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(71) Applicants: **MONASH UNIVERSITY** [AU/AU]; Wellington Road, Clayton, Victoria 3800 (AU). **THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH** [AU/AU]; 1G Royal Parade, Parkville, Victoria 3052 (AU).

(72) Inventors: **NETTER, Hans**; 4/1 Donald Street, Clayton, Victoria 3168 (AU). **CHEONG, Wan-Shoo**; 135 Jalan Tembaga, Island Park, Penang, 11600 (MY). **KINGSTON, Natalie**; 1 Blenham Place, Frankston, Victoria 3199 (AU). **CAMINSCHI, Irina**; 35 Munro Street, Ascot Vale, Victoria 3032 (AU). **LAHOUD, Mireille**; 44/400 Victoria Parade, East Melbourne, Victoria 3002 (AU).

(74) Agent: **FB RICE**; Level 14, 90 Collins Street, Melbourne, Victoria 3000 (AU).

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(57) Abstract: A recombinant VLP-antibody (VLP-Ab) comprising a modified hepadnaviral envelope fusion protein comprising an antibody variable domain. The variable domain may be selected from an antibody heavy chain variable domain and a light chain variable domain. In some embodiments, the variable domain (the first variable domain) is bound to a complementary antibody variable domain of a second variable domain protein and forms the antibody antigen binding site specific for a target molecule. One second variable domain protein may be a modified hepadnaviral envelope fusion protein comprising a complementary antibody variable domain. The VLP-Ab may comprises a modified hepadnaviral envelope fusion protein comprising a heterologous antigen. One modified hepadnaviral envelope fusion protein is a hepatitis B envelope protein or a VLP-forming part or variant thereof. A eukaryotic expression vector comprising a recombinant nucleic acid molecule encoding a hepadnavirus envelope-antibody fusion protein comprising an antibody variable domain wherein the variable domain is selected from an antibody heavy chain variable domain and a light chain variable domain. A eukaryotic expression vector comprising a recombinant nucleic acid molecule encoding a second variable domain protein comprising an antibody variable domain wherein the variable domain is selected from an antibody heavy chain variable domain and a light chain variable domain. The expression vector may encode a single variable domain selected from an antibody heavy chain variable domain and a light chain variable domain. The expression vector may encode an Sc Fv. A eukaryotic expression vector operably linked to a polynucleotide encoding a hepadnavirus envelope-antibody fusion protein comprising and an antibody variable domain (EAFP) wherein the antibody variable domain is selected from an antibody heavy chain variable domain and a light chain variable domain, and further capable of expressing a second polynucleotide encoding a second variable domain protein comprising a complementary antibody variable domain. An isolated eukaryotic cell comprising the expression vector. Pharmaceutical and diagnostic compositions are provided and a wide range of therapeutic or prophylactic uses and methods. The manufacture of a medicament for, the treatment or prevention of an infection of a subject by a pathogen, or an inflammatory, immunity or cancer-related condition in a subject. A method for preparing a VLP-Ab, the method comprising transfecting a eukaryotic cell with an expression vector as described, culturing the cell in vitro for a time and under conditions sufficient to permit VLP-Ab formation and optionally secretion, and at least partly purifying the VLP-Ab from the cell culture material.

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BIOLOGICAL MOLECULES AND METHODS OF USE

FIELD

[0001] The specification describes recombinant viral-like particles (VLP) comprising surface oriented antibodies, and pertains to the fields of molecular medicine, virology and protein including antibody expression. In one illustrative aspect, recombinant hepadnaviral VLPs are modified to comprise antibodies suitable for delivering the VLP-antibody to target cells in a subject in order to thereby facilitate the development of an enhanced immune response. VLPs antibodies may comprise single or multiple antibodies of the same or different specificity. In another illustrative aspect, VLPs presenting functional antigen binding antibodies are proposed for use in passive immunization protocols. Accordingly, the fields of immunology, vaccines and drug delivery are also relevant. The VLP-antibody technology provides an advantageous platform for the delivery of hepadnaviral or heterologous antigens, such as cancer antigens or infectious disease antigens, and further a platform for the delivery of antibodies, to target sites *in situ.* The subject VLP-antibody molecules, and vectors encoding all or part thereof, will have a broad range of applications *inter alia* in treatment and prophylactic protocols, screening assays, diagnostics and medical imaging.

BACKGROUND

[0002] Bibliographic details of references in the subject specification are also listed at the end of the specification.

[0003] Reference to any prior art in this specification is not, and should not be taken as, acknowledgement or any form of suggestion that this prior art forms part of the common general knowledge in any country.

[0004] The use of antibodies in medical therapy and imaging appears to be gaining momentum although their manufacture can be costly and there are problems associated with expression levels, delivery and stability *in vivo.* Particularly useful is the recombinant production of antibodies using a variety of expression hosts. There remains a need for improved methods of producing and delivering biological molecules.

[0005] Another area of interest is the use of VLPs as vaccines. VLPs are structures that retain some of the structural organisation and conformation of native viruses, but lack the viral genome, potentially providing more effective but safe vaccine antigens.

[0006] The present specification describes the production and use of recombinant virus-like particles derived from envelope proteins from eukaryotic viruses. The envelope proteins of hepadnaviruses, such as hepatitis B virus (HBV), are of particular interest as they form highly stable VLPs.

[0007] The infectious HBV virion is a spherical particle 42 nm in diameter consisting of an icosahedral nucleocapsid in which the viral DNA genome is packaged, and a lipoprotein envelope containing three related transmembrane proteins (HBsAg) referred to as HBsAg-large (HBsAgL), HBsAg-middle (HBsAgM) and HBsAg-small (HBsAgS). The synthesis of the envelope proteins is initiated at three different in-frame translation start sites. Consequently, the envelope proteins have a shared region known as the S-domain. HBsAgS is composed only of the S-domain consisting of 226 amino acids (aa); HBsAgM contains an additional N-terminal extensions, the 55 amino acid preS2 domain; and HBsAgL contains the preS2 domain and an additional N-terminal extensions called the preS1 domain.

[0008] The ability of HBsAgS to self-assemble in the presence of lipid at the endoplasmic reticulum (ER) results in the formation of secretion competent subviral particles (VLPs), which do not contain any other HBV viral component. HBsAgS VLPs are 22-25 nanometer (nm) in diameter, highly compact, and it is estimated that one particle contains approximately one hundred HBsAgS molecules. HBsAgM also forms secretion competent VLPs. HBsAgL also forms VLPs which may not be fully secretion competent.

[0009] HBsAg particle formation is an elaborate process. The first step in the particle formation is the cotranslational insertion of the protein into the endoplasmic reticulum (ER) membrane with a short luminal exposed N-terminal sequence, two transmembrane regions separated by a 57aa cytosolic loop, and a luminal external 70 aa domain containing the major B-cell epitopes ('a'-determinant). HBsAgS VLPs represent a highly compact structure due to the large number of intra- and intermolecular disulfide bonds within and between the individual subunits.

[0010] Chimeric VLPs based on the capsid proteins of e.g. HBV, human papilloma virus (HPV), as well as Qβ phage have been engineered to express foreign antigenic sequences

including non-pathogen associated antigens such as nicotine and angiotensin II for smoking cessation and to overcome hypertension, respectively (Ambuhl *et al.* (2007) *J. Hypertens.* 25:63-72; Buonaguro *et al.* (2011) *Exp. Rev.* 10:1569-83; Cornuz *et al.* (2008) *PlosOne* 3:e2547). In contrast to capsid VLPs, which are composed of protein subunits only, HBV based HBsAgS VLPs are composed of envelope proteins and lipid, the ER being the cellular location for assembly. For the presentation of antigenic sequences to the immune system, HBsAgS VLPs have been modified to carry foreign epitopes (Delpeyroux *et al.* (1986) *Science* 233:472-5; Eckhart *et al.* (1996) *J. Gen. Virol.* 77:2001-8; Fomsgaard *et al.* (1998) *Scand. J. Immunol.* 47:289-95; Phogat *et al.* (2008) *Virology* 373:72-84; Netter *et al.* (2001) *J. Virol.* 75:2130-2141). VLPs composed of HBsAgL were developed as a delivery system for genes and drugs to human hepatocytes (Yamada *et al.* (2003) *Nature Biotech.* 21:885-90). Duck and other hepadnaviral envelope proteins have been modified to express antigens of interest as part of VLPs.

[0011] HBsAgS VLPs are the antigenic components of a successful preventative vaccine for Hepatitis B virus (HBV) infection (Jilig *et al.* (1984) *Lancet* 2(8413):1174-5, and Zuckerman J. (2006) *Med. Virol.* 78:169-77). Clinical trials using HBsAgS VLPs to vector malarial antigens have shown that HBsAgS VLPs are a highly successful antigen delivery system (Regules *et al.* (2011) *Exp. Rev. Vaccines* 10:589-99).

[0012] Many vaccine compositions, including VLP-based vaccines such as RTS,S (Regules *et al.* (2011) *Exp. Rev. Vaccines* 10:589-99) include adjuvants, such as alum (aluminium salts) MLP (3-deacetyl monophosphoryl lipid A), oil in water emulsion, QS21 (bark of South American *Quillaja Saponara* tree), CpG oligonucleotides and liposomes formulations. Their purpose is to enhance and prolong immune stimulation *via*, *inter alia*, attraction and maturation of antigen presenting cells at the site of vaccination, migration of APC to draining lymph nodes for effective presentation of antigen T-cells, and priming of an effective adaptive immunity, promoting inflammation, and conversion of antigen to multivalent form.

SUMMARY

[0013] The articles "a" and "an" are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0014] Throughout this specification, unless the context requires otherwise, the words "comprise", "comprises" and "comprising" are understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements. Thus, use of the term "comprising" and the like indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present. By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of". Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present. By "consisting essentially of" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

[0015] Nucleotide and amino acid sequences are referred to by a sequence identifier number (SEQ ID NO:). The SEQ ID NOs: correspond numerically to the sequence identifiers <400>1 (SEQ ID NO:1), <400>2 (SEQ ID NO:2), etc. A summary of sequence identifiers is provided in Table A. A sequence listing is provided after the claims.

[0016] In one embodiment, the present description enables a recombinant VLP-antibody (VLP-Ab) comprising a modified hepadnaviral envelope fusion protein comprising an antibody variable domain (EAFP) wherein the variable domain is selected from an antibody heavy chain variable domain and a light chain variable domain, and wherein the variable domain is bound to a complementary antibody variable domain of a second variable domain protein and forms the antibody antigen binding site specific for a target molecule. The non-limiting examples provided herein relate to the small hepadnaviral envelope protein from hepatitis B virus. VLPs formed from HBsAgS may comprise approximately one hundred HBsAgS subunits. Accordingly, the HBsAgS-based VLPs may comprise a range of

antibodies with different specificities and multiple copies of the same antibody. In some embodiments, therefore, the VLP-Ab comprises more than one functional antibody and optionally between about 5 and about 50 functional antibodies of the same specificity or including antibodies of different specificities.

[0017] In some embodiments, the second variable domain protein is a modified hepadnaviral envelope fusion protein comprising a complementary antibody variable domain (a complementary EAFP). The term "Complementary" refers to antibody light and heavy variable domains forming complementary pairs and thus an antigen-binding site.

[0018] In other embodiments, the second variable domain protein comprising a complementary antibody variable domain, is not a modified hepadnaviral envelope fusion protein. Thus, the complementary antibody variable domain does not require the envelope protein for inclusion in the VLP although it is present in some embodiments.

[0019] Variable domains may be derived from antibodies directed against target antigens receptors or ligands. They may be derived from any source including screening antibody libraries using standard procedures. Antibodies contemplated herein include human antibodies and non-human antibodies. Non-human antibodies may be modified for use in human using known technologies, to be less likely to engender an immune response. Strategies for modifying antibodies include humanisation, superhumanisation, resurfacing and human string content optimisation, as known to the skilled artisan.

[0020] In an illustrative embodiment, the EAFP and/or second variable domain protein comprises an antibody constant domain (such as a VC_H or VC_L domain).

[0021] In other embodiments, the EAFP and/or the second variable domain protein comprise a single antibody variable domain.

[0022] In another aspect, the EAFP comprises a single chain antibody such as an SvFv or a derivative therof.

[0023] The VLP-Ab optionally comprises a domain (tag) suitable for detection of the VLP-Ab.

[0024] In some embodiments, the VLP-Ab or EAFP comprises a heterologous antigen. By "heterologous antigen" is meant that the antigen is not derived from a hepadnaviral envelope protein. In some embodiments, the heterologous antigen is a disease or pathogen

antigen such as a cancer antigen or an infectious disease antigen. In other embodiments the antigen may be any moiety against which an immune response is sought. In some embodiments, the VLP-Ab comprises a modified hepadnaviral envelope fusion protein comprising a heterologous antigen. Thus, in some embodiments the VLP-Ab comprises an EAFP, a second variable domain protein and a modified envelope fusion protein comprising a heterologous antigen. A VLP-Ab comprising an EAFP, and a complementary second variable domain protein, and a modified hepadnavirus envelope fusion protein comprising a foreign (heterologous) antigen, the HIV-1 membrane proximal external region (MPER) sequence is illustrated in Figure 14. The lipid component of the hepadnaviral VLPs are proposed to enhance immunogenicity of decorated antigens that are normally expressed (in nature) on the cell surface.

[0025] In some embodiments, the heterologous antigen is inserted into the "a' determinant of HBsAgS. The heterologous antigen may be introduced internally within the hepadnaviral envelope protein or it may be introduced N-terminally or C-terminally to the envelope protein.

[0026] The modified hepadnaviral envelope fusion protein may be a hepatitis B envelope protein selected from HBsAgL, HBsAgM and HBsAgS or a VLP-forming part or variant thereof. As described in the examples, the presence of wild-type or substantially unmodified HBsAg can enhance VLP secretion in some embodiments.

[0027] In other embodiments, the modified hepadnaviral envelope fusion protein is hyper- or hypo- glycosylated. In one particular embodiment, the HBV envelope protein comprises a deleted glycosylation site at N146, i.e., the S protein comprises an amino acid other than asparagine at position 146 (see SEQ ID NO: 6 starting with MENIT).

[0028] Hepadnaviral envelope based VLP-Abs may comprise two or three or more different modified hepadnaviral envelope fusion proteins comprising envelope protein epitopes from two or more hepadnaviral, such as hepatitis B virus, serotypes. This would be particularly useful when the VLP-Ab is used as an HBV vaccine. This illustrates the capacity of the VLP-Ab to carry a large number of different antibodies and envelope-antibody fusion proteins.

