG TGGATTGGAG

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 841 TGACTGTAGA CAAATCCTCC AGCACAGCCT ACATGCAACT CAGCAGCCTG ACATCTGAGG

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1021 TCTATCCACT GGCCCCTGGA TCTGCTGCCC AAACTAACTC CATGGTGACC CTGGGATGCC

1081 TGGTCAAGGG CTATTTCCCT GAGCCAGTGA CAGTGACCTG GAACTCTGGA TCCCTGTCCA

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1381 TCACCATTAC TCTGACTCCT AAGGTCACGT GTGTTGTGGT AGACATCAGC AAGGATGATC

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4681 CAATAAAATA TCTTTATTTT CATTACATCT GTGTGTTGGT TTTTTGTGTG AATCGTAACT
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4801 CAGTGCAAGT GCAGGTGCCA GAACATTTCT CTATCGAA (SEQ ID NO: 28)

pFUSE2ss-CLIg-mk-B2.1a-vL

B2.1A vL sequence cloned into pFUSEss-CLIg-Mk to generate a full light chain.

Coding sequences highlighted:

Leader sequence (vector-derived)-B2.1A vL-constant light (vector-derived)-STOP

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       1 GGATCTGCGA TCGCTCCGGT GCCCGTCAGT GGGCAGAGCG CACATCGCCC ACAGTCCCCG
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     181 ATATAAGTGC AGTAGTCGCC GTGAACGTTC TTTTTCGCAA CGGGTTTGCC GCCAGAACAC
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     301 GCCATCCACG CCGGTTGAGT CGCGTTCTGC CGCCTCCCGC CTGTGGTGCC TCCTGAACTG
     361 CGTCCGCCGT CTAGGTAAGT TTAAAGCTCA GGTCGAGACC GGGCCTTTGT CCGGCGCTCC
     421 CTTGGAGCCT ACCTAGACTC AGCCGGCTCT CCACGCTTTG CCTGACCCTG CTTGCTCAAC
     481 TCTACGTCTT TGTTTCGTTT TCTGTTCTGC GCCGTTACAG ATCCAAGCTG TGACCGGCGC
     541 CTACCTGAGA TCAACATGTA CAGGATGCAA CTCCTGTCTT GCATTGCACT AAGTCTTGCA

     601 CTTGTCACGA ATTCAGATAT AGTGATGACC CAGTCTCCAA AATTCATGTC CACATCAGTA

     661 GGAGACAGGG TCAGCATCAC CTGCAAGGCC AGTCAGAATG TTCGTACTGC TGTAGCCTGG

     721 TATCAACAGA AACCAGGGCA GTCTCCTAAA GCACTGATTT ACTTGGCATC CAGCCGGCAC

     781 ACTGGAGTCC CTGATCGCTT CACAGGCAGT GGATCTGGGA CAGATTTCAC TCTCACCATT

     841 AGCAATGTGC AATCTGAAGA CCTGGCAGAT TATTTCTGTC TGCAACATTG GAATTATCCG

     901 TACACGTTCG GAGGGGGGAC CAAGCTCGAG ATCAAACGGG CAGATGCTGC ACCAACTGTA

     961 TCCATCTTCC CACCATCCAG TGAGCAGTTA ACATCTGGAG GTGCCTCAGT CGTGTGCTTC

    1021 TTGAACAACT TCTACCCCAA AGACATCAAT GTCAAGTGGA AGATTGATGG CAGTGAACGA

    1081 CAAAATGGCG TCCTGAACAG TTGGACTGAT CAGGACAGCA AAGACAGCAC CTACAGCATG

    1141 AGCAGCACCC TCACGTTGAC CAAGGACGAG TATGAACGAC ATAACAGCTA TACCTGTGAG

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    1681 TCCCAAGGTT TGAACTAGCT CTTCATTTCT TTATGTTTTA AATGCACTGA CCTCCCACAT
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    3361 TCAGAGGTGG CGAAACCCGA CAGGACTATA AAGATACCAG GCGTTTCCCC CTGGAAGCTC
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4081 CAAAACAAAA CGAAACAAAA CAAACTAGCA AAATAGGCTG TCCCCAGTGC AAGTGCAGGT
4141 GCCAGAACAT TTCTCTATCG AA  (SEQ ID NO: 18)
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Example 5: Challenge studies in mice

Mouse model

We use the immunocompetent mouse model developed by Dorner et al (Dorner et al 2011 Hepatology Vol 54 No 5 pages 1873-1875; Dorner et al 2011 Nature Vol 474 pages 208-211; Dorner et al 2013 Methods Vol 59 pages 249-257; Zeisel et al 2011). This is the most appropriate model for testing HCV vaccines.

Commercially available transgenic Gt(ROSA)26Sortm1(Luc)Kaelin mice (Rosa26-Fluc) contain a LoxP-flanked STOP cassette restricting firefly luciferase expression. They are made permissive for HCV entry by infection with adenoviruses encoding essential cell surface receptors (human CD81, occludin, claudin 1 and SR-BI), and then infected with recombinant bicistronic HCVcc expressing cyclization recombination (CRE) recombinase. Upon HCV entry into mouse hepatocytes, the recombinant viral genome is translated and the CRE protein is expressed. The CRE recombinase excises the STOP cassette and activates the luciferase reporter, leading to bioluminescence that can be measured using a using a whole body bioluminescence imager.

Experimental Details

1. Establish a small colony (~30) of the commercially-available transgenic (Rosa26-Fluc CRE reporter mice.
2. Carry out a small-scale vaccination (6-8 animals) with B2.1A Fab-KLH and check the anti-E2 serum titre after each vaccination by ELISA. (Primary vaccination with immunogen in Freund's Complete Adjuvant, followed by 5 boosts with immunogen in Freund's Incomplete Adjuvant).
3. If adequate anti-E2 serum titres are obtained, vaccinate a larger number (24) as above.
4. Genetically humanise the immunised mice by administering adenovirus vectors encoding human CD81 and OCLN, and human or murine SR-BI and CLDN1.

5. After 24 hours administer 2x10^7 TCID50 of HCV-CRE. Use 4 different HCV viruses representing a range of genotypes.

6. After 72 hours measure bioluminescence using a whole body imager, and correlate anti-E2 titre with HCV infection. An inverse correlation indicates that the vaccine protects against HCV challenge

Example 6: B2.1A Structure

A Fab fragment of AP33 was co-crystallised in complex with a single-chain variable fragment (scFv) of B2.1A, and the structure determined to a resolution of 1.8 Å, which unambiguously shows the positions of all the amino acid side-chains and of water molecules at the interface between the two antibodies. The asymmetric unit of this Ab_1-Ab_2 complex was composed of one molecule of AP33 Fab and one molecule of B2.1A scFv. The structural coordinates were determined.

The structure (Figure 13) shows that the CDR loops of B2.1A correspond more closely to the definition of IgG regions described by Chothia et al. than by Kabat et al.

The combining site of B2.1A has an overall concave surface from which the CDR-L1 and CDR-H3 loops protrude outwards, towards the groove formed between the CDR-L2, CDR-L3 and CDR-H3 loops of AP33. The groove on AP33 has an overall negative charge, while the L1 loop on B2.1A has a complementary positive charge. Overall, both combining sites have a hydrophobic nature, due to the presence of numerous aromatic residues. All the heavy and light chain CDRs of B2.1A are involved in interactions with AP33 via hydrogen bonds and other hydrophilic interactions, hydrophobic interactions and van der Waals contacts. The area of the interface is 1069 Å, which is approximately 9% of the total surface of the B2.1a scFv.

Example 7: Antigen mimicry by B2.1A

A comparison of this Ab_1-Ab_2 complex with the Ab_1-Ag complex (i.e. the structure of AP33 in complex with a peptide corresponding to its E2 epitope (Potter et al. 2012; pdb accession code 4gag)) shows that B2.1A docks into the AP33 antigen-binding site (Figure 14). It reveals that CDR-H3 of B2.1A mimics the shape and character of the E2 epitope, even though there is no sequence similarity. The critical E2 residue W420, which is deeply buried in the Ab_1-Ag complex, is mimicked by F_H98 of B2.1A in the Ab_1-Ab_2 complex (Figure 15a).

The other important E2 residues at the Ab_1-Ag interface are G418, N415 and L413. The shape of the antigen around G418 is preserved by the side chain of B2.1A Y_H100A, which forms extensive contacts with W_L96 of AP33 (Figure 15b). The polar character of E2 residue N415, which is deeply buried in the Ab_1-Ag complex, is conferred by N_H100 of B2.1A, while the neighbouring Y_H100A provides a hydrogen bond to Y_H50 of AP33 (Figure 15c). Interestingly, the interactions of L413 with AP33 are mimicked not by an amino acid residue but by five water molecules in the Ab_1-Ab_2 complex (Figure 15d). In keeping with our biochemical and immunisation data (shown in Table 1 and Figs. 9 – 12), this structural analysis confirms that B2.1A is an $Ab_2\beta$, i.e. an anti-idiotypic antibody that fits into the antigen-binding site (paratope) of the Ab_1 precisely enough to be an "internal image" of it, and, by the same token, an effective mimic of the original antigen.

Example 8: B2.1A Binding Affinity

We measured the binding affinity of B2.1A for AP33 by Surface Plasmon Resonance (SPR). B2.1A scFv was immobilised in three different ways: (a) amine coupling to a CM5 chip; (b) amine coupling to a CM4 chip; (c) capture via a histidine tag to a NTA chip. AP33 was then injected over the surface, using single-cycle kinetics. All the data sets were high quality and the three experiments yielded affinity constants of 29 nm, 20 nm and 8 nm, respectively:

Table: Binding affinity of B2.1A for AP33

Expt	Ka (1/Ms)	Kd (1/s)	KD (M)
a)	$1.12*10^4$	$3.21*10^{-4}$	$2.86*10^{-8}$
b)	$1.18*10^4$	$2.43*10^{-4}$	$2.07*10^{-8}$
c)	$4.87*10^4$	$3.9*10^{-4}$	$8.0*10^{-9}$

These values are comparable to the affinity constants of 5.5 – 6.6 nm, measured by SPR, for binding of antibody MRCT10 (humanised AP33 - WO2009/081285) to soluble $E2_{661}$ (Pantua et al 2013).

Example 9: B2.1A Mutagenesis

The crystallographic structure of B2.1A scFv, together with protein-protein interaction prediction servers, inspired the inventors to design point mutations aimed at increasing

its binding affinity for AP33. The inventors reasoned that this might translate into an increased affinity for HCV E2 of Ab3 antibodies elicited by vaccination with B2.1A. The following mutations were introduced into the heavy chain sequence of B2.1A: W33V, E50F, E50Y, F98Y, F98W, N100G, N100del and G100BF, in a wild-type (WT) protein comprising a fusion of B2.1A scFv with maltose binding protein (MBP). The affinity of the mutant proteins for AP33 was assessed by AP33-capture ELISA, using MBP as a detection tag. As shown in Figure 16b, most of the mutants showed little or no binding to AP33. Only two of the mutants, F98W and N100G, retained binding, but it was weaker than WT (Figure 16a).

The EC_{50} values, estimated by fitting a sigmoidal curve to the data, were 1.48 μg/ml for WT and 4.6 μg/ml for F98W.

Thus it seems that it is not possible to improve the affinity of B2.1A for AP33 by mutagenesis.

These results demonstrate that AP33 appears to represent the best possible antibody and additionally show that it is demonstrably superior to rationally designed alternatives and therefore possesses significant technical advantages over other antibody species having different amino acid sequences.

Example 10: Vaccination with B2.1A /Protection from HCV infection

The immunocompetent mouse model developed by Marcus Dorner (Dorner et al 2011; Dorner et al 2013) is used to test whether vaccination with B2.1A can protect against infection by HCV. This is the most appropriate model for testing HCV vaccines. Commercially available transgenic Rosa26-Fluc mice contain a LoxP-flanked STOP cassette restricting firefly luciferase expression. They are made permissive for HCV entry by infection with adenoviruses encoding essential cell-surface receptors (human CD81, occludin, claudin 1 and SR-BI), and then infected with recombinant bicistronic HCVcc expressing cyclisation recombination (CRE) recombinase. Upon HCV entry into mouse hepatocytes, the recombinant viral genome is translated and the CRE protein is expressed. The CRE recombinase excises the STOP cassette and activates the luciferase reporter, leading to bioluminescence that can be measured using a using a whole body bioluminescence imager.

Detailed protocols of immunisation & challenge experiments in mice

Mice

Strain FVB.129S6(B6)-Gt(ROSA)26Sor$^{tm1(Luc)Kael}$/J, (abbreviate to Rosa26-Fluc; Jackson Laboratories stock no 005125). Purchase 2-3 mating pairs and breed the mice to obtain sufficient numbers for immunisation.

Immunisation Protocol 1

Immunogens: (A) B2.1A Fab conjugated to KLH, 1mg/ml

(B) Peptide IQLINTNGSWHINS conjugated to KLH, 1mg/ml

(The peptide corresponds to the AP33 epitope, ie aa 412-423 of HCV E2)

For primary vaccination make up a 1:1 emulsion of immunogen (A) with Freund's Complete Adjuvant (FCA). The final protein concentration is 0.5mg/ml.

For all booster vaccinations make up a 1:1 emulsion of immunogen (A) or (B), as appropriate, with Freund's Incomplete Adjuvant (IFA).

Day 0 Pre-immune bleed.

Day 7 Primary vaccination. Subcutaneous injection of 50 μg in 100 μl per mouse of immunogen (A) in CFA

Day 28 Booster 1. Subcutaneous injection of 50 μg in 100 μl per mouse of immunogen (A) in IFA.

Day 35 Test bleed 1.

Day 42 Booster 2. Subcutaneous injection of 50 μg in 100 μl per mouse of immunogen (B) in IFA.

Day 49 Test bleed 2.

Day 56 Booster 3. Subcutaneous injection of 50 μg in 100 μl per mouse of immunogen (A) in IFA.

Day 63 Test bleed 3.

Day 70 Booster 4. Subcutaneous injection of 50 μg in 100 μl per mouse of immunogen (B) in IFA.

Day 77 Test bleed 4.

Day 84 Booster 5. Subcutaneous injection of 50 μg in 100 μl per mouse of immunogen (A) in IFA.

Day 91 Test bleed 5.

The timing does not have to be exactly as above. The first boost should be at least three weeks after the primary immunisation, and the subsequent boosters should be at least two weeks apart. A test bleed should be taken 7 – 10 days after the booster.

Immunisation Protocol 2

Immunogen: B2.1A Fab conjugated to KLH, 1mg/ml

For primary vaccination make up a 1:1 emulsion of immunogen with Freund's Complete Adjuvant (FCA). The final protein concentration is 0.5mg/ml.

For all booster vaccinations make up a 1:1 emulsion of immunogen with Freund's Incomplete Adjuvant (IFA).

Day 0 Pre-immune bleed.

Day 7 Primary vaccination. Subcutaneous injection of 50 μg in 100 μl per mouse of immunogen in CFA

Day 28 Booster 1. Subcutaneous injection of 50 μg in 100 μl per mouse of immunogen in IFA.

Day 35 Test bleed 1.

Day 42 Booster 2. Subcutaneous injection of 50 μg in 100 μl per mouse of immunogen in IFA.

Day 49 Test bleed 2.

Day 56 Booster 3. Subcutaneous injection of 50 μg in 100 μl per mouse of immunogen in IFA.

Day 63 Test bleed 3.

Day 70 Booster 4. Subcutaneous injection of 50 μg in 100 μl per mouse of immunogen in IFA.

Day 77 Test bleed 4.

Day 84 Booster 5. Subcutaneous injection of 50 μg in 100 μl per mouse of immunogen in IFA.

Day 91 Test bleed 5.

The timing does not have to be exactly as above. The first boost should be at least three weeks after the primary immunisation, and the subsequent boosters should be at least two weeks apart. A test bleed should be taken 7 – 10 days after the booster.

If the test bleeds show that the mice have developed HCV E2-specific antibodies, proceed with genetic humanisation and challenge according the protocol below. If the test bleeds show that the mice have developed a high titre (>1:10,000) of HCV E2-specific antibodies after two or three boosters, there is no need to give all the boosters.

We have described two immunisation protocols. The first protocol includes boosters with a peptide corresponding to the E2 epitope that is mimicked by the CDRs of B2.1A. This aims to focus the immune response on the desired region of B2.1A. The second protocol boosts with B2.1A Fab alone. Our data show that we can definitely elicit E2-specific antibodies using B2.1A Fab alone. Boosting with peptide may or may not confer an advantage. The skilled worker may choose the protocol according to their needs.

Test bleeds are processed as is known in the art, i.e. by taking the test bleed, clotting it, taking the supernatant, centrifuging it to pellet any cells not removed with the clot, adding 1mM sodium azide and storing it at 4 degrees Celsius until needed.

Titration[1] of mouse serum by sE2 by ELISA

1. Coat the wells of a 96-well Immulon 2 HB plate with 0.2μg/well of purified soluble HCV E2[2] in 100ul PBS. Incubate overnight at RT.
2. Discard sE2 and block with 2% skimmed milk powder in PBST[3], 200μl/well. Incubate for 2 hours at RT.
3. Wash 3x with PBST. The plate can be stored at this stage at -20°C or 4°C.
4. Add two-fold dilutions of serum in 100μl of PBST. Incubate for 2 hours at RT.
5. Wash 3x with PBST.
6. Add 100μl/well of anti-mouse HRP conjugate (Sigma A4416) diluted 1/3000 in PBST. Incubate for 1 hour at RT.
7. Wash 4x PBST.

8. Add 100µl/well of TMB substrate. Incubate at RT for 30 mins.
9. Stop the reaction by adding 50µl/well of 0.5M H_2SO_4.
10. Read the absorbance at 450 nm in a microplate reader.