[0029] The present description is not limited to any particular antibody or antigen. The skilled person will be aware that the illustrative embodiments provide a sound basis for

expecting that many different antibody specificities may be employed in the subject VLP-Abs. In some embodiments, the antibody is specific for a target molecule which is a self or a non-self antigen. In other applications, the antibody specifically binds to a target molecule on a host cell such as an immune competent or antigen presenting cell. In some embodiments, the antibody binds specifically to a C-type lectin expressed on antisense presenting cells and up on antibody binding they promote internalisation of VLP-Ab. Illustrative C-type lectins include without limitation Clec12A, Clec9a, Clec1a, DEC-205 and Dectin 2. In some embodiments, the antibody specifically binds to Clec12a receptor. In other embodiments, the antibody specifically binds to Clec9a receptor. Alternatively, the antibody specifically binds to a target molecule on an infectious agent, such as a virus, such as HIV. In some embodiments, the VLP-Ab comprises antibody antigen binding sites for two or more different antigen specificities.

[0030] As shown in the Examples, the modified hepadnaviral envelope fusion protein may comprise a spacer domain between the antibody variable domain and the envelope protein.

[0031] In some embodiments, the VLP-Ab comprises a packaged hepatitis delta (i.e., HDV or dAgL). Hepatitis delta requires HBV, and is associated with HBV infections.

[0032] In another aspect, the description contemplates an expression vector comprising a recombinant nucleic acid molecule encoding a hepadnavirus envelope-antibody fusion protein comprising an antibody variable domain (EAFP) wherein the variable domain is selected from an antibody heavy chain variable domain and a light chain variable domain. Conveniently, the expression vector comprises a eukaryotic promoter and encodes an ER signal sequence.

[0033] In some embodiments, the expression vector comprises a recombinant nucleic acid molecule encoding a second variable domain protein comprising an antibody variable domain wherein the variable domain is selected from an antibody heavy chain variable domain and a light chain variable domain. Conveniently, the vector comprises a eukaryotic promoter (e.g. mammalian or yeast) and encodes ER signal sequence. In some embodiments the second variable domain protein comprises a constant antibody domain.

[0034] In some embodiments, the vectors encode a single variable domain selected from an antibody heavy chain variable domain and a light chain variable domain.

[0035] In some embodiments, the eukaryotic expression vector is capable of expressing (is operably linked to) a polynucleotide encoding a hepadnavirus envelope-antibody fusion protein comprising an ER signal sequence and an antibody variable domain (EAFP) wherein the variable domain is selected from an antibody heavy chain variable domain and a light chain variable domain, and further capable of expressing a second polynucleotide encoding a second variable domain protein comprising a complementary antibody variable domain.

[0036] In another aspect the description enables an isolated eukaryotic cell comprising one or more of the herein described expression vectors.

[0037] Compositions, including medical and diagnostic compositions are expressly contemplated. In some embodiments, the composition comprises a VLP-Ab as described herein. In other embodiments, the composition comprises a pharmaceutically or physiologically acceptable carrier and/or diluent. Such compositions are for use *inter alia* in medical therapy or imaging. Vaccine compositions, including those directed against more than one pathogen or disease associated antigen are expressly contemplated.

[0038] The description provides for the use of a composition comprising a VLP-Ab as described herein in, or in the manufacture of a medicament for, the treatment or prevention of an infection of a subject by a pathogen, or an inflammatory, immunity or cancer-related condition in a subject.

[0039] The description enables a method for eliciting an immune response against HBV or for treating or preventing an HBV infection in a subject, the method comprising administering to the subject an effective amount of a composition comprising a VLP-Ab as described herein, wherein the modified hepadnaviral envelope fusion protein is a hepatitis B envelope protein is selected from HBsAgL, HBsAgM and HBsAgS or a VLP-forming part or variant thereof. In some embodiments, the target molecule is selected from a Clec9a receptor and/or a Clec12a receptor.

[0040] The description also contemplates methods for treating or preventing an HIV infection in a subject. In some embodiments the method comprises administering to a subject an effective amount of a composition comprising a VLP-Ab as described herein, wherein the modified hepadnaviral envelope fusion protein is optionally a hepatitis B envelope protein or a VLP-forming part or variant thereof, and the target molecule an HIV antigen. In some embodiments, the antibody is an HIV neutralising antibody. The invention is not limited to

HIV, and neutralising antibodies determined by the antigens of any infectious agent may be employed in the present VLP-Abs.

[0041] The description also contemplates a method for eliciting an immune response against an antigen or for treating or preventing a condition or disease or infection in a subject related to the antigen, the method comprising administering to the subject an effective amount of a composition comprising a VLP-Ab as described herein, wherein the modified hepadnaviral envelope fusion protein comprises the antigen, wherein optionally the EAFP is a hepatitis B envelope protein or a VLP-forming part or variant thereof, and the target molecule is on the surface of an immune or antigen presenting cell or in a tissue comprising same. By directing the recombinant VLP-Ab to an antigen presenting cell, the VLP-Ab is internalised by the antigen presenting cell and antigens are presented in the context of MHC class I and MHC class II proteins. It will be appreciated that any hepadnaviral or heterologous antigen or epitope of interest (such as medically or diagnostically) may be introduced into the subject VLP-Abs. By such routes it is proposed that the subject VLP-Abs may be useful in inducing tolerance or inducing effective immune responses in subjects against cancer antigens and chronic infections with infective agents. For example, in this context, decorated HBsAgS VLPs can be directed to immune competent cells, for instance expressing Clec12a receptor. The decorated HBsAgS VLPs contain an additional foreign HIV-1 specific antigenic sequence (inserted within or either end of the HBsAgS domain) facilitating an efficient anti-HIV-1 immune response because of targeting immune competent cells. Decorated VLPs as carriers/platforms for foreign antigenic sequences are illustrated in Figure 13 and 14.

[0042] In other embodiments, the description provides methods for preparing a VLP-Ab comprising providing or transfecting a eukaryotic cell with one or more expression vectors, culturing the cell *in vitro* for a time and under conditions sufficient to permit VLP-Ab formation, and optionally secretion, and at least partly purifying the VLP-Ab from the cell culture material. Typically, light and heavy variable domains are selected which heterodimerize under physiological conditions. VLP-Ab may be purified to homogeneity by methods known in the art.

[0043] As described herein, the inventors have arrayed antigen-binding domains of antibodies on the surface of hepanaviral VLPs. In a further aspect, a further antigen binding molecule of interest is a single chain fused variable (ScFv) antigen binding domain, as known

in the art. As illustrated in Figure 30 and the legend thereto, in this aspect, such VLP-Abs comprising fused variable regions are also encompassed. Here, the combination of hepadnaviral VLP, ScFv and optionally heterologous antigen are contemplated for therapy or diagnosis.

[0044] Each embodiment described herein is to be applied *mutatis mutandis* to each and every other embodiment unless specifically stated otherwise.

[0045] This summary is not an exhaustive recitation of all embodiments of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0046] **Figure 1** is a diagrammatic representation of illustrative components of the herein described VLP-Abs. (A): Small hepatitis B surface wild-type (wt) protein (HBsAgS). (B): HBsAgS protein fused to the γ variable antibody (Ab) domain (γV-S). (C): HBsAgS protein fused to the κ variable Ab domain (κV-S). (D):κ variable and constant (C) light chain Ab domains, not fused to HBsAgS (κVC). (E): κ variable Ab domain fused to myc-tag, not fused to HBsAgS (κVmyc). Illustrative hybrid VLP-Ab combinations - (F) VLPs composed of wtHBsAgs, γV-S, and κV-S and (G) VLPs composed of wtHBsAgS, γV-S and complemented *in trans* with κVC or κVmyc.

[0047] **Figure 2** is a representation of separated protein after immunoprecipitation of 35S-labelled VLPs with anti-HBsAgS antibodies. A: Mock;B: wildtype (wt) HBsAgS; C: wtHBsAgS + κVC; D: γV-HBsAgS (γV-S) fusion + κVC; E: wtHBsAgS + γV-S fusion + κVC; F: wtHBsAgS + κVmyc; G: γV-S fusion + κVmyc; H: wtHBsAgS + γV-S fusion +κVmyc. Asteric: position of κVC chain; Diamond: position of the γV-S un/glycosylated fusion proteins; Arrows: positions of the 24 and 27kD HBsAgS proteins (un/glycosylated).

[0048] **Figure 3** is a representation of separated proteins after ultracentrifugation of cell culture supernatant to pellet VLPs, followed by anti-myc Western blot. Lane 1: Unrelated protein with myc tag. Transfection with plasmids expressing 2: Mock;3: wildtype (wt) HBsAgS; 4: wtHBsAgS + κVmyc;5: γV-S fusion + κVmyc; and6: wtHBsAgS + γV-S fusion + κVmyc; [κVmyc (14kDa) detected].

[0049] **Figure 4** is a representation of separated proteins after immunoprecipitation of 35S-labelled VLPs with anti-HBsAgS antibodies. Lane 1: Mock; Lane 2: HBsAgS(N146Q); Lane 3: γV-S(N146Q); Lane 4: κVmyc; Lane 5: γV-S(N146Q) + κVmyc; Lanes 6-9: γV-S(N146Q) + κVmyc + HBsAgS(N146Q) [increasing amounts of HBsAgS(N146Q), 1:1, 1:3, 1:5, 1:10] (1:1 ratio in this experiment already in plateau)κ/γV-domains derived from anti-Clec12a antibody. Note: single bands at 24kD and 42kD in contrast to Figure 2; all subunits are not glycosylated.

[0050] **Figure 5** is an electron micrographic representation of decorated VLP-Abs. HEK293T cells were transfected with expression plasmids encoding for γV-S(N146Q)*and κVmyc (no plasmid added encoding for wildtype HBsAgS). Cell culture supernatant was

harvested, concentrated via ultracentrifugation, then the pellet separated via a CsCl gradient; fractions with the same density as wtHBsAgS VLPs analysed by electron microscopy (shown are γV-S(N146Q)/κVmyc VLPs). The results show that VLP-Abs can be formed in the absence of wildtype HBsAgS subunits. γV-S (HBsAgS fused to the variable domain from the anti-Clec12a heavy (γ) chain). The average size of the decorated VLP-Abs is larger than wildtype VLPs (22-25nm, composed of wildtype HBsAgS). Please note, size range may vary if VLPs contain fusion HBsAgS (such as γV-S(N146Q)) and in addition wildtype HBsAgS.

[0051] **Figure 6** is a graphical representation of results from an enzyme immunosorbent assay (EIA) using as a target the Clec12a protein. Samples:A: VLPs with wtHBsAgS + γV-S + κV-S. B: VLPs with wtHBsAgS + γV-S + κVC. C: VLPs with wtHBsAgS + γV-S + κVmyc. D: anti-Clec12a antibody. E: VLPs composed of wtHBsAgS. The γV and κVC sequences are derived from the Clec12a antibody. A-E, x-axis: 50ng Clec12a protein was used to coat each well, then incubated with different VLP amounts, as indicated (A,B,C,E), or different dilutions of the Clec12a antibody (D). One set of wells was coated with 1μg bovine serum albumin (BSA).

[0052] **Figure 7** is a representation of flow cytometry data showing binding of wildtype (wt) HBsAgS VLPs or VLPs composed of wtHBsAgS + gV-S fusion + kVmyc (anti-Clec12a VLPs) to CHO cells or CHO cells expressing Clec12a receptor, followed by analysis by flow cytometry. VLPs decorated with anti-Clec12a variable domain bind to CHO cells expressing Clec12a receptor; no binding to CHO cells in the absence of Clec12a receptor. Wildtype VLPs do not bind to CHO cells in the presence or absence of Clec12a receptor.

[0053] **Figure 8** is a graphical representation of enzyme linked immunosorbent assay (EIA) results. A/B (panel A and B represent the same data): EIA to assay for VLP binding to Clec12a receptor protein. Wildtype (wt) and decorated VLPs were pre-incubated with different dilutions of anti-Clec12a antibody, as indicated in panel A. At dilutions 1:1,000, 1:2,000, 1:4,000 the presence of the Clec12a antibody competes with the VLPs decorated with the anti-Clec12a specific antigen-binding domains for a constant amount of target Clec12aprotein. Higher dilutions of anti-Clec12a antibody (reduced amount of anti-Clec12a antibody) allows increased binding of decorated VLPs to Clec12a protein. No binding of wildtype VLPs. Experiment was repeated with an additional control antibody which is of the

same isotype as the anti-Clec12a antibody but with an unrelated binding target. Control antibody does not compete with binding of decorated VLPs (Figure 9).

[0054] **Figure 9** is a graphical representation of data showing decorated VLPs (VLP-Abs) binding to Clec12a protein in the presence of rat anti-Clec12a antibodies (A) or rat antibodies with unrelated specificity (B). Both rat antibodies are from the same isotype. At lower dilutions (1:1000 and 1:2000) the anti-Clec12a antibody competes with decorated VLPs for binding to Clec12a protein, not the antibodies with unrelated specificity.

[0055] **Figure 10** is a graphical representation of data showing the results of an assessment of an anti-HBsAgS immune response by EIA. Groups of 7 mice were injected subcutaneously at the base of the tail with 2μg of wtVLPs (A) or VLPs with targeting capability (wtHBsAgS + γV-S + κVmyc) (B) in the presence of 5nmol CpG. Two weeks after the third immunisation, blood samples were collected and assessed for anti-HBsAg responses at dilutions 1:250, 1:500, 1:1000, 1:2000, 1:4000. (C) Pre-immune sera, (D) anti-HBsAgS monoclonal antibody as EIA control.

[0056] **Figure 11** is a graphical representation of data showing enhanced immune responses to VLP-Abs. Two groups of mice (n=7) are immunised with decorated VLPs or wildtype VLPs, then serum samples taken at different time points, and measured for the presence of anti-HBsAgS antibodies. The average titre for each group is shown. Following established immunisation protocols for anti-Clec12a antibody (chemically coupled to a protein), VLPs were immunised in the presence of adjuvant CpG. See also Figure 16.