[1]The titre of a serum is defined as the lowest concentration that gives a positive antigen-specific signal. In this assay, a positive signal is defined as an A_{450} reading that is three times higher than that produced by non-immune, control serum at the same dilution. The mean signal from several non-immune sera is used as the control.
[2]soluble E2 (sE2) expressed and purified from insect cells. It comprises aa 384–661 of the HCV polyprotein, ie the ectodomain without the membrane-proximal and trans-membrane regions.
[3]PBST = PBS + 0.05% Tween 20

Infection of genetically humanised Rosa26-Fluc mice with HCV-CRE

The preparation of adenoviruses and recombinant HCV-CRE and the analysis of HCV entry by in vivo bioluminescence imaging are carried out exactly as known in the art, for example as described in sections *2.2.1, 2.2.2* and *2.3.2* of Dorner et al, 2013 which is incorporated herein by reference specifically for the detailed description of performing this technique.

In this example we show data for six mice. Three mice were given a primary vaccination with B2.1A Fab coupled to KLH, followed by five booster vaccinations. This elicited robust anti-E2 titres of 1:12,800 in two mice and 1:1,600 in the third mouse (Figure 17).

The E2 reactivity is inhibited by a peptide containing the AP33 epitope, showing that the Ab3 antibodies elicited in the Rosa26-Fluc mice have the same specificity as AP33 (Figure 18). This shows that the binding to E2 of AP33 and of Ab3 in the Rosa26 Fluc immune sera is specifically inhibited in a concentration-dependent manner by the WT peptide that contains the AP33 epitope. There is no inhibition by the peptide in which W420, an essential contact residue for AP33, has been replaced by R. As expected, ALP98, which binds to a different linear epitope on E2 (aa residues 644 – 651), is not inhibited by either of the peptides.

Thus the Rosa26Fluc mice showed a good immune response.

The vaccinated and unvaccinated mice are made permissive for HCV infection as described above, and then challenged with $2x10^7$ $TCID_{50}$ of HCV-CRE.

References to Examples

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Although illustrative embodiments of the invention have been disclosed in detail herein, with reference to the accompanying drawings, it is understood that the invention is not limited to the precise embodiment and that various changes and modifications can be effected therein by one skilled in the art without departing from the scope of the invention as defined by the appended claims and their equivalents.

2015310671 06 Aug 2018

CLAIMS

1. An antibody or antigen binding fragment thereof capable of binding to the antigen binding pocket of the AP33 antibody, wherein said antibody or antigen binding fragment thereof comprises VL CDR1 (L1), VL CDR2 (L2), and VL CDR3 (L3) consisting of the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:23 respectively, and comprises VH CDR1 (H1), VH CDR2 (H2), and VH CDR3 (H3) consisting of the amino acid sequences of SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26 respectively.

2. An antibody or antigen binding fragment thereof according to claim 1 wherein said antibody or antigen binding fragment thereof comprises VL amino acid sequence consisting of the amino acid sequence of SEQ ID NO:20.

3. An antibody or antigen binding fragment thereof according to claim 1 wherein said antibody or antigen binding fragment thereof comprises VH amino acid sequence consisting of the amino acid sequence of SEQ ID NO:22.

4. An antibody or antigen binding fragment thereof according to claim 1 wherein said antibody or antigen binding fragment thereof comprises VL amino acid sequence consisting of the amino acid sequence of SEQ ID NO:20 and wherein said antibody or antigen binding fragment thereof comprises VH amino acid sequence consisting of the amino acid sequence of SEQ ID NO:22.

5. An antibody or antigen binding fragment thereof according to any preceding claim, wherein the antigen binding fragment thereof is selected from the group consisting of a Fab fragment, a Fab' fragment, a F(ab')2 fragment, a scFv, a Fv, a rIgG, and a diabody.

6. An antibody or antigen binding fragment thereof according to claim 5 wherein said antigen binding fragment is a scFv and wherein said scFv comprises the amino acid sequence of SEQ ID NO:11 or SEQ ID NO:12 or SEQ ID NO:13.

7. A nucleic acid comprising a nucleotide sequence encoding the variable heavy chain domain and/or the variable light chain domain of the antibody or antigen binding fragment according to any preceding claim.

8. The nucleic acid of claim 7, wherein the nucleic acid comprises one or more nucleotide sequences selected from the group consisting of SEQ ID NO:19 and SEQ ID NO:21.

9. A nucleic acid comprising a nucleotide sequence complementary to the nucleotide sequence of claim 7 or claim 8.

10. A vector comprising the nucleic acid of claim 7 or claim 8.

11. The vector of claim 10, wherein the vector further comprises an expression control sequence operatively linked to the nucleic acid encoding the variable heavy chain domain and/or the variable light chain domain.

12. A host cell containing the vector of claim 10 or claim 11.

13. The host cell of claim 12, wherein the cell is a eukaryotic cell.

14. The host cell of claim 13, wherein the eukaryotic cell is a Chinese Hamster Ovary (CHO) cell or a human embryonic kidney (HEK) cell.

15. A method of producing an antibody or antigen binding fragment thereof, comprising incubating a host cell according to any of claims 12 to 14 such that the encoded variable heavy chain domain and/or variable light chain domain is expressed by the cell; and recovering the expressed the antibody or antigen binding fragment thereof.

16. The method of claim 15, which further comprises isolating and/or purifying the recovered antibody or antigen binding fragment thereof.

17. A composition comprising the antibody or antigen binding fragment thereof according to any of claims 1 to 6 and a pharmaceutically acceptable carrier or excipient.

18. A composition according to claim 17 further comprising a carrier protein, the carrier protein preferably selected from the group consisting of tetanus toxoid and CRM 197 mutant diphtheria toxin.

19. A composition according to claim 17 or claim 18 further comprising an adjuvant.

20. A composition according to any of claims 17 to 19 formulated for use in humans.

21. An antibody or antigen binding fragment thereof capable of inducing in a mammal an immune response against the hepatitis C virus E2 protein, wherein said antibody or antigen binding fragment thereof is capable of binding to the antigen binding pocket of the monoclonal AP33 antibody.

22. An antibody or antigen binding fragment thereof capable of inducing in a mammal an immune response against the hepatitis C virus E2 protein, wherein said antibody or antigen binding fragment thereof comprises VL CDR1 (L1), VL CDR2 (L2), and VL CDR3 (L3) consisting of the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:23 respectively, and comprises VH CDR1 (H1), VH CDR2 (H2), and VH CDR3 (H3) consisting of the amino acid sequences of SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26 respectively.

23. An antibody or antigen binding fragment thereof capable of binding to the AP33 antibody wherein said antibody or antigen binding fragment thereof exhibits binding to AP33 antibody mutants FL32A, NL91A, WL96A, YH33A, YH50A, YH58A, IH95A and YH100A of less than 50% of its binding to the AP33 antibody.

24. A method of inducing in a mammal an immune response against the hepatitis C virus E2 protein, the method comprising administering to said mammal an antibody or antigen binding fragment thereof according to any of claims 1 to 6 or 21 to 23, a nucleic acid according to any of claims 7 to 9, a vector according to claim 10 or claim 11, or a composition according to any of claims 17 to 20.

25. An antibody or antigen binding fragment thereof according to any of claims 1 to 6 or 21 to 23, a nucleic acid according to any of claims 7 to 9, a vector according to claim 10 or claim 11, or a composition according to any of claims 17 to 20, when used to induce an immune response against the hepatitis C virus E2 protein in a mammal.

FIGURE 1

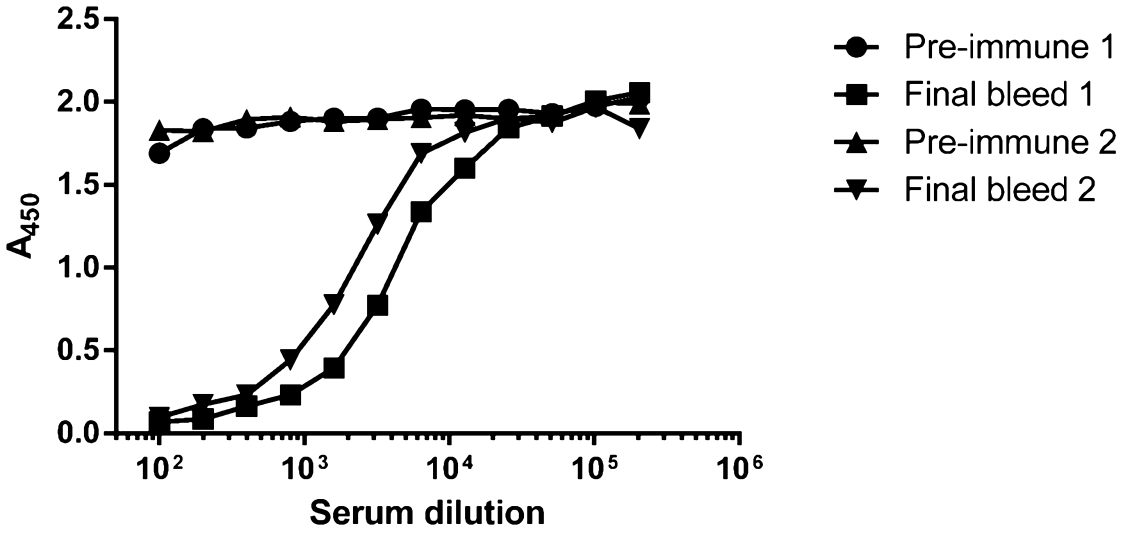

FIGURE 2

Absorbance (A450)

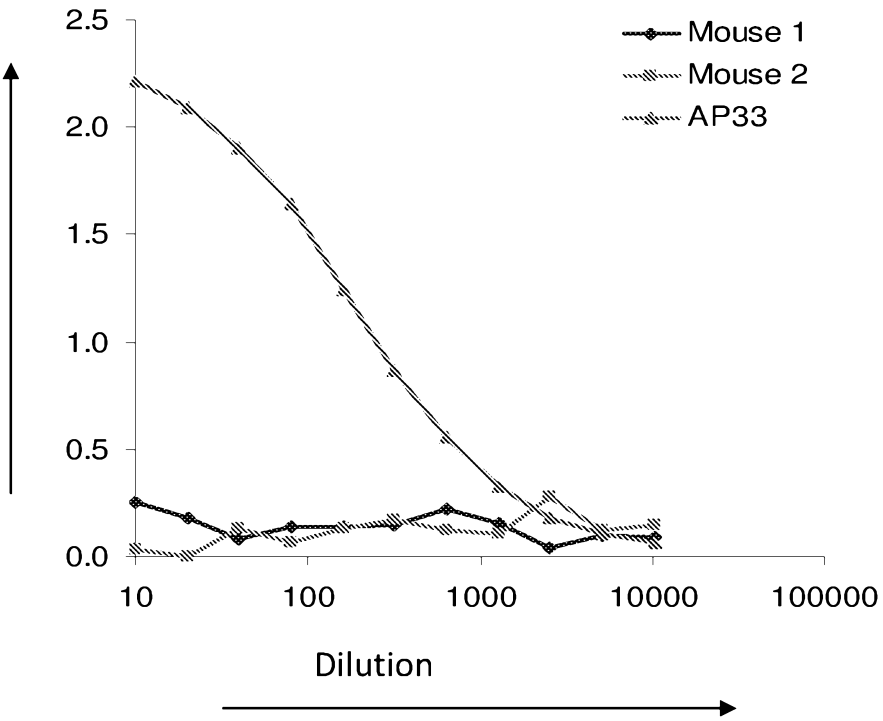

FIGURE 3

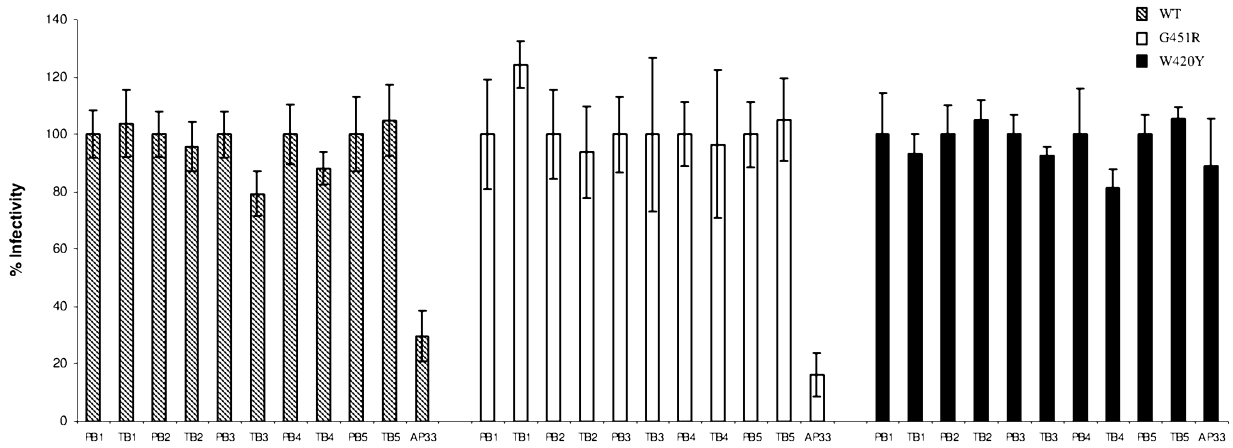

FIGURE 4

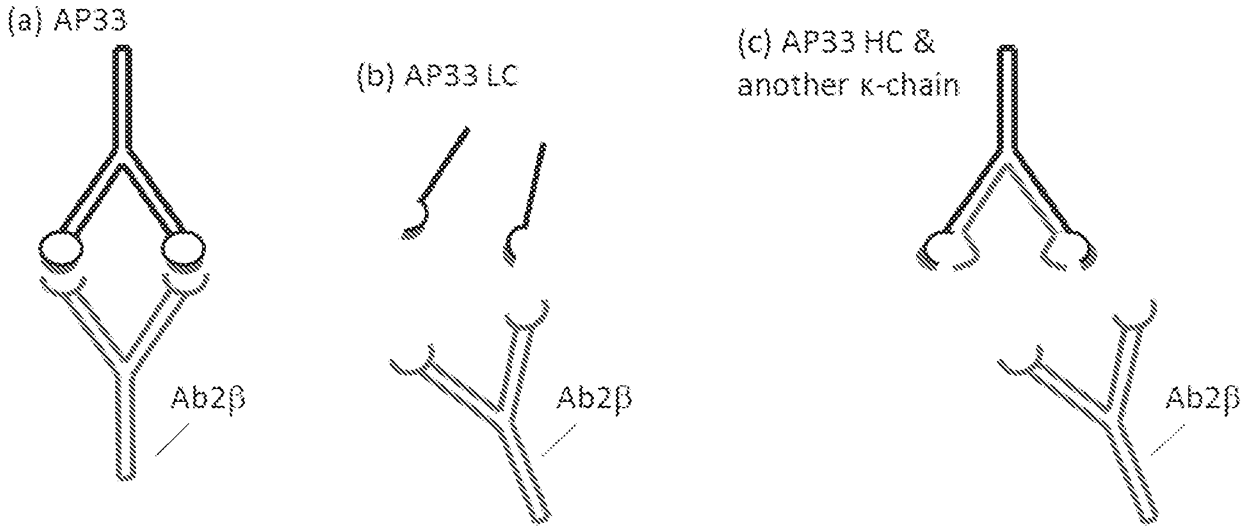

FIGURE 5

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GCCAGCCCCCTGATGGGGGCGACACTCCACCATGAATCACTCCCCTGTGAGGAAC   55

TACTGTCTTCACGCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAG  110

CCTCCAGGACCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTA  165

CACCGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATAAACCCGCTCAATGCCT  220

GGAGATTTGGGCGTGCCCCCGCAAGACTGCTAGCCGAGTAGTGTTGGGTCGCGAA  275

AGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCCCCGGGAGGTCTCGTA  330
                 ┌──► Core
                 M   S   T   N   P   K   P   Q   R   K   T    11
GACCGTGCACC ATG AGC ACG AAT CCT AAA CCT CAA AGA AAA ACC   374

 K   R   N   T   N   R   R   P   Q   D   V   K   F   P    25
AAA CGT AAC ACC AAC CGT CGC CCA CAG GAC GTC AAG TTC CCG   416

 G   G   G   Q   I   V   G   G   V   Y   L   L   P   R    39
GGT GGC GGT CAG ATC GTT GGT GGA GTT TAC TTG TTG CCG CGC   458

 R   G   P   R   L   G   V   R   A   T   R   K   T   S    53
AGG GGC CCT AGA TTG GGT GTG CGC GCG ACG AGG AAG ACT TCC   500

 E   R   S   Q   P   R   G   R   R   Q   P   I   P   K    67
GAG CGG TCG CAA CCT CGA GGT AGA CGT CAG CCT ATC CCC AAG   542

 A   R   R   P   E   G   R   T   W   A   Q   P   G   Y    81
GCA CGT CGG CCC GAG GGC AGG ACC TGG GCT CAG CCC GGG TAC   584

 P   W   P   L   Y   G   N   E   G   C   G   W   A   G    95
CCT TGG CCC CTC TAT GGC AAT GAG GGT TGC GGG TGG GCG GGA   626

 W   L   L   S   P   R   G   S   R   P   S   W   G   P   109
TGG CTC CTG TCT CCC CGT GGC TCT CGG CCT AGC TGG GGC CCC   668

 T   D   P   R   R   R   S   R   N   L   G   K   V   I   123
ACA GAC CCC CGG CGT AGG TCG CGC AAT TTG GGT AAG GTC ATC   710

 D   T   L   T   C   G   F   A   D   L   M   G   Y   I   137
GAT ACC CTT ACG TGC GGC TTC GCC GAC CTC ATG GGG TAC ATA   752

 P   L   V   G   A   P   L   G   G   A   A   R   A   L   151
CCG CTC GTC GGC GCC CCT CTT GGA GGC GCT GCC AGG GCC CTG   794