[0057] **Figure 12** is a representation of separated proteins after immunoprecipitation of 35S-labelled VLPs with anti-HBsAgS antibodies to identify incorporated γV-HBsAgS (NIH-45-46) (gV-S[NIH]) fusion proteins. 1: Mock; 2: wildtype (wt) HBsAgS; 3+4: γV-S[NIH]; 5: γV-S[NIH) + wtHBsAgS (1:5), 6: γV-S[NIH] + wtHBsAgS (1:10). Arrows: 24kD and 27kD HBsAgS proteins (unglycosylated, glycosylated). ◊ indicates the position of the γV-S[NIH] un/glycosylated fusion proteins. The variable antibody domain of the NIH45-46 γ heavy chain was fused to HBsAgS, and similar to the anti-Clec12a γV-antibody domain, was co-packed in the presence of wtHBsAgS, and secreted into the cell culture supernatant.

[0058] **Figure 13** is a diagrammatic representation of VLPs decorated with anti-Clec12a variable domain co-assembled with HBsAgS proteins containing a foreign antigenic sequence

I. Targeted delivery of antigenic sequences for inducing anti-foreign immune responses (viral antigens, cancer antigens etc.). Insertion of HIV-1 neutralising epitope (MPER) into HBsAgS subunit (in centrally in red (darker grey), shown for the un-fused HBsAgS subunit). Chimeric HBsAgS proteins containing an HIV-1 epitope are described herein and in PCT/AU2012/000614.

[0059] **Figure 14** is a representation of separated proteins after VLPs decorated with anti-Clec12a variable domain are co-assembled with HBsAgS proteins containing a foreign antigenic sequence. VLPs were synthesised composed of γV-S(N146Q) + κVmyc + HBsAgS(N146Q)-MPER (γV and κV sequences derived from anti-Clec12a antibody). Mock (lane 1); HBsAgS(N146Q)-MPER (lane 2); γV-S(N146Q) (lane 3); κVmyc (lane 4); γV-S(N146Q)+ κVmyc (lane 5); γV-S(N146Q)+ κVmyc + HBsAgS(N146Q)-MPER at ratios 1:1:1 (lane 6), 1:1:3 (lane 7), 1:1:5 (lane 8). Note: High amounts of HBsAgS(N146Q)-MPER versus γV-S(N146Q) (lane 7 versus lane 8) results in a somewhat reduced incorporation of γV-S(N146Q) (HBsAgS(N146Q)-MPER is abundant). Similar observations were made with γV-S and wildtype HBsAgS: high amounts of wtHBsAgS results in somewhat reduced levels of γV-S in the VLPs.

[0060] **Figure 15** is a representation of data showing successful packaging of large-delta antigen by VLPs containing γV-S fusion proteins. Hepatitis delta virus (HDV) is a satellite virus and needs the hepatitis B virus envelope (HBV) proteins for assembly and release. HDV replication is independent of HBV, and is not liver-specific. The HBsAgS protein is sufficient for HDV packaging. The large delta antigen (dAgL) is essential for HDV packaging to facilitate the contact to HBsAgS. In the absence of HBsAgS, dAgL cannot be packaged and released; HBsAgS is required. The presence of the HBsAgS fusion proteins will allow dAgL packaging and release. This will allow HDV targeting into cells of interests such as for example cancer cells as dictated by the specificity of the antibody domains arrayed on the decorated VLPs.

[0061] **Figure 16** is a graph showing that mice immunised with decorated VLPs recall a response more efficiently than mice immunised with wildtype VLPs. Groups of 7 mice were vaccinated three times at two week intervals with 2 μg VLP (wildtype or decorated) in the presence of 10 nmol CpG (ODN1668). The antibody variable domain used for generating the decorated VLPs are derived from the anti-Clec12a antibody targeting the Clec12a receptor

expressed on immune competent cells (dendritic cells, macrophages). Blood samples were taken at the days indicated along X-axis. Serum was collected and tested for anti-HBsAg-specific antibody responses. Both groups of mice were boosted at day 221 with 2μg wildtype VLPs only (no decorated VLPs used) in the presence of 10 nmol CpG (ODN1668) to recall memory responses. Antibody responses were assessed 7-days post 'boost' at day 228. Graphed is the antibody titre as determined by anti-HBsAg ELISA. Titres are considered positive of OD 405-490 nm is >than 2x OD 405-490nm of pre-immune serum. Depicted is the mean +/- SEM of 7 mice performed in duplicate. Arrows below X-axis indicate times if immunisation. See also Figure 11.

[0062] **Figure 17** is a plasmid map of vectors expressing antibody-HBV envelope fusion proteins. Expression is driven by the presence of an eukaryotic promoter. The open reading frame contains the information for an antibody variable domain derived from the heavy or light chain only (V_H or V_L); or antibody variable domain including constant region (derived from heavy or light chain, VC_H or VC_L). The antibody derived sequence is expressed in frame to the wildtype or mutant HBV envelope protein (middle [HBsAgM] or small [HBsAgS] or any combination thereof. This covers separated domains (via linker region) or those directly fused to the envelope proteins.

[0063] **Figure 18** is a plasmid map of vectors expressing antibody domains only. The plasmids are co-transfected with plasmids expressing the antibody chain-HBV envelope fusion proteins to provide the second antibody chain (variable domain, partner domain) in trans. The plasmids contain an eukaryotic promoter which drives the transcription of a messenger RNA encoding the antibody heavy or light chain variable domain (V_H or V_L), or variable domain in combination with the antibody constant region (VC_H or VC_L). Antibody sequence can be expressed with a tag sequence.

[0064] **Figure 19** provides nucleotide and amino acid sequences of an antibody sequence derived from anti-Clec12a antibody κ chain (light chain) including signal sequence: variable region fused to the myc tag (NH2-EQKLISEEDL-Cterm (SEQ ID NO: 2)).

[0065] **Figure 20** provides nucleotide and amino acid sequences of an antibody sequence derived from anti-Clec12a antibody κ chain (light chain) including signal sequence: variable region and constant region (ADAA).

[0066] **Figure 21** provides nucleotide and amino acid sequences of an antibody sequence (variable domain) derived from anti-Clec12a antibody κ chain (light chain) fused to a linker sequence (GAS) then a short sequence belonging to the middle domain of the envelope protein (-SRIGDPALN-), then the small HBV envelope protein (HBsAgS) region starting with (MENIT----) ending with (---VYI). HBsAgS glycosylation site (wildtype) present: N146. (-- PSDGNCT --). N146: amino acid 146 in the HBsAgS sequence (starting with MENIT ----).

[0067] **Figure 22** provides nucleotide and amino acid sequences of an antibody sequence (variable domain) derived from anti-Clec12a antibody κ chain (light chain) fused to a linker sequence (GAS) then a short sequence belonging to the middle domain of the envelope protein (-SRIGDPALN-), then the small HBV envelope protein (HBsAgS) region starting with (MENIT----) ending with (---VYI). HBsAgS glycosylation site removed: N146 changed to Q. (-- PSDGQCT --).

[0068] **Figure 23** provides nucleotide and amino acid sequences of an antibody sequence (variable domain) derived from anti-Clec12a antibody γ chain (heavy chain) fused to a linker sequence (GAS) then a short sequence belonging to the middle domain of the envelope protein (-SRIGDPALN-), then the small HBV envelope protein (HBsAgS) region starting with (MENIT----) ending with (---VYI). HBsAgS glycosylation site (wildtype) present: N146. (-- PSDGNCT --).

[0069] **Figure 24** provides nucleotide and amino acid sequences of an antibody sequence (variable domain) derived from anti-Clec12a antibody γ chain (heavy chain) fused to a linker sequence (GAS) then a short sequence belonging to the middle domain of the envelope protein (-SRIGDPALN-), then the small HBV envelope protein (HBsAgS) region starting with (MENIT----) ending with (---VYI). HBsAgS glycosylation site removed: N146 changed to Q. (-- PSDGQCT --).

[0070] **Figure 25** provides nucleotide and amino acid sequences of an antibody sequence (variable domain and constant region 1) derived from anti-Clec12a antibody γ chain (heavy chain) fused to a linker sequence (GAS) then a short sequence belonging to the middle domain of the envelope protein (-SRIGDPALN-), then the small HBV envelope protein (HBsAgS) region starting with (MENIT----) ending with (---VYI). HBsAgS glycosylation site (wildtype) present: N146. (-- PSDGNCT --).

[0071] **Figure 26** provides nucleotide and amino acid sequences of an NIH45-46 Anti-HIV-1 Heavy chain with signal sequence. An antibody sequence (variable domain) is derived from the anti-HIV-1 antibody (NIH45-46) heavy chain fused to a linker sequence (GAS) then the small HBV envelope protein (HBsAgS) region starting with (MENIT----) ending with (---VYI). HBsAgS glycosylation site (wildtype) present: N146. (-- PSDGNCT --). N146: amino acid 146 in the HBsAgS sequence (starting with MENIT ----).

[0072] **Figure 27** provides nucleotide and amino acid sequences of a NIH45-46 Anti-HIV-1 Light chain with signal sequence. An antibody sequence (variable domain) is derived from the anti-HIV-1 antibody (NIH45-46) light chain fused to a linker sequence (GAS) then the small HBV envelope protein (HBsAgS) region starting with (MENIT----) ending with (---VYI). HBsAgS glycosylation site (wildtype) present: N146. (-- PSDGNCT --). N146: amino acid 146 in the HBsAgS sequence (starting with MENIT ----).

[0073] **Figure 28** provides a representation of separated proteins after VLPs decorated with functional anti-Clec12A variable domains are co-assembled with either wild-type (wt), or mutant HBsAg subunits. VLPs were synthesised composed of γV-S (N146Q) + κVmyc + wt-HBsAgS or myc-HBsAgS (γV and κV sequences are derived from anti-Clec12A antibody). A) mock transfection; B) wt HBsAg; C) myc-HBsAg (myc-S); D) γV-S + κVmyc; E) γV-S + κVmyc +wt-HBsAg; F) γV-S + κVmyc +myc-S. [ug = unglycosylated, g = glycosylated].

[0074] **Figure 29** illustrates that different antibodies can be incorporated into secreted VLPs. This is a representation of separated proteins that are secreted and co-assembled with glycosylation mutant HBsAgS. A) Mock transfection; B) HBsAg (N146Q); C) anti-Clec12A γV-S + κVmyc; D) anti-Clec12A γV-S + κVmyc + HBsAgS (N146Q); E) HVR-antibody γV-S + κVmyc; F) HVR-antibody γV-S + κVmyc + HBsAgS (N146Q).

[0075] **Figure 30:** Different antibodies and different fusion-techniques can be used to incorporate antibody domains into VLPs. This figure is a representation of separated proteins secreted and assembled with wild-type HBsAg. A) Mock transfection; B) wt-HBsAg; C) anti-DEC205 scFv-HBsAg fusion protein; D) scFv-HBsAg + wt HBsAg (1:1 ratio); E) scFv-HBsAg + wt HBsAg (1:2 ratio).

[0076] **Figure 31:** Immunisation outcomes of mice aged to one year prior to initiation of vaccine trial. Mice were immunised subcutaneously at the base of the tail with 400 ng of VLP

in the presence of 5 nmol CpG. VLP were either wt HBsAg, Clec12A-targeted or HVR VLP (which contain antibody domains that do not target immune receptors). Blood samples were collected and tested for anti-HBsAg antibody responses *via* EIA.

[0077] **Figure 32** illustrates the ability for the parental 5D3 antibody to prevent the binding of the Clec12A targeted VLPs from binding to the Clec12A target protein was assessed by EIA. 25 ng of Clec12A protein was coated onto EIA plates, 100 μl of 500 ng/ml 5D3 antibody, isotype control antibody or PBS was added to wells. 100 μl of 1ug/ml T-VLP (Clec12A targeted VLP), UT-VLP (wt HBsAg VLP) or PBS was then added to wells. VLPs were detected using anti-HBsAg antibodies.

[0078] **Figure 33** is a representation of the animo acid and nucleotide sequence of Dec 205 scFv-HBsAg.

TABLE A

BRIEF DESCRIPTION OF THE SEQUENCES

SEQUENCE ID NUMBER	SEQUENCE
SEQ ID NO: 1	Nucleotide sequence encoding Myc tag (NH2-EQKLISEEDL-Cterm).
SEQ ID NO: 2	Amino acid sequence of Myc tag (NH2-EQKLISEEDL-Cterm).
SEQ ID NO: 3	Nucleotide sequence encoding middle domain of HBV envelope protein (-SRIGDPALN-).
SEQ ID NO: 4	Amino acid sequence of middle domain of HBV envelope protein (-SRIGDPALN-).
SEQ ID NO: 5	Nucleotide sequence encoding HBsAgS [= virus HBV hepatitis B] as published by Galibert *et al.*(1979)*Nature* 281:646-650. The HBsAgS sequence was derived from the full length genome. Serotype ayw, Genotype D. The sequence (HBV full length genome) is available: GenBank V01460.1.
SEQ ID NO: 6	Amino acid sequence HBsAgS and encoded by SEQ ID NO: 5including wild-type glycosylation site N146(-- PSDGNCT --).
SEQ ID NO: 7	Nucleotide sequence of illustrative linker domain (GAS).
SEQ ID NO: 8	Antibody sequence of illustrative linker domain (GAS).

SEQUENCE ID NUMBER	SEQUENCE
SEQ ID NO: 9	Nucleotide sequence encoding kappa light variable chain from anti-Clec12a antibody including signal sequence (rat).
SEQ ID NO: 10	Amino acid sequence of kappa light variable chain from anti-Clec12a antibody including signal sequence (rat).
SEQ ID NO: 11	Nucleotide sequence encoding kappa light constant region from anti-Clec12a antibody including signal sequence (rat).
SEQ ID NO: 12	Amino acid sequence of kappa light constant region from anti-Clec12a antibody including signal sequence (rat).
SEQ ID NO: 13	Nucleotide sequence encoding heavy variable chainderived from anti-Clec12a antibody γ chain (rat).
SEQ ID NO: 14	Amino acid sequence of heavy variable chainderived from anti-Clec12a antibody γ chain (rat).
SEQ ID NO: 15	Nucleotide sequence encoding κ12V-HBsAgS (N146Q) (-- PSDGQCT --).
SEQ ID NO: 16	Amino acid sequence of κ12V-HBsAgS (N146Q) antibody sequence (variable domain) derived from anti-Clec12a antibody κ chain (light chain) fused to a linker sequence (GAS) then a short sequence belonging to the middle domain of the envelope protein (-SRIGDPALN-), then the small HBV envelope protein (HBsAgS) region starting with (MENIT----) ending with (---VYI). HBsAgS glycosylation site removed: N146 changed to

SEQUENCE ID NUMBER	SEQUENCE
	Q. (-- PSDGQCT --). See also Figure 22.
SEQ ID NO: 17	Nucleotide sequence encoding γ12V-HBsAgS (N146Q).
SEQ ID NO: 18	Amino acid sequence of γ12V-HBsAgS (N146Q). Antibody sequence (variable domain) derived from anti-Clec12a antibody γ chain (heavy chain) fused to a linker sequence (GAS) then a short sequence belonging to the middle domain of the envelope protein (-SRIGDPALN-), then the small HBV envelope protein (HBsAgS) region starting with (MENIT----) ending with (---VYI). HBsAgS glycosylation site removed: N146 changed to Q. (-- PSDGQCT --). See also Figure 24.
SEQ ID NO: 19	Nucleotide sequence encodingγ12VC-HBsAg (N146N).
SEQ ID NO: 20	Amino acid sequence of γ12VC-HBsAg (N146N). Antibody sequence (variable domain and constant region 1) derived from anti-Clec12a antibody γ chain (heavy chain) fused to a linker sequence (GAS) then a short sequence belonging to the middle domain of the envelope protein (-SRIGDPALN-), then the small HBV envelope protein (HBsAgS) region starting with (MENIT----) ending with (---VYI). HBsAgS glycosylation site (wildtype) present: N146. (-- PSDGNCT --). All antibody sequences contain a signal sequence which allows translation at the endoplasmic reticulum (ER). See also Figure 25.
SEQ ID NO: 21	Nucleotide sequence encoding NIH45-46 Anti-HIV-1 Heavy chain with signal sequence

SEQUENCE ID NUMBER	SEQUENCE
SEQ ID NO: 22	Amino acid sequence of NIH45-46 Anti-HIV-1 Heavy chain with signal sequence. Antibody sequence (variable domain) derived from the anti-HIV-1 antibody (NIH45-46) heavy chain fused to a linker sequence (GAS) then the small HBV envelope protein (HBsAgS) region starting with (MENIT----) ending with (---VYI). HBsAgS glycosylation site (wildtype) present: N146. (-- PSDGNCT --). N146: amino acid 146 in the HBsAgS sequence (starting with MENIT ----). See also Figure 26.
SEQ ID NO: 23	Nucleotide sequence encoding NIH45-46 Anti-HIV-1 Light chain with signal sequence.
SEQ ID NO: 24	Amino acid sequence of NIH45-46 Anti-HIV-1 Light chain with signal sequence. Antibody sequence (variable domain) derived from the anti-HIV-1 antibody (NIH45-46) light chain fused to a linker sequence (GAS) then the small HBV envelope protein (HBsAgS) region starting with (MENIT----) ending with (---VYI). HBsAgS glycosylation site (wildtype) present: N146. (-- PSDGNCT --). N146: amino acid 146 in the HBsAgS sequence (starting with MENIT ----). See also Figure 27.
SEQ ID NO: 25	Dec 205 ScFv-HBsAs nucleotide sequences.
SEQ ID NO: 26	Dec 205 ScFv-HBsAs amino acid sequence.

DETAILED DESCRIPTION

[0079] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Practitioners are particularly directed to Sambrook *et al.* (1989) (*supra*), Coligan *et al.*, *Current Protocols In Protein Science*, John Wiley & Sons, Inc., 1995-1997, in particular Chapters 1, 5 and 6 and Ausubel *et al., Current Protocols in Molecular Biology, Supplement 47*, John Wiley & Sons, New York, 1999; Colowick and Kaplan, eds., *Methods In Enzymology*, Academic Press, Inc.; Weir and Blackwell, eds., *Handbook of Experimental Immunology, Vols. I-IV*, Blackwell Scientific Publications, 1986; Joklik ed., *Virology, 3rd Edition*, 1988; Fields and Knipe, eds, *Fundamental Virology, 2nd Edition*, 1991; Fields *et al.*, eds, *Virology, 3rd Edition*, Lippincott-Raven, Philadelphia, Pa, 1996, for definitions and terms of the art and other standard methods known to the person skilled in the art. Reference is made in addition to Baumal *et al.* (1971) *J. Exp. Med.* 134:1316-34; Bergman & Kuehl (1979) *J. Biol. Chem.* 254:8869-76; Feige *et al.* (2010) *Sci.*35:189-198; Mangold *et al.* (1997) *Arch. Virol.* 142:2257-67; Seeger *et al.* (2007) *Fields Virology* 5th edition p2977-3029; Stirk *et al.* (1992) *Intervirology* 33:148-58; Wunderlich & Bruss (1996) *Arch. Virol.* 141:1191-205; Zhao *et al.* (2006) *Human Vaccines* 2:174-80, Chailan *et al.*(2011) *FEBs Journal* 278 2858-2866.

[0080] Like hepadnaviral envelope proteins, antibodies are formed in the endoplasmic reticulum (ER) compartment of eukaryotic cells. Antibody heavy and light chains are translocated into the ER and folding of the antibody domains typically begins before the polypeptide chains are completely translated. In general, antibodies such as immunoglobulin G (IgG) antibodies, for example, assemble first as a heavy chain dimer to which the light chain is added. Then, a covalent disulfide bond between the light constant domain and heavy constant domain stabilises the antibody molecule. Both antibody binding site formation and hepadnaviral VLP assembly depend on the ER. The present inventors conceived that a hepadnaviral envelope protein fused to an antibody antigen binding domain might be produced with correct folding of the individual domains and that a VLP-Ab with functional surface exposed antigen-binding domains might be producible.

[0081] This description enables targeted delivery of a VLP within a subject. The description also enables delivery of antibodies to a subject as part of a VLP.

[0082] As discussed herein, the term "antibody" or "immunoglobulin" includes full length antigen-binding antibodies and antigen-binding fragments thereof as known in the art. An antibody, or an antigen binding fragment of an antibody, comprises an antigen-binding domain. The antigen binding site or domain of naturally occurring antibodies is formed by the precise juxtaposition of typically six hypervariable loops (also regarded as complementary determining regions or CDR) provided by the light chain variable region and the heavy chain variable region and aided by more conserved framework regions of the variable domains. Illustrative fragments known to the skilled addressee include Fab, Fab', Fd, Fd', Fv, dAb, isolated CDR region, $F(ab')_2$ bivalent fragments, diabodies and liner antibodies. As used herein, antibodies are not referred to an antigens.

[0083] Hepadnaviruses are a family of enveloped, double-stranded viruses which can cause infections humans and animals. The family includes orthohepadnoviruses such as hepatitis B virus (HBV) which infects man, and avian hepadnaviruses including species infecting one of duck, snow goose, heron and crane, among others. The envelope proteins from a range of hepadnavirus species, including HBV and duck hepatitis virus, share a high level of sequence similarity.

[0084] The term "hepadnaviral envelope protein" or "modified hepadnaviral envelope protein" or "HEP" includes all or part of an hepadnavirus envelope protein or a VLP-forming variant or part thereof. In some embodiments, the HEP comprises epitopes of one or more hepadnaviral serotypes, strains or isotypes etc. The term includes native or non-native forms, including homologs or their derivatives or sequences of hepadnaviral envelope proteins. Modified (variant) or chimeric hepadnaviral envelope proteins are known in the art. Some are described herein and include envelope proteins such as HBsAgS having one or more of a modified cysteine bonding pattern, a modified signal sequence, a modified glycosylation pattern, a codon optimised encoding sequence, and one or more amino acid deletions including biolocially active i.e. VLP forming, fragments, truncations, substitutions and additions. Substitution may be conservative or non-conservative as known in the art and described further herein. As contemplated herein, the HEP may be employed to vector a heterologous antigen using procedures well understood by the skilled addressee.

[0085] Also as discussed herein, the term "VLP" or "lipoprotein particle" or "particle" is used broadly to refer to small groups of lipid and protein molecules similar to the viral-like particles produced by certain viruses that infect eukarytokes.

[0086] Accordingly, in one broad aspect, the specification describes a hepadnaviral envelope protein-based VLP displaying a functional antibody comprising a complementary pair of antibody variable domains. In some embodiments, the VLP is produced in a eukaryotic cell in conjunction with a complementary pair of antibody variable domains wherein at least one of the variable domains is expressed as a nucleic acid encoded fusion protein together with a hepadnaviral envelope protein, and the complementary variable domain is expressed as a nucleic acid encoded fusion protein together with a hepadnaviral envelope protein or is expressed in the same cell unfused to a hepadnaviral envelope protein.

[0087] In another broad aspect, the present specification enables a VLP-antibody (VLP-Ab) which comprises a recombinant modified hepadnaviral envelope fusion protein comprising an antibody variable domain of the antibody (EAFP).

[0088] In some embodiments the EAFP comprising a variable domain is able to bind antigen (the target protein or epitope) in the absence of a complementary variable domain. In these embodiments, the VLP-antibody does not comprise a second variable domain protein. In some embodiments, the EAFP comprises a heavy chain variable domain that serves to bind antigen (target) in the absence of a complementary light variable domain.

[0089] In another embodiments, the present specification enables a VLP-antibody (VLP-Ab) which comprises a recombinant modified hepadnaviral envelope fusion protein comprising an antibody variable domain wherein the antibody variable domain of the fusion protein is bound to its complementary variable domain to form an antigen binding site of the antibody (VLP-Ab).

[0090] In one embodiment, the specification provides a recombinant modified hepadnaviral envelope protein directly or indirectly fused to an antibody variable domain of an antibody selected from a heavy chain variable domain and a light chain variable domain. By "indirect" is meant that intervening amino acids, such as a spacer domain may be employed.

[0091] The modified hepadnaviral envelope antibody fusion protein has particle forming ability and antibody forming ability. A fusion protein, as referred to herein, comprises two or

more heterologous proteins that are expressed as a single RNA and single protein from an expression plasmid encoding the fusion protein. The "recombinant" molecules described are produced by genetic modification of a parent molecule, such as a envelope protein whose genetic sequence is altered to facilitate expression of a fusion protein comprising all or part of the envelope protein and all or part of an antigen-binding protein.

[0092] By "fusion protein" is meant two separate proteins produced intracellularly as one protein, optionally linked together by a sequence of amino acids forming a spacer domain by expression from a single nucleic acid molecule encoding the fusion protein. Spacer or linker domains are short peptides that are optionally introduced between protein domains. They often comprise flexible residues such as glycine and serine so that adjacent protein domains are able to move relative to each other. Longer linkers are used to reduce steric hindrance between domains. Linkers may be as short as 4-6 amino acids or as long as about 300 amino acids.

[0093] By "particle forming ability" "VLP-forming variant or part" is meant that the derivative, variant or part is able, like its parent molecule at least to some extent, to form a virus-like particle. Typically, such a molecule will comprise at least about 30%, 40%, 50%, 60%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to a parent molecule or reference or native hepadnaviral envelope protein such as HBsAgS, or at least about 30%, 40%, 50%, 60%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or the entire length of a reference or native hepadnaviral envelope protein such as HBsAgS.

[0094] VLP-forming derivatives (variants) are made and tested using established methods by introducing increasing amounts of mutations. Function domains may be retained or they may retain a higher level of sequence identity to a wild-type (native) VLP-forming hepadnaviral envelope protein, than non-functional domains. Illustrative functional domains include the first internal loop (amino acids 29-77 of HBsAgS) and the first transmembrane region (internal, not exposed)(amino acids 8-28 of HBsAgS). These regions also tolerate some mutation.

[0095] By "target" is meant the protein, antigen, cell or tissue comprising the epitope specifically determined by the antigen-binding site of an antibody variable domain.

[0096] During expression, the modified hepadnaviral envelope-antibody fusion protein (EAFP) is inserted into an endoplasmic reticulum membrane and forms a VLP with surface oriented antibody variable domains.

[0097] In some embodiments, the antibody variable domain of the EAFP is bound to a complementary antibody variable domain provided by a complementary EAFP in the VLP. In an illustrative embodiment, a first EAFP comprises a heavy chain variable domain and a second EAFP comprises a complementary light chain variable domain. Binding, in the form of the non-covalent heterodimerisation, between the light chain variable domain and the heavy chain variable domain may enhance the antigen-binding ability of the antibody.

[0098] In other embodiments, the antibody variable domain of the EAFP is bound to a second variable domain protein that is not fused to a HEP and which comprises a complementary antibody variable domain to form with the EAFP an antigen binding site comprising heavy and light antibody domains. In an illustrative embodiment, the EAFP comprises a heavy chain variable domain and the second variable domain protein comprises a complementary light chain variable domain.

[0099] During expression, the modified hepadnaviral envelope antibody fusion protein (EAFP) is inserted into an endoplasmic reticulum membrane and forms a VLP with surface oriented antibody variable domains. The surface oriented antibody variable domain (either a heavy or a light chain variable domain) is, in the context of the HEP, capable of binding to a complementary (heavy or light chain) variable domain provided either by a second EAFP in the VLP or by a second variable domain protein comprising the complementary variable domain. In some embodiments, the second variable domain protein (SVDP) is expressed endogenously within a cell that also expresses the EAFP. In some embodiments, complementary variable domains are expressed by the same or a different expression plasmid within the same or different cells. As required in some embodiments, expression vectors comprise an exogenous signal sequence allowing for translation at the ER as well as or in place of that provided by the envelope or antibody sequence.

[00100] A dual expression construct is conveniently employed (such as pTandem-1 and pBudCE4.1) for expression of the EAFP ± a second variable protein in mammalian cells and

expression of genes in transfected cells tested by Western blotting and IF with suitable monoclonal antibodies. Assembly of VLP-Abs may be assessed by sedimentation through sucrose gradients and Western blotting and heterodimerisation of EAFP and second variable domain assessed by co-immunoprecipitation and binding Mabs in a VLP EIA.

[00101] Accordingly, in some embodiments, the disclosure enables a recombinant VLP-antibody (VLP-Ab) which comprises a modified hepadnaviral envelope fusion protein comprising an antibody heavy chain variable domain or a light chain variable domain, wherein the variable domain is bound to a complementary antibody variable domain of a second protein and forms the antibody antigen binding site.

[00102] In some embodiments, the disclosure enables a recombinant VLP-antibody (VLP-Ab) which comprises a modified hepadnaviral envelope fusion protein comprising an antibody heavy chain variable domain or an antibody light chain variable domain (EAFP), wherein the variable domain is bound to the complementary variable domain of a second protein and forms the antibody antigen binding site substantially on the surface of the VLP.