 A   H   G   V   R   V   L   E   D   G   V   N   Y   A   165
GCG CAT GGC GTC CGG GTT CTG GAA GAC GGC GTG AAC TAT GCA   836

 T   G   N   L   P   G   C   S   F   S   I   F   L   L   179
ACA GGG AAC CTT CCT GGT TGC TCT TTC TCT ATC TTC CTT CTG   878
                                                 ┌──► E1
 A   L   L   S   C   L   T   V   P   A   S   A   Y   Q   193
GCC CTG CTC TCT TGC CTG ACT GTG CCC GCT TCA GCC TAC CAA   920
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 V   R   N   S   S   G   L   Y   H   V   T   N   D   C   207
GTG CGC AAT TCC TCG GGG CTT TAC CAT GTC ACC AAT GAT TGC  962

 P   N   S   S   I   V   Y   E   A   A   D   A   I   L   221
CCT AAC TCG AGT ATT GTG TAC GAG GCG GCC GAT GCC ATC CTG 1004

 H   T   P   G   C   V   P   C   V   R   E   G   N   A   235
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 S   R   C   W   V   A   V   T   P   T   V   A   T   R   249
TCG AGG TGT TGG GTG GCG GTG ACC CCC ACG GTG GCC ACC AGG 1088

 D   G   K   L   P   T   T   Q   L   R   R   H   I   D   263
GAC GGC AAA CTC CCC ACA ACG CAG CTT CGA CGT CAT ATC GAT 1130

 L   L   V   G   S   A   T   L   C   S   A   L   Y   V   277
CTG CTT GTC GGG AGC GCC ACC CTC TGC TCG GCC CTC TAC GTG 1172

 G   D   L   C   G   S   V   F   L   V   G   Q   L   F   291
GGG GAC CTG TGC GGG TCT GTC TTT CTT GTT GGT CAA CTG TTT 1214

 T   F   S   P   R   R   H   W   T   T   Q   D   C   N   305
ACC TTC TCT CCC AGG CGC CAC TGG ACG ACG CAA GAC TGC AAT 1256

 C   S   I   Y   P   G   H   I   T   G   H   R   M   A   319
TGT TCT ATC TAT CCC GGC CAT ATA ACG GGT CAT CGC ATG GCA 1298

 W   D   M   M   M   N   W   S   P   T   A   A   L   V   333
TGG GAT ATG ATG ATG AAC TGG TCC CCT ACG GCA GCG TTG GTG 1340

 V   A   Q   L   L   R   I   P   Q   A   I   M   D   M   347
GTA GCT CAG CTG CTC CGG ATC CCA CAA GCC ATC ATG GAC ATG 1382

 I   A   G   A   H   W   G   V   L   A   G   I   A   Y   361
ATC GCT GGT GCT CAC TGG GGA GTC CTG GCG GGC ATA GCG TAT 1424

 F   S   M   V   G   N   W   A   K   V   L   V   V   L   375
TTC TCC ATG GTG GGG AAC TGG GCG AAG GTC CTG GTA GTG CTG 1466
                                  E2
 L   L   F   A   G   V   D   A   E   T   H   V   T   G   389
CTG CTA TTT GCC GGC GTC GAC GCG GAA ACC CAC GTC ACC GGG 1508

 G   N   A   G   R   T   T   A   G   L   V   G   L   L   403
GGA AAT GCC GGC CGC ACC ACG GCT GGG CTT GTT GGT CTC CTT 1550

 T   P   G   A   K   Q   N   I   Q   L   I   N   T   N   417
ACA CCA GGC GCC AAG CAG AAC ATC CAA CTG ATC AAC ACC AAC 1592

 G   S   W   H   I   N   S   T   A   L   N   C   N   E   431
GGC AGT TGG CAC ATC AAT AGC ACG GCC TTG AAT TGC AAT GAA 1634

 S   L   N   T   G   W   L   A   G   L   F   Y   Q   H   445
AGC CTT AAC ACC GGC TGG TTA GCA GGG CTC TTC TAT CAA CAC 1676

 K   F   N   S   S   G   C   P   E   R   L   A   S   C   459
```

AAA	TTC	AAC	TCT	TCA	GGC	TGT	CCT	GAG	AGG	TTG	GCC	AGC	TGC	1718
R	R	L	T	D	F	A	Q	G	W	G	P	I	S	473
CGA	CGC	CTT	ACC	GAT	TTT	GCC	CAG	GGC	TGG	GGT	CCT	ATC	AGT	1760
Y	A	N	G	S	G	L	D	E	R	P	Y	C	W	487
TAT	GCC	AAC	GGA	AGC	GGC	CTC	GAC	GAA	CGC	CCC	TAC	TGC	TGG	1802
H	Y	P	P	R	P	C	G	I	V	P	A	K	S	501
CAC	TAC	CCT	CCA	AGA	CCT	TGT	GGC	ATT	GTG	CCC	GCA	AAG	AGC	1844
V	C	G	P	V	Y	C	F	T	P	S	P	V	V	515
GTG	TGT	GGC	CCG	GTA	TAT	TGC	TTC	ACT	CCC	AGC	CCC	GTG	GTG	1886
V	G	T	T	D	R	S	G	A	P	T	Y	S	W	529
GTG	GGA	ACG	ACC	GAC	AGG	TCG	GGC	GCG	CCT	ACC	TAC	AGC	TGG	1928
G	A	N	D	T	D	V	F	V	L	N	N	T	R	543
GGT	GCA	AAT	GAT	ACG	GAT	GTC	TTC	GTC	CTT	AAC	AAC	ACC	AGG	1970
P	P	L	G	N	W	F	G	C	T	W	M	N	S	557
CCA	CCG	CTG	GGC	AAT	TGG	TTC	GGT	TGT	ACC	TGG	ATG	AAC	TCA	2012
T	G	F	T	K	V	C	G	A	P	P	C	V	I	571
ACT	GGA	TTC	ACC	AAA	GTG	TGC	GGA	GCG	CCC	CCT	TGT	GTC	ATC	2054
G	G	V	G	N	N	T	L	L	C	P	T	D	C	585
GGA	GGG	GTG	GGC	AAC	AAC	ACC	TTG	CTC	TGC	CCC	ACT	GAT	TGC	2096
F	R	K	H	P	E	A	T	Y	S	R	C	G	S	599
TTC	CGC	AAA	CAT	CCG	GAA	GCC	ACA	TAC	TCT	CGG	TGC	GGC	TCC	2138
G	P	W	I	T	P	R	C	M	V	D	Y	P	Y	613
GGT	CCC	TGG	ATT	ACA	CCC	AGG	TGC	ATG	GTC	GAC	TAC	CCG	TAT	2180
R	L	W	H	Y	P	C	T	I	N	Y	T	I	F	627
AGG	CTT	TGG	CAC	TAT	CCT	TGT	ACC	ATC	AAT	TAC	ACC	ATA	TTC	2222
K	V	R	M	Y	V	G	G	V	E	H	R	L	E	641
AAA	GTC	AGG	ATG	TAC	GTG	GGA	GGG	GTC	GAG	CAC	AGG	CTG	GAA	2264
A	A	C	N	W	T	R	G	E	R	C	D	L	E	655
GCG	GCC	TGC	AAC	TGG	ACG	CGG	GGC	GAA	CGC	TGT	GAT	CTG	GAA	2306
D	R	D	R	S	E	L	S	P	L	L	L	S	T	669
GAC	AGG	GAC	AGG	TCC	GAG	CTC	AGC	CCG	TTG	CTG	CTG	TCC	ACC	2348
T	Q	W	Q	V	L	P	C	S	F	T	T	L	P	683
ACA	CAG	TGG	CAG	GTC	CTT	CCG	TGT	TCT	TTC	ACG	ACC	CTG	CCA	2390
A	L	S	T	G	L	I	H	L	H	Q	N	I	V	697
GCC	TTG	TCC	ACC	GGC	CTC	ATC	CAC	CTC	CAC	CAG	AAC	ATT	GTG	2432
D	V	Q	Y	L	Y	G	V	G	S	S	I	A	S	711
GAC	GTG	CAG	TAC	TTG	TAC	GGG	GTA	GGG	TCA	AGC	ATC	GCG	TCC	2474

```
  W   A   I   K   W   E   Y   V   V   L   L   F   L   L   725
 TGG GCC ATT AAG TGG GAG TAC GTC GTT CTC CTG TTC CTT CTG 2516