[00103] In some embodiments, the second variable domain protein is a modified hepadnaviral envelope antibody fusion protein comprising an antibody variable domain complementary to the variable domain of the EAFP.

[00104] In some embodiments, the second variable domain protein is unfused to a HEP (is not a fusion protein comprising a HEP) and comprises an antibody variable domain complementary to the variable domain of the first EAFP.

[00105] In some embodiments, the EAFP or the second protein comprises an antibody constant domain.

[00106] In some embodiments, the EAFP is a hepatitis B virus envelope protein directly or indirectly fused to the antibody variable domain of an antibody.

[00107] In some embodiments, the antibody or antigen binding site thereof binds specifically to an immune or antigen presenting cell, such as a dendritic cell.

[00108] In some embodiments, the antibody is an anti-C-type lectin antibody (e.g. Clec9a or Clec12a).

[00109] Antigen-presenting cells include both professional and facultative types of antigen-presenting cells. Professional antigen-presenting cells include, but are not limited to,

macrophages, monocytes, B lymphocytes, cells of myeloid lineage, including monocytic-granulocytic-DC precursors, marginal zone Kupffer cells, microglia, T cells, Langerhans cells and dendritic cells including interdigitating dendritic cells and follicular dendritic cells. Examples of facultative antigen-presenting cells include but are not limited to activated T cells, astrocytes, follicular cells, endothelium and fibroblasts. In some embodiments, the antigen-presenting cell is selected from monocytes, macrophages, B-lymphocytes, cells of myeloid lineage, dendritic cells or Langerhans cells.

[00110] The present invention is not limited to Clec12a and Clec9a receptors on antigen presenting cells. As the skilled person will appreciate, many suitable receptors or epitopes are expressed on the surface of antigen preventing cells suitable for facilitating targeting of VLPs to antigen presenting cells and facilitating internalisation and antigen presentation.

[00111] Notably, the Cleg12A protein is highly expressed and upregulated in cases of acute myeloid leukemia and acute lymphoid leukemia. Accordingly, the subject VLP-Abs are delivered directly to these cells.

[00112] There are many suitable target molecules/receptors expressed on antigen presenting cells. These include the Toll-like receptors, C-type lectins in general including DEC-205 (CD205) which is predominantly expressed on CD8+ dendritic cells and is a particularly an attractive target molecule (Bonifaz *et al.* (2004). *J. Experimental Medicine* 199:815-824). DEC-205 is a type I C-type lectin. Also, Dectin-2; DC-associated C-type lectin 2, the C-type lectin receptor 1(Clec-1), Clec12a and Clec9a. Further, the macrophage mannose receptor and DC-SIGN (CD209) provide attractive target molecules.

[00113] In some embodiments, the antibody of the VLP-Ab binds specifically to a target cell or tissue within a subject.

[00114] In some embodiments, the antibody is a neutralising antibody that is able to reduce viral infectivity. In the example of HIV, illustrative broad-acting neutralising antibodies include antibodies NIH45-46 and 10E8.

[00115] Immunoglobulins are typically multi-chain proteins typically comprising two pairs of light chains and two pairs of heavy chains. So called "heavy chain antibodies" lacking light chains are found in camelids and in a number of fish species. In higher vertebrates, there are two types of light chain –κ and λ– whereas heavy chains can be of five types: μ, δ, γ, ε and α. The type of heavy chain defines the class of immunoglobulin: IgM,

IgD, IgG, IgE and IgA, respectively. Each chain typically contains four (heavy chains) or two (light chains) intrachain disulfide bonds and is composed of multiple variants of a basic domain (two for the light and usually four for the heavy chain) assuming the characteristic immunoglobulin fold, in which two β-sheets are packed face to face and linked together by conserved interchain disulfide bridges and by interstrand loops.

[00116] Given their modular nature and the conservation of their scaffold structure, antibodies are particularly suitable candidates for protein engineering. It is possible to 'transplant' the antigen-binding property from a 'donor' to an 'acceptor' antibody by exchanging either fragments or antigen-binding regions. The specificity of an antibody against a given antigen, obtained for example in the mouse, can, in principle, be transferred to a human antibody, thereby obtaining a molecule with the desired specificity and less likely to elicit an immune response.

[00117] Several strategies have been devised to reach this goal, including antibody chimerisation, humanisation, superhumanisation, resurfacing and human string content optimisation as known in the art.

[00118] The canonical structure method to predict the structure of the hypervariable loops is based on the observation that, in spite of their high sequence variability, five of the six loops of the antigen-binding site, and part of the sixth, can assume a small repertoire of main-chain conformations, called 'canonical structures', determined by the length of the loops and by the presence of key residues at specific positions, inside and outside of the loops themselves. The other loop residues are free to vary to modify the topography and physicochemical properties of the antigen-binding site. Most of the hypervariable regions of known structures have conformations very close to the described canonical structures. The method is implemented in the publicly available web server "PIGS" and has been extended to allow the prediction of the structure of loops from immunoglobulin λ chains. Previous studies have shown that changes in the heavy chain variable domain–light chain variable domain (VH–VL) association can modify the relative positions of the hypervariable loops, which, in turn, can alter the general shape of the antigen-binding site, as well as the disposition of side-chains that interact directly with the antigen.

[00119] Modified forms of murine monoclonal antibodies may be produced by replacing the nucleotides encoding selected murine heavy or light chain constant domains with

nucleotides encoding human heavy or light chain constant domains, such as is described in U.S. Patent No. 4,816,567 and by Morrison *et al.*, (1984) *Proc. Nat. Acad. Sci.* 81: 6851.

[00120] Antibodies contemplated herein include humanised antibodies. In general, humanised antibodies are human antibodies (the recipient antibody) in which the complementarity determining (CDR) region residues have been replaced by CDR region residues from a non-human species (the donor antibody), such as from a mouse, rat, rabbit or non-human primate. In some cases, certain framework region (FR) residues of the human antibody may also be replaced by corresponding non-human residues, or the humanised antibodies may comprise residues which are not found in the recipient antibody or in the donor antibody. These modifications are made to enhance antibody performance and affinity. In general, the humanised antibody will comprise substantially all of at least one, and typically two, variable regions, in which all or substantially all of the CDR regions correspond to those of a non-human antibody, and all or substantially all of the FRs are those of a human antibody sequence. The humanised antibody may also optionally comprise at least a portion of an antibody constant region (Fc), typically that of a human antibody (Jones *et al.* (1986) *Nature* 321:522-525; Reichmann *et al.* (1988) *Nature* 332:323-329; Presta (1992) *Curr. Op. Struct. Biol.* 2:593-596; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439; Larrick *et al.* (1989) *Bio/Technology* 7:934; Winter and Harris (1993) *TIPS* 14:139; Carter *et al.* (1992) *Proc. Nat. Acad. Sci.* 89:4285). Similarly, to create a primatised antibody the murine CDR regions can be inserted into a primate framework using methods known in the art (see e.g. WO 93/02108 and WO 99/55369).

[00121] Alternatively, a humanised antibody may be created by a process of 'veneering'. A statistical analysis of unique human and murine immunoglobulin heavy and light chain variable regions revealed that the precise patterns of exposed residues are different in human and murine antibodies, and most individual surface positions have a strong preference for a small number of different residues (see Padlan *et al.* (1991) *Mol. Immunol.* 28:489- 498, and Pedersen *et al.* (1994) *J. Mol. Biol.* 235:959-973). Therefore, it is possible to reduce the immunogenicity of a non-human Fv by replacing exposed residues in its framework regions that differ from those usually found in human antibodies. Because protein antigenicity may be correlated with surface accessibility, replacement of the surface residues may be sufficient to render the mouse variable region 'invisible' to the human immune system. This procedure

of humanization is referred to as 'veneering' because only the surface of the antibody is altered, the supporting residues remain undisturbed.

[00122] International publication No. WO 2004/006955 describes methods for humanizing antibodies, based on selecting variable region framework sequences from human antibody genes by comparing canonical CDR structure types for CDR sequences of the variable region of a non-human antibody to canonical CDR structure types for corresponding CDRs from a library of human antibody sequences, e.g. germline antibody gene segments. Human antibody variable regions having similar canonical CDR structure types to the non-human CDRs form a subset of member human antibody sequences from which to select human framework sequences. The subset members may be further ranked by amino acid similarity between the human and the non-human CDR sequences. In the method of WO 2004/006955, top ranking human sequences are selected to provide the framework sequences for constructing a antibody that functionally replaces human CDR sequences with the non-human CDR counterparts using the selected subset member human frameworks, thereby providing a humanised antibody of high affinity and low immunogenicity without need for comparing framework sequences between the non-human and human antibodies. The CDRs of a given antibody may be readily identified, for example using the system described by Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed., US Department of Health and Human Services, PHS, NIH, NIH Publication No. 91-3242, 1991.

[00123] In some embodiments, the EAFP is glycosylated within a host eukaryotic cell.

[00124] In some embodiments the EAFP is modified to remove one or more glycosylation sites (hypoglycosylated).

[00125] In some embodiments, the EAFP is modified to allow for hyperglycosylation.

[00126] In some embodiments, the EAFP comprises a heterologous antigen or protein.

[00127] In some embodiments, the EAFP comprises antibody heavy and light chain variable domains.

[00128] In some embodiments, the EAFP comprises an antibody heavy variable domain.

[00129] In some embodiments, the EAFP does not include an antibody Fc region.

[00130] In some embodiments, the EAFP comprises a spacer domain between the HEP and the antibody variable domain. In some embodiments, the antibody variable domain is N-terminal to the HEP or replaces an N-terminal portion of an HEP.

[00131] In some embodiments, the VLP-Ab comprises further HEP-based fusion proteins whereby one or more antigens of interest are expressed and displayed on the surface of the VLP.

[00132] In other embodiments, the VLP-Ab comprises hepatitis delta virus or hepatitis delta antigen.

[00133] In other embodiments, the VLP-Ab comprises drugs, nucleic acids, or other agents within the VLP.

[00134] In some embodiments, the HEP comprises one of HBsAgL, HBsAgS, HBsAgM or combinations or parts thereof. The L polypeptide of HBV is typically excluded from VLP of HBV but is present in duck hepatitis virus, for example, and may be used to vector antigens, as described in WO 2004/092387.

[00135] In some embodiments the hepadnavirus is hepatitis B virus (HBV).

[00136] In some embodiments, the antibody binds specifically to a target antigen or target receptor expressed on a target cell, such as an antigen presenting cell.

[00137] In other embodiments, the antibody binds specifically to a cell bound or soluble receptor or self or non-self antigen or to an infectious agent such as but not limited to a virus, bacterium or fungus.

[00138] In some embodiments, the VLP comprises antibodies of more than one specificity directed to one or more targets. Different antibodies having different specificities may be displayed on the same VLP providing enhanced binding to a target antigen, cell or tissue. Multiple antibodies of one or more specificities may be expressed in this way on the VLP surface.

[00139] In another aspect, the present description provides a recombinant nucleic acid molecule encoding a hepadnavirus envelope-antibody fusion protein wherein the antibody comprises a single antibody variable domain of an antibody. In some embodiments, expression vectors encoding the fusion protein and a second protein as described herein are provided. As used herein, an expression vector is capable of directing the expression of the

nucleic acid molecules to which they are operatively linked. In one embodiment, the expressed fusion proteins are encompassed and may be made available in kit form.

[00140] In another aspect, the present description provides a recombinant nucleic acid molecule encoding a hepadnavirus envelope-antibody fusion protein wherein the antibody comprises an antigen binding derivative of an antibody such as an ScFv. In one embodiment, the expressed fusion proteins are encompassed and may be made available in kit form. One illustrative ScFv fusion is set out in Figure 33.

[00141] In one embodiment, any one or more the novel amino acid or nucleotide sequences of fusion molecules set out in Table A are encompassed.

[00142] In another embodiments, the present description enables a eukaryotic expression vector capable of expressing a recombinant nucleic acid molecule encoding a hepadnavirus envelope-antibody fusion protein comprising an antibody variable domain of the antibody. Any such vector will typically comprise a UTR, an appropriate signal sequence, a start codon, Kozak consensus sequences etc.

[00143] In some embodiments, the present description enables a recombinant eukaryotic cell comprising the VLP-Ab as described herein.

[00144] In some embodiments, the present description enables a nucleic acid encoding the EAFP and or the second variable domain (second protein) as described herein.

[00145] In some embodiments, the present description enables a nucleic acid encoding the EAFP and a nucleic acid encoding a second protein comprising a complementary antibody variable domain. In some embodiments, the description provides for the use of the herein described nucleic acid molecules or expression vectors comprising same in the manufacture of a medicament for the treatment or prophylaxis of a condition or disease such as a cancer, an autoimmune condition, or inflammatory condition or an infectious disease

[00146] A composition comprising the VLP-Ab as described herein is also provided. In some embodiments the compositions are for use or when used in the manufacture of a medicament such as for the treatment or prophylaxis of a condition or disease such as a cancer, an autoimmune condition, or inflammatory condition or an infectious disease. In other embodiments, the compositions are for use in medical imaging.

[00147] A pharmaceutical/vaccine composition comprising the VLP-Ab and various uses thereof are described herein.

[00148] VLP-Abs comprising an hepadnaviral (such as HBV) envelope protein fused to the variable domain of an antibody and comprising a second protein comprising a complementary antibody variable domain of an antibody that targets the VLP to an antigen presenting cell is a preferred embodiment of the present description. In an illustrative embodiment, anti-Clec12a antibodies are employed. Clec12a is a receptor expressed on the surface of antigen presenting cells including dendritic cells which are efficient in cross-presentation. Thus HBV VLP-decorated with antibodies that bind to selected antigen presenting cells are proposed for use in the treatment of HBV infections.

[00149] A method of eliciting an immune response comprising administering the VLP-Ab is described herein.

[00150] A method for preparing a composition comprising a VLP-Ab complex as described herein is also contemplated.

[00151] Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC). These include, *inter alia*, BHK, VERO, HT1080, 293, 293T, 293F, RD, COS-7, CHO, Jurkat, HUT, SUPT, C8166, HepG2, MOLT4/clone8, MT-2, MT-4, H9, PM1, CEM, myeloma cells (e.g., SB20 cells) and CEMX174, HEK293T, NSO, SP2 cells, HeLa cells, A549 cells, 3T3 cells, and a number of other cell lines. Other cells that may be used include insect cell lines, such as Sf9cells, avian, amphibian cells, plant cells, yeast and fungal yeast cells.

[00152] In one embodiment, a non-mammalian cell may be employed such as a yeast cell, such as *Hansenula polymorpha*. Such cells are useful for providing controlled levels of expression.

[00153] When recombinant expression vectors are introduced into mammalian host cells, the VLP-antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the VLP- antibody in the host cells or, more preferably, secretion of the VLP-antibody into the culture medium in which the host cells are grown. VLP-antibodies can be recovered from the culture medium using standard protein purification methods. Further, expression of VLP-antibodies of the invention from host cell lines can be enhanced

using a number of known techniques. Secretion competence is not essential although preferred and VLPs can be purified from host cellular material, if required.

[00154] Vectors available for cloning and expression in host cell lines are well known in the art, and include but are not limited to vectors for cloning and expression in mammalian or yeast cell lines, vectors for cloning and expression in bacterial cell lines, vectors for cloning and expression in phage and vectors for cloning and expression insect cell lines. The VLP-antibodies can be recovered using standard protein purification methods.

[00155] By "control element" or "control sequence" is meant nucleic acid sequences (*e.g.*, DNA) necessary for expression of an operably linked coding sequence in a particular host cell. Control sequences that are suitable for eukaryotic cells include transcriptional control sequences such as promoters, polyadenylation signals, transcriptional enhancers, translational control sequences such as translational enhancers and internal ribosome binding sites (IRES), nucleic acid sequences that modulate mRNA stability, as well as targeting sequences that target a product encoded by a transcribed polynucleotide to an intracellular compartment within a cell or to the extracellular environment.

[00156] The term "sequence identity" as used herein refers to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, I) or the identical amino acid residue (*e.g.*, Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. For the purposes of the present invention, "sequence identity" are understood to mean the "match percentage" calculated by the DNASIS computer program (Version 2.5 for windows; available from Hitachi Software engineering Co., Ltd., South San Francisco, California, USA) using standard defaults as used in the reference manual accompanying the software.

[00157] The terms "signal sequence" or "signal peptide refers to a short (about 3 to about 60 amino acids long) peptide that directs co- or post-translational transport of a protein from

the cytosol to certain organelles such as the nucleus, mitochondrial matrix, and endoplasmic reticulum, for example. For proteins having an ER targeting signal peptide, the signal peptides are typically cleaved from the precursor form by signal peptidase after the proteins are transported to the ER, and the resulting proteins move along the secretory pathway to their intracellular (*e.g.*, the Golgi apparatus, cell membrane or cell wall) or extracellular locations. ER targeting signal peptides as used herein include amino-terminal hydrophobic sequences which are usually enzymatically removed following the insertion of part or all of the protein through the ER membrane into the lumen of the ER. Thus, it is known in the art that a signal precursor form of a sequence can be present as part of a precursor form of a protein, but will generally be absent from the mature form of the protein. When a protein is said to comprise an ER targeting signal sequence, it is to be understood that, although a precursor form of the protein does contain the signal sequence, a mature form of the protein will likely not contain the signal sequence. Examples of ER targeting signal peptides or sequences that are functional in mammalian cells include the following: the signal sequence for interleukin-7 (IL-7) described in U.S. Patent No. 4,965,195; the signal sequence for interleukin-2 receptor described in Cosman *et al.* ((1984), *Nature* 312:768); the interleukin-4 receptor signal peptide described in EP Patent No. 0 367 566; the type I interleukin-1 receptor signal sequence described in U.S. Patent No. 4,968,607; the type II interleukin-1 receptor signal peptide described in EP Patent No. 0 460 846; the signal sequence of human IgG (METDTLLLWVLLLWVPGSTG); and the signal sequence of human growth hormone (MATGSRTSLLLAFGLLCLPWLQEGSA). Many other ER-targeting signal sequences are known in the art, including ones from prokaryotes (*e.g.*, bacteriophages), insects (copepods, ostracods, *etc.*), reptilians and avians as well as artificial ER targeting signal sequences such as: LLLVGILFWA and MRLLLLLLLLLLPQAQA(Lobigs *et al.*, 1990. *J Virol.* 64(9):4346-55). Reference also may be made to several reviews by Gunnar von Heijne, which describe ER targeting signal peptides and methods to predict them (*see*, for example, von Heijne, G. 1990. *J. Membrane Biol.* 115:195-201; von Heijne, G. 1990. *Current Opinion in Cell Biology* 2:604-608; Nielsen *et al.*, 1999. *Protein Engineering*12(1):3-9; and Emanuelsson *et al.*, 2001. *Biochimica et Biophysica Acta* 1541:114-119). HBsAgS has an internal signal sequence which is not cleaved. However, a long foreign sequence (antibody variable domain) fused to HBsAgS makes the HBsAgS-specific internal signal sequence not operational, and therefore a

signal sequence may be provided upstream of the variable domain. Antibody chains have signal sequences which allow antibody secretion via ER.

[00158] By "corresponds to" or "corresponding to" is meant a nucleic acid sequence that displays substantial sequence identity to a reference nucleic acid sequence (*e.g.*, at least about 30, 35, 40, 45, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 97, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or even up to 100% sequence identity to all or a portion of the reference nucleic acid sequence) or an amino acid sequence that displays substantial sequence similarity or identity to a reference amino acid sequence (*e.g.*, at least 30, 35, 40, 45, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 97, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or even up to 100% sequence similarity or identity to all or a portion of the reference amino acid sequence).

[00159] "Subjects" contemplated in the present description include humans or animals including laboratory animals or art accepted test animals. Patients include human subjects in need of treatment or prophylaxis.

[00160] As used herein, the terms "treatment", "treating", and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment", as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, *i.e.*, arresting its development; and (c) relieving the disease, *i.e.*, causing regression of the disease.

[00161] The present invention also relates to a vaccine comprising the herein described VLPs, in admixture with a suitable pharmaceutically acceptable diluent or carrier. The vaccine may be lyophilized prior to use and may furthermore be admixed with suitable adjuvants. Accordingly the vaccine may be in kit form. In some embodiments, the present invention provides a vaccine having confirmed VLP production in cell culture, the DNA construct will be used to immunise BALB/c mice, such that the VLPs are expressed *in vivo*. The use of this DNA vaccine construct to assess immune responses is two-fold: (1) it will

enable a more rapid assessment of its immunogenicity before VLP-Ab expression is performed in yeast or cell line and (2) it will provide the potential to use it as part of a DNA-VLP-Ab prime-boost vaccine strategy.

[00162] By "pharmaceutically acceptable" carrier, or diluent is meant a pharmaceutical vehicle comprised of a material that is not biologically or otherwise undesirable, i.e. the material may be administered to a subject along with the selected active agent without causing any or a substantial adverse reaction. Carriers may include excipients and other additives such as diluents, detergents, coloring agents, wetting or emusifying agents, pH buffering agents, preservatives, and the like. Conventional pharmaceutically acceptable carriers, excipients, buffers or diluents, may be included in vaccine compositions of this invention. Generally, a vaccine composition in accordance with the present invention will comprising an immunologically effective amount of the VLP-Ab, and optionally an adjuvant, in conjunction with one or more conventional pharmaceutically acceptable carriers and/or diluents. An extensive though not exhaustive list of adjuvants can be found in Coulter and Cox, "Advances in Adjuvant Technology and Application", in *Animal Parasite Control Utilizing Biotechnology*, Chapter 4, Ed. Young, W.K., CRC Press 1992, and in Cox and Coulter, "Adjuvants – A Classification and Review of Their Modes of Action", *Vaccine***15(3)**, 248-256, 1997. As used herein "pharmaceutically acceptable carriers and/or diluents" include any and all solvents, dispersion media, aqueous solutions, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art and is described by way of example in *Remington's Pharmaceutical Sciences*, 18th Edition, Mack Publishing Company, Pennsylvania, U.S.A.

[00163] The terms “composition” "compound", "active agent", "pharmacological agent" or "physiological agent", "medicament", "agent" and "drug" are used to refer to a chemical compound that induces a desired pharmacological and/or physiological effect. The terms also encompass pharmaceutically acceptable and pharmacologically active ingredients of those active agents specifically mentioned herein including but not limited to salts, esters, amides, prodrugs, active metabolites, analogs and the like. When the terms "compound", "active agent", "pharmacologically active agent", "medicament", "active" and "drug" are used, then it is to be understood that this includes the active agent *per se* as well as pharmaceutically acceptable, pharmacologically active salts, esters, amides, prodrugs, or pro-forms,

enantiomers, metabolites, analogs, etc. The term "agent" is not to be construed as a chemical compound only but extends to peptides, polypeptides and proteins as well as genetic molecules such as RNA, DNA and chemical analogs thereof.

[00164] An "effective amount" means an amount necessary to at least partially attain the desired immunological response. An effective amount for a human subject lies in the range of about 0.1ng/kg body weight/dose to about 1g/kg body weight/dose. In some embodiments, the range is about 0.1μg to 1g, about 1mg to 1g, 1mg to 500mg, 1mg to 250mg, 1mg to 50mg, or 1μg to 1mg/kg body weight/dose. Illustrative dosages include 20-75 μg/adult and 15-30 μg/child. Dosage regimes are adjusted to suit the exigencies of the situation and may be adjusted to produce the optimum therapeutic or prophylactic dose. For example, several doses may be provided daily, weekly, monthly or other appropriate time intervals.

[00165] The VLPs, and polypeptide nucleic acid molecules of the present invention can be formulated in pharmaceutical compositions which are prepared according to conventional pharmaceutical compounding techniques. See, for example, Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing, Company, Easton, PA, U.S.A.). The composition may contain the active agent or pharmaceutically acceptable salts of the active agent. These compositions may comprise, in addition to one of the active substances, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g. topical, intravenous, oral, intrathecal, epineural or parenteral.

[00166] For oral administration, the compounds can be formulated into solid or liquid preparations such as capsules, pills, tablets, lozenges, powders, suspensions or emulsions. In preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, suspending agents, and the like in the case of oral liquid preparations (such as, for example, suspensions, elixirs and solutions); or carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations (such as, for example, powders, capsules and tablets). Because of their ease in administration, tablets and capsules represent the most

advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be sugar-coated or enteric-coated by standard techniques.

[00167] For parenteral administration, the compound may be dissolved in a pharmaceutical carrier and administered as either a solution of a suspension. Illustrative of suitable carriers are water, saline, dextrose solutions, fructose solutions, ethanol, or oils of animal, vegetative or synthetic origin. The carrier may also contain other ingredients, for example, preservatives, suspending agents, solubilizing agents, buffers and the like. When the compounds are being administered intrathecally, they may also be dissolved in cerebrospinal fluid.

[00168] The active agent is preferably administered in a therapeutically effective amount. The actual amount administered and the rate and time-course of administration will depend on the nature and severity of the condition being treated. Prescription of treatment, e.g. decisions on dosage, timing, etc. is within the responsibility of general practitioners or specialists and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of techniques and protocols can be found in Remington's Pharmaceutical Sciences, *supra*.

[00169] As used herein, the term "about" refers to a quantity, level, value, percentage, dimension, size, or amount that varies by as much as 30%, 20% or 10% to a reference quantity, level, value, percentage, dimension, size, or amount.

[00170] An "antigen" or "immunogen" refers to a molecule containing one or more epitopes (either linear, conformational or both) that will stimulate an immune system to make a humoral and/or cellular antigen-specific response. Generally, a B-cell epitope will include at least about 5 amino acids but can be as small as 3-4 amino acids. A T-cell epitope, such as a cytolytic T-cell (CTL) epitope, will include at least about 7-9 amino acids, and a helper T-cell epitope at least about 12-20 amino acids. Normally, an epitope will include between about 7 and 15 amino acids, such as, 9, 10, 12 or 15 amino acids. The term "antigen" denotes both subunit antigens, (i.e., antigens which are separate and discrete from a whole organism with which the antigen is associated in nature). An oligonucleotide or polynucleotide which expresses an antigen or antigenic determinant *in vivo*, such as in gene therapy and DNA immunization applications, is also included in the definition of antigen herein.

[00171] An "immunological response" to an antigen or composition is the development in a subject of a humoral and/or a cellular immune response to an antigen present in the composition of interest. The ability of a particular antigen to stimulate a cell-mediated immunological response may be determined by a number of assays, such as by lymphoproliferation (lymphocyte activation) assays, CTL cytotoxic cell assays, or by assaying for T-lymphocytes specific for the antigen in a sensitized subject. Such assays are well known in the art. Methods of measuring cell-mediated immune response include measurement of intracellular cytokines or cytokine secretion by T-cell populations, or by measurement of epitope specific T-cells. The immune response may serve to neutralize infectivity, and/or mediate antibody-complement, or antibody dependent cell cytotoxicity (ADCC) to provide protection to an immunized host. Such responses can be determined using standard immunoassays and neutralization assays, as known in the art. An "immunogenic composition" is a composition that comprises an antigenic molecule where administration of the composition to a subject results in the development in the subject of a humoral and/or a cellular immune response to the antigenic molecule of interest.

[00172] "Operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus, a given promoter operably linked to a coding sequence is capable of effecting the expression of the coding sequence when the proper enzymes are present. The promoter need not be contiguous with the coding sequence, so long as it functions to direct the expression thereof.

[00173] "Recombinant" as used herein to describe a nucleic acid molecule means a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which, by virtue of its origin or manipulation: (1) is not associated with all or a portion of the polynucleotide with which it is associated in nature; and/or (2) is linked to a polynucleotide other than that to which it is linked in nature. The term "re-combinant" as used with respect to a protein or polypeptide means a polypeptide produced by expression of a recombinant polynucleotide. "Recombinant host cells," "host cells," "cells," "cell lines," "cell cultures," and other such terms denoting prokaryotic microorganisms or eukaryotic cell lines cultured as unicellular entities, are used interchangeably, and refer to cells which can be, or have been, used as recipients for recombinant vectors or other transfer DNA, and include the progeny of the original cell which has been transfected.

[00174] Generally, a viral polypeptide is "derived from" a particular polypeptide of a virus (viral polypeptide) if it is (i) encoded by an open reading frame of a polynucleotide of that virus (viral polynucleotide), or (ii) displays sequence identity to polypeptides of that virus as described herein.

[00175] By "expression vector" is meant any autonomous genetic element capable of directing the transcription of a polynucleotide contained within the vector and suitably the synthesis of a peptide or polypeptide encoded by the polynucleotide. Such expression vectors are known to practitioners in the art.