  L   A   D   A   R   V   C   S   C   L   W   M   M   L   739
 CTT GCA GAC GCG CGC GTC TGC TCC TGC TTG TGG ATG ATG TTA 2558
                                 ⌐→ p7
  L   I   S   Q   A   E   A   A   L   E   N   L   V   I   753
 CTC ATA TCC CAA GCG GAG GCG GCT TTG GAG AAC CTC GTA ATA 2600
```

FIGURE 6

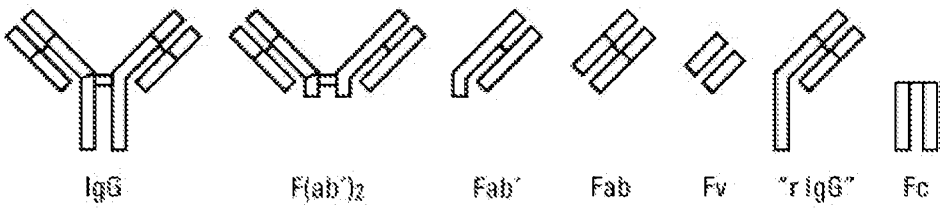

FIGURE 7

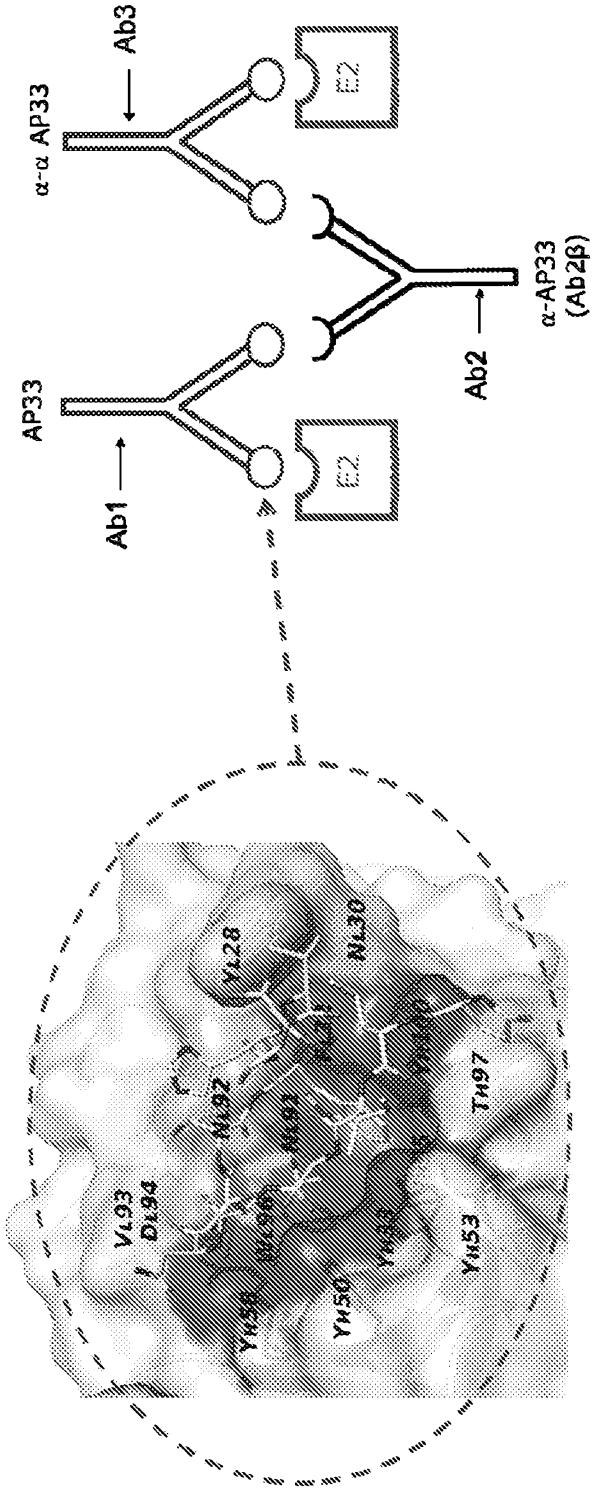
Ab3
α-α AP33