[00176] The term "isolated" means material that is substantially or essentially free from components that normally accompany it in its native state. For example, an "isolated nucleic acid molecule", as used herein, refers to a nucleic acid or polynucleotide, isolated from the sequences which flank it in a naturally-occurring state, e.g., a DNA fragment which has been removed from the sequences that are normally adjacent to the fragment. Alternatively, an "isolated modified protein" or an "isolated polypeptide" and the like, as used herein, refer to *in vitro* isolation and/or purification of a protein from its natural cellular environment, and from association with other components of the cell. Without limitation, an isolated nucleic acid, polynucleotide, peptide, or polypeptide can refer to a native sequence that is isolated by purification or to a sequence that is produced by recombinant or synthetic means.

[00177] The terms "polypeptide" "protein" and "peptide" and "glycoprotein" are used interchangeably and mean a polymer of amino acids not limited to any particular length. The term does not exclude modifications such as myristylation, glycosylation, phosphorylation and addition of signal sequences.

[00178] "Synthetic" sequences, as used herein, includes polynucleotides whose expression has been optimized as described herein, for example, by codon substitution, deletions, replacements and/or inactivation of inhibitory sequences. "Wild-type" or "native" sequences, as used herein, refers to polypeptide encoding sequences that are essentially as they are found in nature.

[00179] The terms "expression" or "gene expression" refer to either production of RNA message or translation of RNA message into proteins or polypeptides. Detection of either types of gene expression in use of any of the methods described herein are part of the invention.

[00180] By "biologically active portion" or "biologically active part" or "functional part or portion" is meant a portion of a full-length polypeptide which portion retains at least part of the activity of the full length molecule at least in so far as it retains the structural and functional abilities to for example, form VLPs or, in the case of an antibody, to bind antigen or in the case of an antigen to elicit an immune response. As used herein, the term "biologically active portion" includes deletion mutants and peptides, for example of at least about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150, 300, 350 contiguous amino acids (and every integer in between), which retains activity. Variants of this type may be obtained through the application of standard recombinant nucleic acid techniques or synthesized using conventional or state of the art liquid or solid phase synthesis techniques. Recombinant nucleic acid techniques can also be used to produce such portions. The biological activities of portions are tested *in vivo* and/or *in vitro*.

[00181] "Hybridization" is used herein to denote the pairing of complementary nucleotide sequences to produce a DNA-DNA hybrid or a DNA-RNA hybrid. Hybridization can occur under varying circumstances as known to those of skill in the art. The phrase "hybridizing specifically to" and the like refer to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex mixture (e.g., total cellular) DNA or RNA.

[00182] In some embodiments VLP-Abs comprises one or more detectable or purification tags or markers to facilitate detection or purification. In some embodiments, the second protein and/or the EAFP comprises a detection domain.

[00183] The description herein is not limited to a particular detection marker and extends to qualitative or quantitative detection using any of the commonly used reporter molecules in detection assays, such as enzymes, fluorophore and radionuclide containing molecules and chemilluminescent molecules. By 'detection marker' or 'detection tag' is meant a molecule or particle which, by its chemical nature, provides an analytically identifiable signal which allows the detection of an VLP-Ab particle. Tags are well recognised, a wide variety of different reporter systems are available and those allowing rapid visual detection are clearly the most useful in the context of, for example, point of care diagnostics.

[00184] In some embodiments, the detection marker is a visually detectable reporter molecule such as a colloidal particle or microparticle. Colloidal metal and metalloid particles include those comprising gold, silver, platinum, iron, copper, selenium; metal complexes such as cyclopentadienylmanganese(I) tricarbonyl, gold cluster; and microparticles such as latex and dyed latex particles. The detectable modification is conveniently selected from: a fluorescence molecule, a chromogen, a catalyst, an enzyme, a dye such as an infrared dye, a fluorochrome, a chemiluminescent, bioluminescent or phosphorescent moiety, a lanthanide ion, a radioisotope or a visual label such as gold or silver nanoparticles. Fluorescent molecules are particularly well established however, this is a rapidly moving field and the present invention is in no way limited to the use of any particular detectable modification. In some embodiments distinguishable compounds such as fluorophores, dyes or particles are used to facilitate combinatorial analyses. In the case of a direct visual label, use may be made of a colloidal metallic or non-metallic particle, a dye particle, bioluminescent enzymes, an enzyme or a substrate, an organic polymer, a latex particle, a liposome, or other vesicle containing a signal producing substance and the like. Especially preferred labels of this type include large colloids, for example, metal colloids such as those from gold, selenium, silver, tin and titanium oxide. In one embodiment in which an enzyme is used as a direct visual label, biotinylated residues are incorporated. Suitable fluorochromes include, but are not limited to, fluorescein isothiocyanate (FITC), tetramethylrhodamine isothiocyanate (TRITC), R-Phycoerythrin (RPE), and Texas Red. Other exemplary fluorochromes include those discussed by Dower *et al.* (International Publication WO 93/06121). Reference also may be made to the fluorochromes described in U.S. Patents 5,573,909 (Singer *et al*), 5,326,692 (Brinkley *et al*). Alternatively, reference may be made to the fluorochromes described in U.S. Patent Nos. 5,227,487, 5,274,113, 5,405,975, 5,433,896, 5,442,045, 5,451,663, 5,453,517, 5,459,276, 5,516,864, 5,648,270 and 5,723,218. Commercially available fluorescent labels include, for example, fluorescein phosphoramidites such as Fluoreprime (Pharmacia), Fluoredite (Millipore) and FAM (Applied Biosystems International), Texas Red, NBD, coumarin, dansyl chloride and rhodamine. Radioactive reporter molecules include, for example, ^{32}P, which can be detected by an X-ray or phosphoimager techniques.

[00185] The present invention extends to qualitative or quantitative detection using any of the commonly used reporter molecules in detection assays known in the art such as enzymes, fluorophores or radionuclide containing molecules, chemilluminescent molecules and binding

molecules such as binding pairs. In the case of an enzyme immunoassay, an enzyme is conjugated to a second antibody generally by means of glutaraldehyde or periodate. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates which yield a fluorescent product rather than the chromogenic substrates listed above. In all cases, the enzyme labelled antibody is added to the first antibody antigen complex, allowed to bind, and the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantified, usually spectrophotometrically, to give an indication of the amount of antigen which is present in the sample. Alternatively, fluorescent compounds, such as fluorescein and rhodamine are chemically coupled to antibodies without altering their binding capacity. When activated by a illumination with light of a particular wave length, the fluorochrome labelled antibody absorbs the light energy inducing a state of excitability in the molecule followed by emission of the light at a characteristic wavelength visually detectable with a microscope. The term 'binding partner' or 'binding pair' is a reference to complementary molecules which bind or interact with each other *via* a reversible non-covalent or covalent attachment determined by their structure. Other binding relationships are known to those skilled in the art, such as for example those employing glutathione, nickel-chelators and leucine zipper binding pairs (c-Jun and vFos) and any such binding relationship is included herein. Binding pairs may be used in detection and/or purification. Affinity chromatography typically uses binding pairs or ligand substrate interactions to purify a polypeptide of interest.

[00186] In order that the invention may be readily understood and put into practical effect, particular preferred embodiments will now be described by way of the following non-limiting examples.

EXAMPLE 1

Design and synthesis of VLPs decorated with antibody domains

[00187] To demonstrate that VLPs with exposed functional antibody domains can be designed and produced, the antigen-binding domains derived from a characterised rat monoclonal anti-mouse Clec12a antibody were utilised (Lahoud *et al.* (2009) *J. Immunol.* 182:7587-94).

[00188] Clec12a is a member of the C-type lectin receptors, which comprise a heterogeneous family of soluble and transmembrane proteins defined by a characteristic C-type lectin domain. C-type lectins have multiple roles, they recognise microbial carbohydrate moieties, sense products from dying cells, and transduce inflammatory signals to modulate the immune response. The C-type lectin Clec12a is expressed on monocytes, dendritic cells and various macrophage cell lines on human and mouse cells. Clec12a engagement enhances LPS responses and the expression of the CCR7 chemokine. Clec12a can be activated by antibody-binding and supports the internalisation of bound anti-Clec12 antibody making it an attractive target molecule for the purposes of immunotherapies. Monoclonal antibodies targeting human and mouse Clec12a were generated, and utilised for the induction of potent immune responses. The cDNA sequence of the rat IgG2a 1/06-5D3 (anti-mouse Clec12a) monoclonal antibody (mAb) (Lahoud *et al.* (2009) *J. Immunol.* 182:7587-94)was used to generate expression vectors encoding the variable domains of the heavy chain (γV) or light chain (κV) fused to the HBsAgS envelope protein (Figure 1).

[00189] To generate VLPs with arrayed antigen-binding domains, the cDNAs specific for the variable regions of γ heavy and κ light chains of the rat anti-mouse Clec12a monoclonal antibody were amplified and inserted upstream of the HBsAgS open reading frame. The sequences for the antibody binding domain and HBsAgS envelope protein are separated by a glycine-serine linker sequence (Figure 1 B,C). To develop a particulate delivery system decorated with functional antibody binding domains, the γV-HBsAgS- (γV-S)and κV-HBsAgS-fusion (κV-S) proteins are co-expressed in the HEK293T cell line. Alternatively, γV-S fusion proteins are complemented *in trans* by co-expressing the partner κ variable antibody domain in the absence of the HBsAgS fusion partner, κVC or κVmyc (Figure 1D,

E). Co-expression of wtHBsAgS assists VLP formation, allowing the co-packaging of secretion-defective mutant HBsAgS proteins into secretion-competent VLPs.

[00190] The γV-S fusion protein was expressed in HEK293T cells in the presence or absence of the κVC or κVmyc proteins, then the presence of HBsAgS activity in the cell culture supernatant determined. HBsAgS activity in the supernatant proves that the chimeric protein is secretion competent, and strongly indicates that the chimeric HBsAgS protein is functional and forms VLPs. The co-expression of γV-S fusion and κVC allows the detection of the expressed proteins in the cell culture supernatant, 42/45kDa γV-S fusion (un/glycosylated) and the 26kDa κVC protein (Figure 2, lane D, κVC highlighted by asterisk, γV-S fusion proteins with a diamond). As a negative control, the presence of wtHBsAgS does not support co-packaging of κVC (Figure 2, lane C).

[00191] To assess whether the variable antibody domains arrayed on VLPs form a functional antigen-binding domain, an enzyme immunosorbent assay (EIA) was performed with the Clec12a protein as target (Figure 6). The VLPs bound to Clec12a were monitored by anti-HBsAgS antibodies. Three different types of decorated VLPs were assessed for binding to the Clec12a protein, VLPs composed of both γV-S and κV-S fusion proteins in the presence of wtHBsAgS (Figure 6A), VLPs composed of the γV-S fusion protein in the presence of wtHBsAgS, and complemented *in trans* with the κVC or κVmyc (Figure 6B, C). Wildtype VLPs served as negative control, and the anti-Clec12a antibody as a positive control (Figure 6D, E). Bovine serum albumin (BSA) was included as an unspecific binding target. Preliminary data indicate that VLPs containing γV-S complemented *in trans* with the κVC or κVmyc proteins showed more efficient binding than VLPs containing both γV- and κV-S fusion proteins (Figure 6A-C) possibly due to incorrect positioning of both fusion proteins within the particle as depicted in Figure 1F. The decorated VLPs did not bind to BSA excluding non-specific binding. Consistently, wtVLPs did not bind to Clec12a (Figure 6E).

[00192] To demonstrate that the decorated VLPs bind to the native Clec12a receptor expressed on cells, binding studies were performed with Chinese Hamster Ovary (CHO) cells in the presence of Clec12a expression (Figure 7). Wildtype VLPs and VLPs composed of γV-S,κVmyc and wtHBsAgs were incubated with CHO and CHO/Clec12a cells, and binding assessed with anti-HBsAgS antibodies. The analysis by flow cytometry revealed that the

decorated VLPs bound to CHO cells expressing Clec12a. Wildtype VLPs did not show any binding to CHO or CHO/Clec12a cells.

[00193] The EIA studies with Clec12a coated onto plates and the flow cytometry studies with cells expressing the Clec12a receptor protein strongly indicate that the heavy and light chains fold and pair correctly to form a functional antigen-binding site.

The density of antibody domains on the VLP surface may influence the binding ability. To identify VLPs with optimal binding parameters, different sets of VLPs distinguished by different ratios of wtHBsAgS versus γ/κV-S fusion proteins are assessed in the binding assays. This includes a repetition of the experiments shown in Figure 6 to allow statistical analyses. To demonstrate that the authenticity of the antigen-binding domain is retained in the VLP context, competitive binding assays of decorated VLPs to the Clec12a receptor in the presence of anti-Clec12a antibodies are performed (Figure 8 and 9). As negative control, a rat antibody with the same isotype as anti-Clec12a but non-related antigen-binding specificity is included.

[00194] The secretion competence of the HBsAgS fusion proteins strongly indicates that the HBsAgS domain has retained functionality and hybrid particles are formed. To confirm that chimeric HBsAgS proteins have retained their ability to package particles, packaging studies with the hepatitis delta antigen large (HDAg-L) are performed (Figure 15). Correctly folded HBsAgS protein domains have the ability to package HDAg-L into particles, and to assist HDAg-L secretion (Wang *et al.*(1991)*J. Virol.*65:6630-36). Co-transfection experiments in the presence of expression plasmids encoding for HDAg-L, κV-, γV-S-fusion proteins and wtHBsAgS are performed, secretion of HDAg-L in the supernatant are quantified by immunoblot analysis with anti-HDAg-L antibodies. To confirm that HDAg-L is packaged within hybrid particles composed of wtHBsAgS proteins and κV- or γV-S-fusion proteins, the VLPs are affinity purified using the Clec12a target protein. The HDAg-L packaging experiments are complemented by determining the density of the generated VLPs using a 10 to 40% (wt/wt) CsCl step gradient. The fractions containing VLPs are identified by using an HBsAgS specific EIA. The positive fractions are desalted, concentrated, and the VLPs visualised by electron microscopy using a negative staining procedure with 2% uranyl acetate.

[00195] To assess that the decorated VLPs have a higher avidity and favourable on/off rates compared to the parent antibody, binding of decorated VLPs and anti-Clec12a antibodies

are performed using plasmon resonance measurements (BIAcore). Clec12a protein are immobilised on the CM5 or CM4 sensor chips using established procedures (Veiga *et al.* (2009) *Chem. Biochem.* 10:1032-1044). VLPs composed of both κV- and γV-S fusions, and VLPs composed of γV-S fusions complemented *in trans* with the unfused κVmyc or κVC proteins are assessed for association and dissociation rates, and the binding affinity are determined using BIAevaluation software (GE Healthcare). These studies will determine the biophysical parameters and binding affinities of the different decorated VLP sets compared to the parent anti-Clec12a antibody.