E2
α-AP33
(Ab2β)
Ab2
AP33
Ab1
E2
VL28
NL30
NL92
AL91
TH97
VL93
DL94
YH58
YH50
YH33
YH53

FIGURE 8

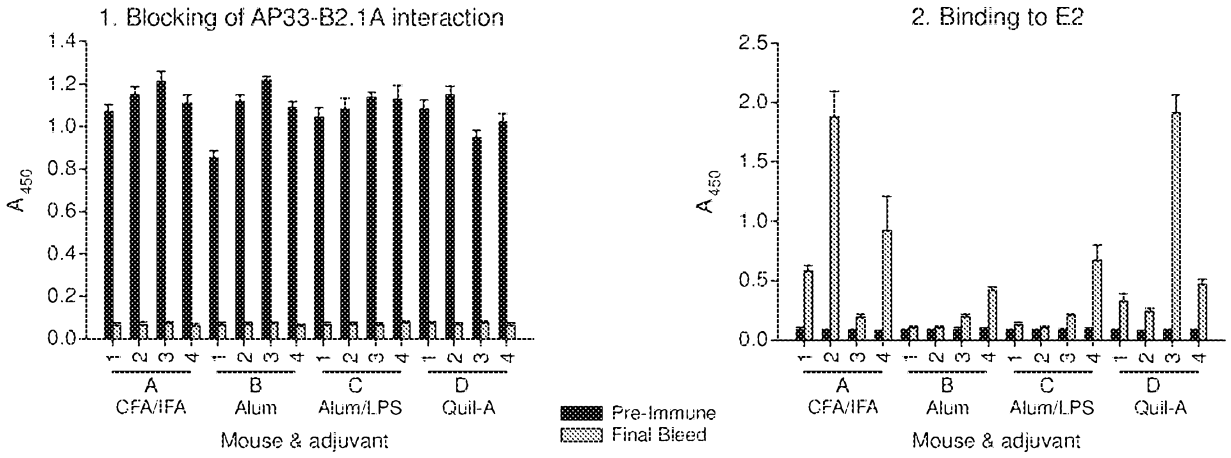

FIGURE 9

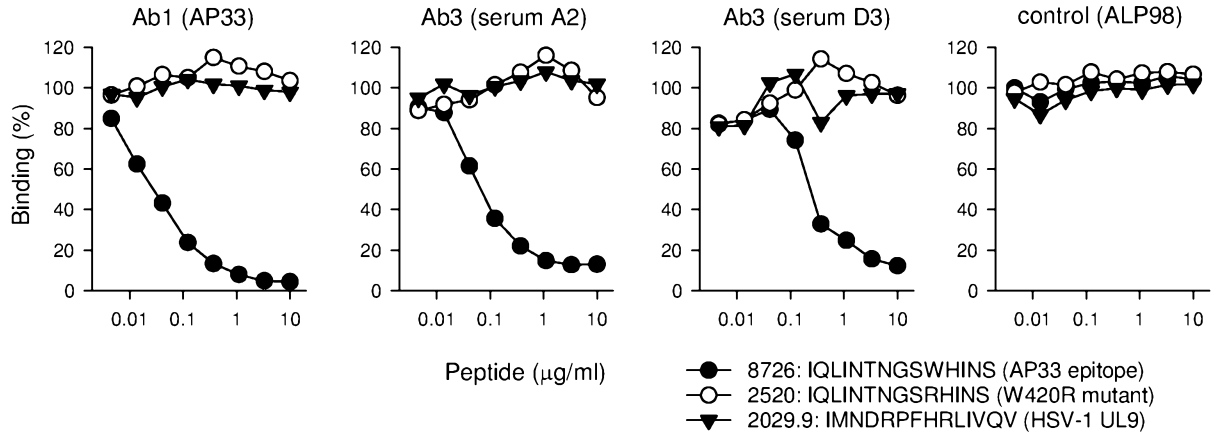

FIGURE 10

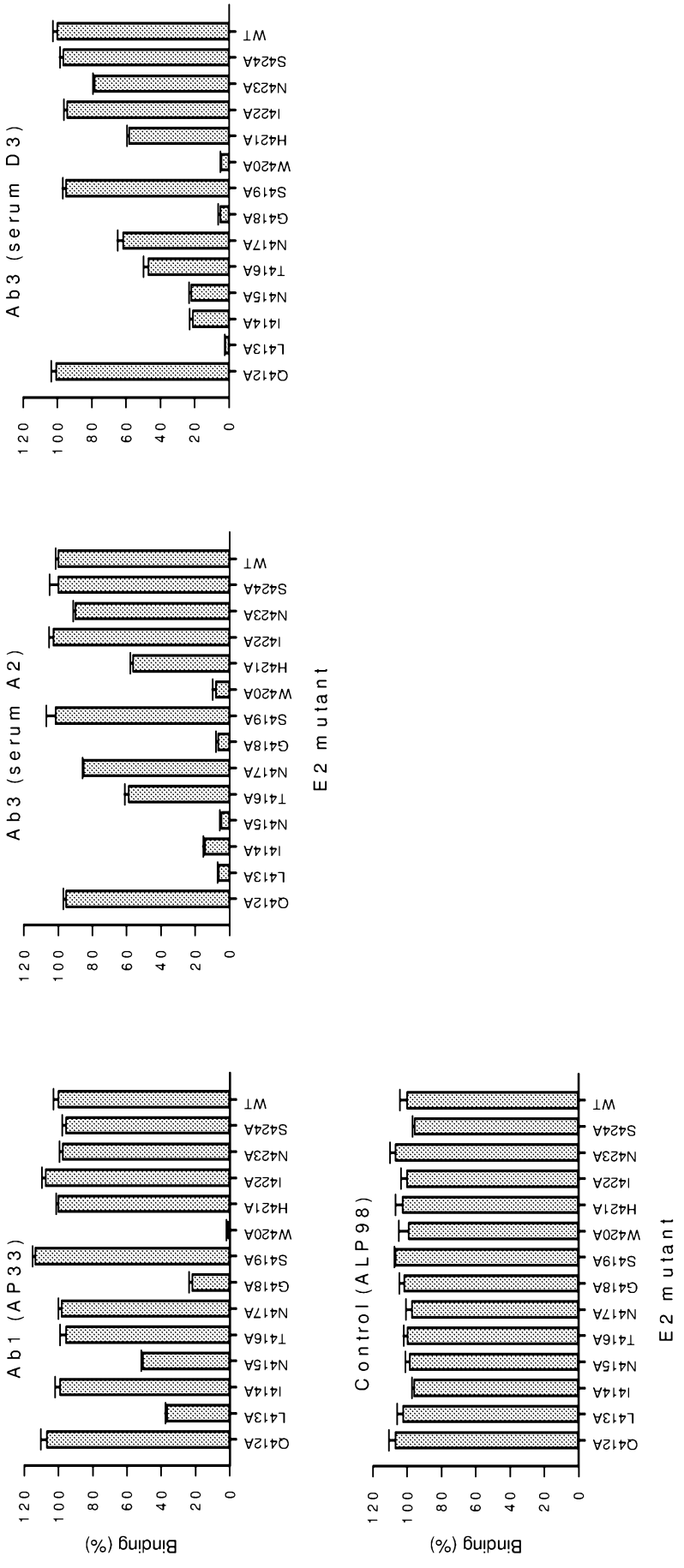
Ab1 (AP33)
Ab3 (serum A2)
Ab3 (serum D3)
Control (ALP98)
Binding (%)
E2 mutant
Q412A
L413A
I414A
N415A
T416A
N417A
G418A
S419A
W420A
H421A
I422A
N423A
S424A
WT
0
20
40
60
80
100
120

FIGURE 11

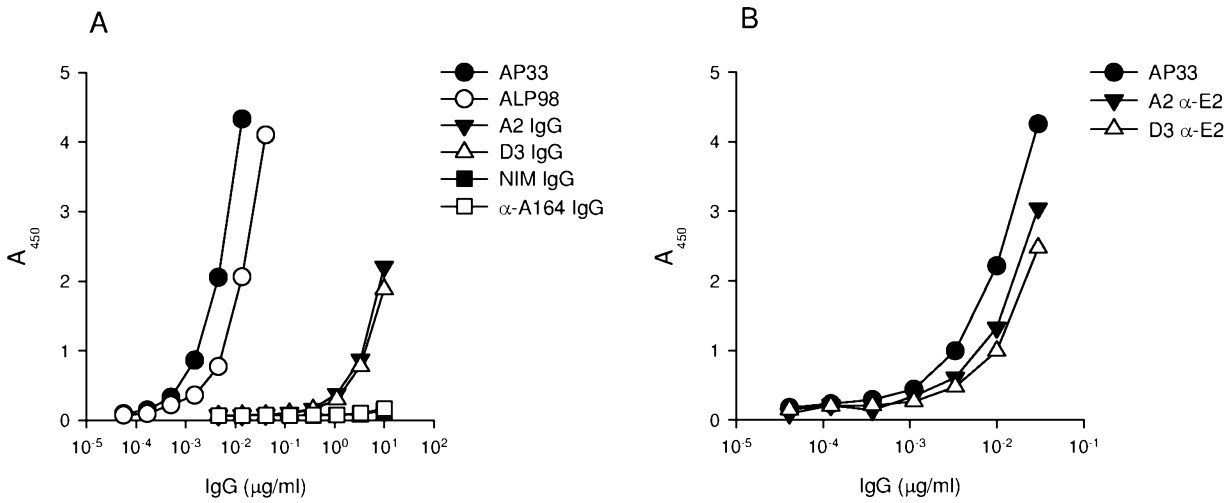

FIGURE 12

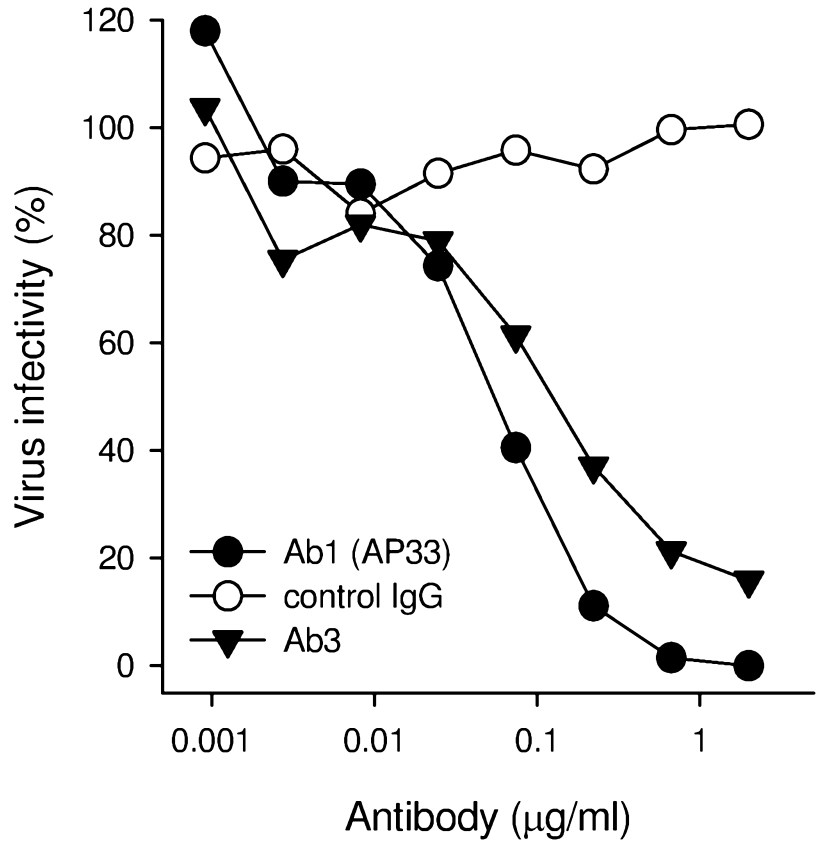

FIGURE 13

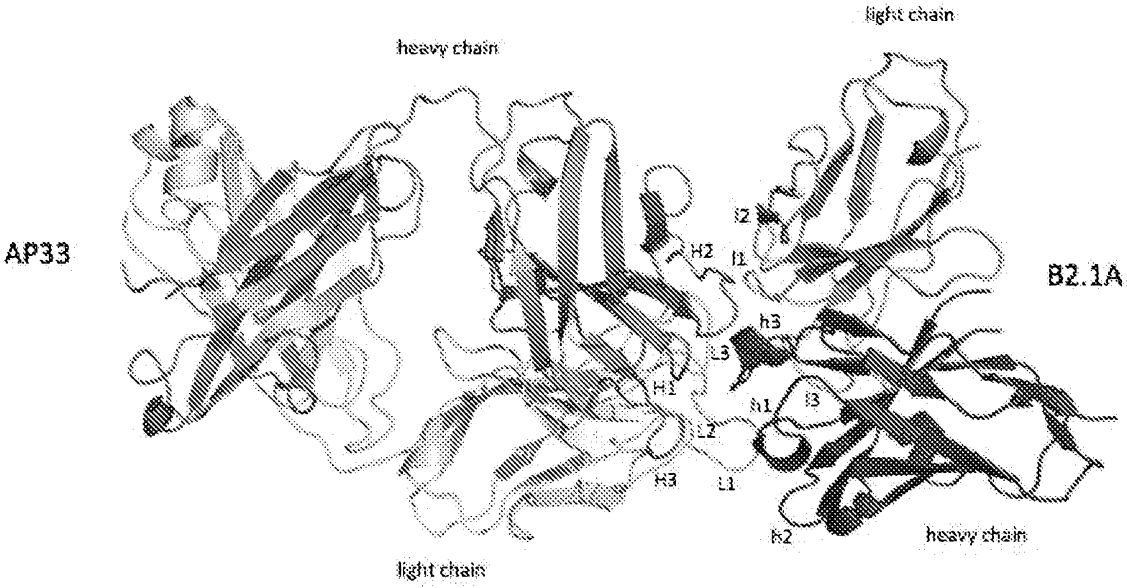

FIGURE 14

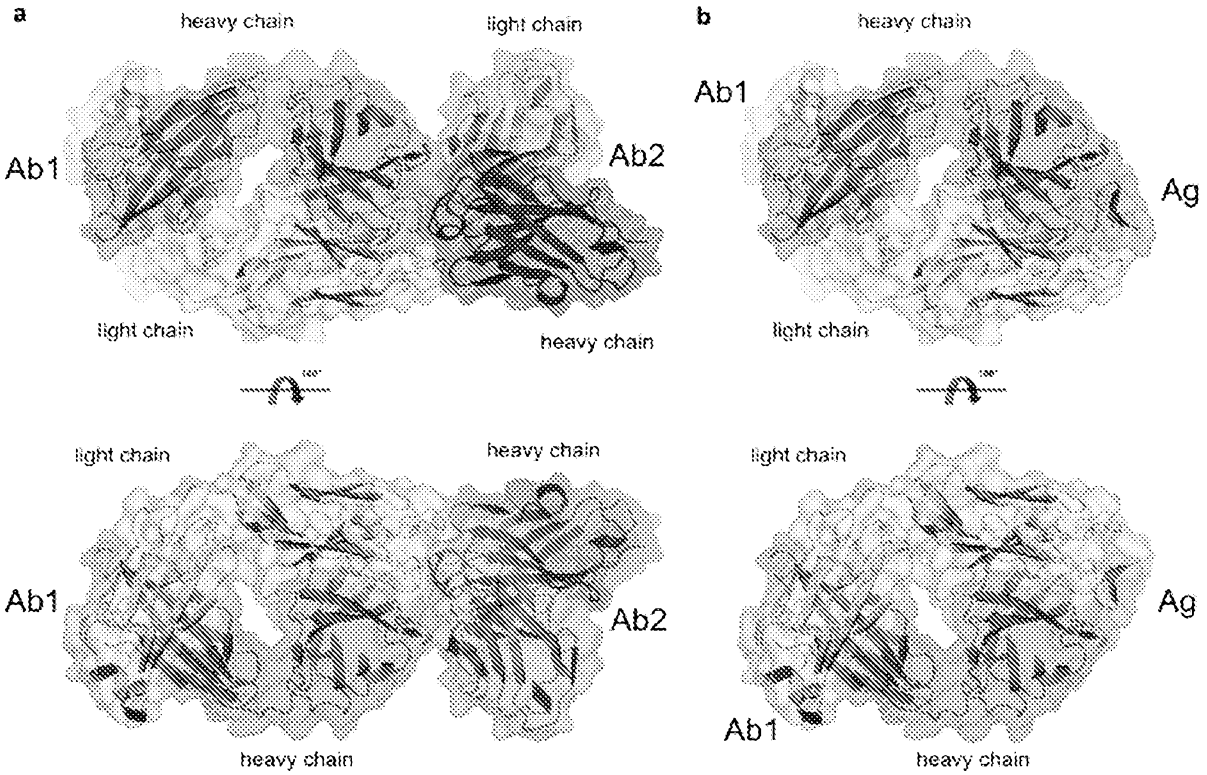

FIGURE 15

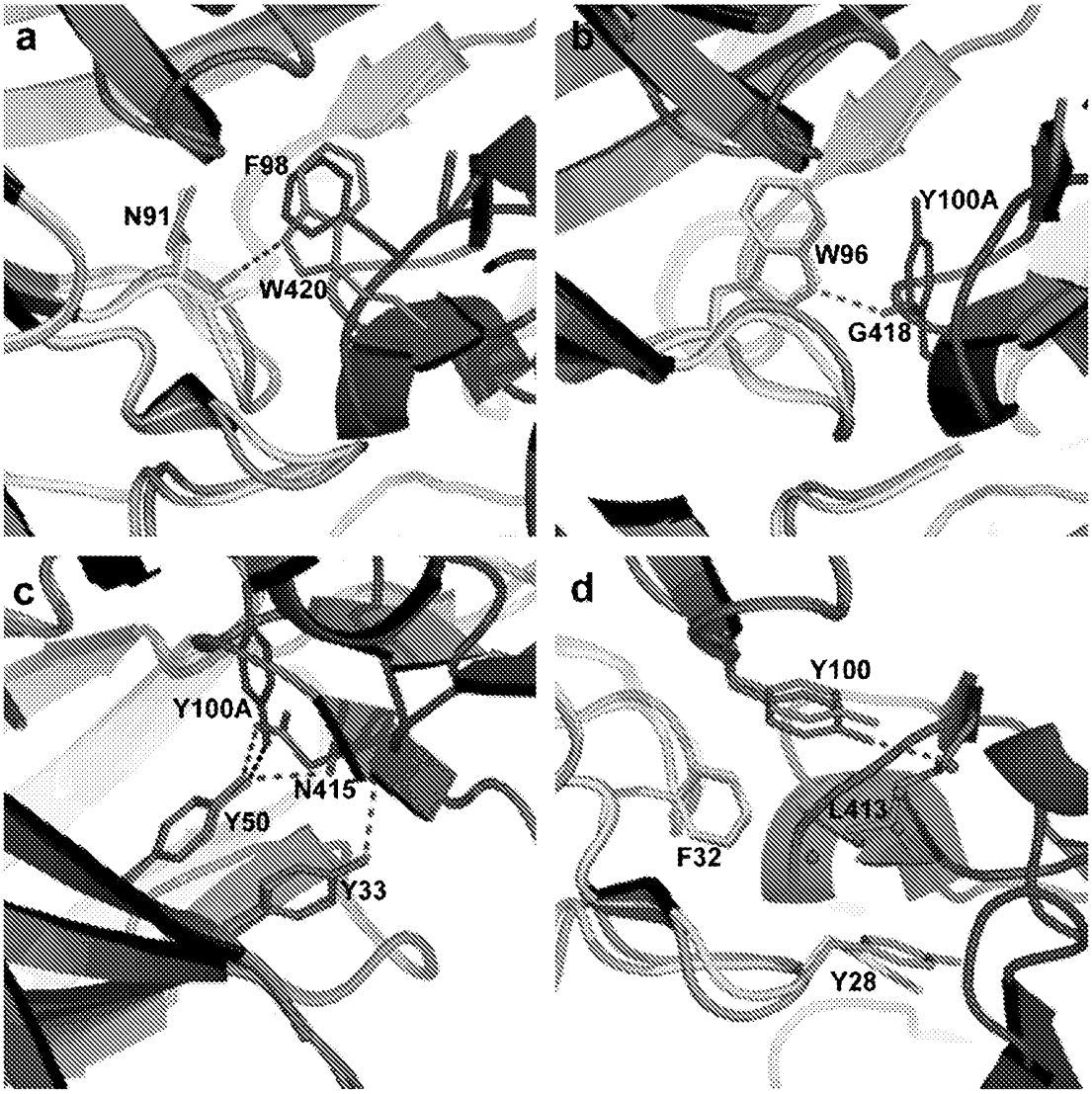

FIGURE 16

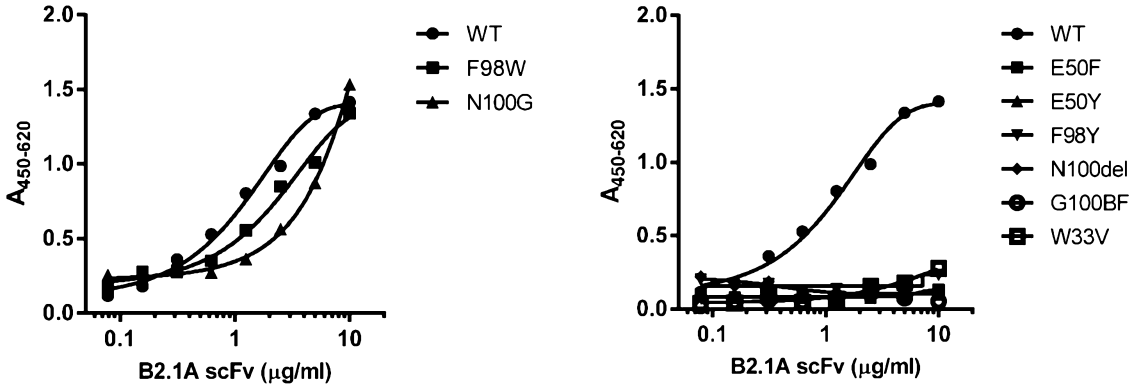

FIGURE 17

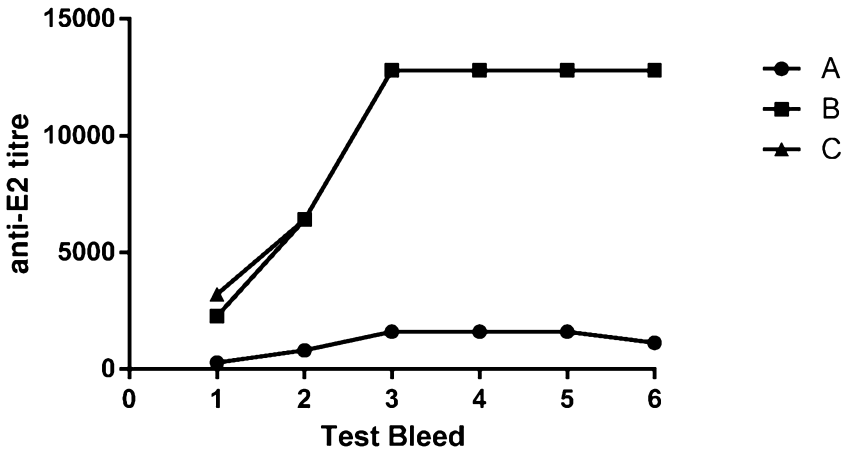

FIGURE 18