[00196] To determine whether the decorated VLPs (wtHBsAgS + γV-S + kVmyc) exhibit an enhanced immunogenicity compared to wtVLPs, immunisation studies in C57/Bl6 mice were performed (Figure 10). Immunisations of mice with a rat anti-mouse Clec12a mAb (1/06-5D3) resulted in enhanced anti-rat immunoglobulin antibody responses compared to immunisations with a control rat mAb (Lahoud *et al.* (2009) *J. Immunol.* 182:7587-94). Following protocols established with the 1/06-5D3 mAb, mice were subcutaneously immunised three times at two week intervals with wtVLPs or decorated VLPs in the presence of the adjuvant CpG. Serum samples were taken after the second and third immunisation. Higher anti-HBsAgS antibody responses were detected in serum samples from mice immunised with the decorated VLPs compared to mice immunised with wtVLPs after the second immunisation. The data shown are derived from samples taken after the third immunisation, the differences are statistically highly significant (Figure 10).

[00197] The immunisation studies as shown in Figure 10 are continued with VLPs containing different levels of anti-Clec12 binding domains. The antibody titres are determined, and monitored over a time period of at least 6 months after the last immunization (Figure 11 and 16). Model epitopes derived from ovalbumin (Ova) are utilised to show VLPs decorated with anti-Clec12a variable domain have the capability to induce an enhanced activation of naive T cells, (Robertson *et al.* (2000) *J. Immunol.* 164:4706-12; Lahoud *et al.*(2009) *J. Immunol.*182:7587-94) showed that Ova conjugated to anti-Clec12a mAb induced Ova-specific T cells to proliferate. To assess MHC class II antigen presentation, γV-S fusion proteins expressing $Ova_{323-339}$ peptide (γV-VLP-Ova_{323}) are generated. MHC class I antigen presentation are measured using γV-S fusion proteins containing $Ova_{257-264}$ peptide (γV-VLP-Ova_{257}). The model epitopes are introduced into the γV-S fusion proteins, and the

immunogenicity of resulting hybrid VLPs determined. To measure the activation of naive T cells, CFSE-labelled Ova-specific CD8 (OT-I) or CD4 (OT-II) transgenic T cells are adoptively transferred into mice, which are immunised with VLPs decorated with the anti-Clec12a specific variable domains. Non-decorated VLPs with the Ova model epitopes are used as control immunogens. After three days, spleens are harvested, and the proliferative response of the transgenic T cells counted by flow cytometry. All of the methodologies are established to determine the cellular immunogenicity associated with the decorated VLPs compared to the control non-decorated VLPs.

[00198] VLPs decorated with the anti-Clec9a specific antigen-binding domains are administered, and the anti-HBsAgS immune response measured. The Clec9a receptor with a selective expression pattern allows specific antigen targeting to the $CD8^+$ subset, and therefore represents a useful target for the induction of antiviral or antitumor CTLs. A highly immunogenic decorated VLP can possibly represent a successful tool for therapeutic purposes to overcome chronic hepatitis B. VLPs engineered with foreign antigenic sequences and therefore, decorated VLPs with the ability to deliver disease relevant antigens of interest are proposed as effective tools to induce potent immune responses against cancer cells or chronic infectious diseases.

[00199] Humoral and cellular immunity obtained by using VLPs decorated with Clec9a-specific antibody domains are compared to outcomes obtained with wtVLPs. The assays to detect anti-HBsAgS antibodies are established, as well as to determine anti-HBsAgS specific CTL immune responses. HBsAgS-specific CTL epitopes presented by murine MHC-I molecules have been identified. Three different $H\text{-}2^d$-restricted CTL-epitopes have been used (Env362, Env364, Env28) to assess anti-HBsAgS specific cellular immune responses (Cheong *et al.* (2012) *Antiviral Res.* 93: 209-218). In addition to the utilisation of the wtHBsAgS protein as fusion partner for the antibody chains, chimeric HBsAgS proteins are used containing the $Ova_{323\text{-}339}$ and $Ova_{257\text{-}264}$ model epitopes for a detailed analysis of antigen presentation via MHC class I and class II molecules. The mouse models and tools to measure $Ova_{323\text{-}339}$ and $Ova_{257\text{-}264}$ specific T cell activation are available. The immunogenicity of VLPs decorated with anti-Clec12a and anti-Clec9a specific antigen-binding regions may be tested in a mouse model transgenic for HBsAg.

[00200] The isolation and characterisation of human monoclonal antibodies derived from B cells of HIV-1 infected patients has provided a considerable understanding of the

specificities of neutralising antibody responses to HIV-1 and the underlying mechanisms (Kwong & Mascola (2012) *Immunity Rev.* 37:412-25). The neutralising antibodies are directed against the HIV-1 envelope proteins, which are derived from a glycosylated gp160 precursor protein gp160 is cleaved by a host protease into the surface protein gp120 and the membrane-anchored protein gp41. Both proteins form heterotrimeric spikes and remain associated in the viral envelope by non-convalent interactions. gp41 contains the fusion domain, which mediates virus-to-cell membrane interaction. Broadly neutralising antibodies have been identified targeting gp120 and gp41, however the induction of their synthesis *in vivo* by active immunisation has been proven difficult as the target sites show structural complexities. Exceptionally broad neutralisation of diverse HIV-1 strains is achieved by recently discovered antibodies targeting the CD4 binding site of gp120. The monoclonal antibodies VRC01, VRC02, and VRC03 have the ability to neutralise over 90% of circulating HIV-1 isolates. An even more potent clonal variant of VRC01 was synthesised (NIH45-46), which was modified by rational design to increase contact with gp120 (Figure 12). Passive immunisation with neutralising antibodies prevented infection in non-human primates, protects neonatal primates.

[00201] As proposed herein, VLPs decorated with a highly effective and cross-neutralising antibody are used as an advanced tool for passive immunisation procedures. VLPs with anti-HIV-1 specific antibody domains have been successfully generated as shown herein. The variable antibody domain of the NIH45-46 γ heavy chain was fused to HBsAgS, and similar to the anti-Clec12a γV-antibody domain, was co-packed in the presence of wtHBsAgS, and secreted into the cell culture supernatant (Figure 12). Expression vectors specific for the variable domain of the NIH45-46 κ light chain are generated and provided *in trans* to allow the assembly of functional antibody binding domains. The 10E8 antibody targets an epitope located in the gp41 envelope protein. In addition to VLPs decorated with the NIH45-46 neutralising antibody domains, VLPs specific for the 10E8 antibody variable domain are generated, and assessed for their neutralising capability.

[00202] The neutralising capacity of the VLPs decorated with the antigen-binding domains from the 10E8 or NIH45-46 antibodies are tested by a single cycle infectivity assay employing gp120gp41-pseudotyped luciferase reporter HIV-1 particles as described (Dhillon *et al.* (2007) *J. Virol.* 81: 6548-62). The reporter viruses are produced in HEK293T cells by co-transfecting the luciferase reporter virus vector pNL4.3.Luc.R–E– and pcDNA3.1-based

HIV-1 gp120.gp41 expression vectors derived from a variety of primary HIV-1 clade A, B, C and D isolates. Pseudotypes containing the envelope proteins of amphotropic murine leukemia virus are used as a negative control for neutralisation assays. The functionality of the constructs in single cycle entry assays has been confirmed. The ability of the decorated VLPs to neutralise pseudovirions is determined using JC53 cells (a HeLa cell derivative that stably expresses CD4, CCR5 and CXCR4). The utility of these cells for neutralisation assays has been confirmed using HIV-1 pseudoparticles.

[00203] VLPs with optimal γV-S/κV-S to wtHBsAgS ratios, including optimised VLPs complemented with κVC or κVmyc with best binding results are identified. Binding kinetics, affinity constants and on/off rates are determined. Competitive binding studies in the presence of the Clec12a antibody are completed, including the biochemical characterisation of the decorated VLPs (e.g. density), EM studies finalised, HDAg-L packaging assessed. Alb1HBV transgenic mice are imported and bred. VLPs decorated with anti-HIV-1 and anti-Clec9a antigen-binding domains are designed and their production optimised followed by functionality tests, for anti-Clec9a VLPs: interaction with DCs, immunisation studies, for anti-HIV-1 VLPs: neutralisation of entry assays, binding kinetics.

Example 2

Clec12A-targeted VLPs can co-package and secrete mutant HBsAg subunits

[00204] Figure 2 shows that the Clec12A-targeted VLPs can be formed incorporating γV-S and either κV-S, κCV or κVmyc with wild-type HBsAgS. Figure 4 also shows that mutant HBsAg subunits (HBsAg N146Q; hypoglycosylated mutant) can also be incorporated into the VLPs. Figure 14 illustrates the ability to incorporate subunits which contain a mutant HBsAg-subunit which contains a neutralising epitope of HIV-1. In this Example, an additional mutant subunit has been shown to incorporate into targeted VLPs (Figure 28). The mutant subunits incorporate a component of the gene c-myc. C-myc is an oncogene and commonly used tag sequence.

Different antibody variable domains can be incorporated into secreted VLPs

[00205] Figure 2 and Figure 4, illustrate how a functional anti-Clec12A antibody variable domain (figure 2, figure 4), as well as an anti-HIV variable domain (Figure 12) can be incorporated into VLP and As further illustrated in Figure 29, it is possible to incorporate different antibody domains into the VLPs.

Different antibody and different fusion-protein type of antibody domains can be incorporated into VLPs.

[00206] As described herein in one aspect, the VLP-Ab comprises a single antibody variable domain fused to the HBsAg subunit and the partner (complementary binding) chain will be co-expressed within the VLP and find its way to bind to the fused antibody variable domain.

In a further aspect of the invention, the antibody heavy and light chain can be incorporated into a single chain scFv (single-chain fused-variable) fused to the HBsAg subunit in place of the single antibody domain previously used. The data illustrated in Figure 30 shows that these VLPs can be secreted. Additionally, this data shows the use of a further different antibody (anti-DEC205) specific for a different immune receptor.

Targeted VLPs induce better anti-HBsAg responses in aged mice

[00207] The immune system shows a reduced ability to induce immune responses after puberty, this is primarily due to sex hormones resulting in thymic atrophy and reducing the ability to responds to infection or vaccination. The inventors hypothesised that vaccines targeted to the immune system will result in better immune outcomes than vaccines that are not targeted to the immune system.

Figure 31 illustrates results of an immunisation trial performed in C57/Bl6 mice that were immunised with either wt HBsAg VLPs, Clec12A-targeted VLPs or HVR VLPs (HVR VLPs outlined in Figure 29 these VLPs contain antibody domains similar to the targeted VLPs, but they do not bind to the immune receptor).

Competitive binding assay performed with Clec12A-tageted VLPs and the parental antibody clone 5D3.

[00208] To show that the functionality and the specificity of the decorated Clec12A-targeted VLP has been retained competitive binding assays with the 5D3 antibody and the decorated VLP were performed. The experimental procedure and results are illustrated in figure 32 and the legend to Figure 32. The ability of the 5D3 antibody to prevent the binding of targeted VLPs along with the inability of the isotype control antibody and the PBS control to prevent binding of the targeted VLPs to the Clec12A protein indicates again that the identical antibody domain is retained and forms the correct conformation.

[00209] Many modifications will be apparent to those skilled in the art without departing from the scope of the present invention.

[00210] The disclosure of every patent, patent application, and publication cited herein is hereby incorporated herein by reference in its entirety.

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Zuckerman J. (2006) *Med. Virol.* 78:169-77

WHAT IS CLAIMED IS:

1. A recombinant VLP-antibody (VLP-Ab) comprising a modified hepadnaviral envelope fusion protein comprising an antibody variable domain (EAFP) wherein the variable domain is selected from an antibody heavy chain variable domain and a light chain variable domain, and wherein the variable domain is bound to a complementary antibody variable domain of a second variable domain protein and forms the antibody antigen binding site specific for a target molecule.

2. The VLP-Ab of claim 1, wherein the second variable domain protein is a modified hepadnaviral envelope fusion protein comprising a complementary antibody variable domain.

3. The VLP-Ab of claim 1, wherein the second variable domain protein is not a modified hepadnaviral envelope fusion protein.

4. The VLP-Ab of any one of claims 1 to 3, wherein the EAFP comprises an antibody constant domain.

5. The VLP-Ab of any one of claims 1 to 3, wherein the second variable domain protein comprises an antibody constant domain.

6. The VLP-Ab of any one of claims 1 to 5, wherein the EAFP comprises a single antibody variable domain.

7. The VLP-Ab of any one of claims 1 to 5, wherein the second variable domain protein comprises a single antibody variable domain.

8. The VLP-Ab of any one of claims 1 to 7 comprising a domain suitable for detection of the VLP-Ab.

9. The VLP-Ab of any one of claims 1 to 7 wherein the EAFP further comprises a heterologous antigen.

10. The VLP-Ab of any one of claims 1 to 9 wherein the VLP-Ab comprises a modified hepadnaviral envelope fusion protein comprising a heterologous antigen.

11. The VLP-Ab of any one of claims 1 to 10 wherein the modified hepadnaviral envelope fusion protein is a hepatitis B envelope protein or a VLP-forming part or variant thereof.

12. The VLP-Ab of any one of claims 1 to 11, wherein the EAFP and or modified hepadnaviral envelope fusion protein comprising a heterologous antigen comprises a deleted glycosylation site at N146 of S domain.

13. The VLP-Ab of any one of claims 1 to 12, comprising two or more different EAFPs comprising envelope protein epitopes from two or more hepadnaviral, such as hepatitis B virus, serotypes.

14. The VLP-Ab of any one of claims 1 to 13, wherein the antibody is specific for a target molecule which is a self or a non-self, antigen receptor or ligand.

15. The VLP-Ab of any one of claims 1 to 13, wherein the antibody specifically binds to a target molecule on a host cell, such as an antigen presenting cell.

16. The VLP-Ab of any one of claims 1 to 13, wherein the antibody specifically binds to a target molecule on an infectious agent.

17. The VLP-Ab of any one of claims 1 to 16, comprising antibody antigen binding sites of two or more different antigen specificities.

18. The VLP-Ab of any one of claims 1 to 17, wherein the modified hepadnaviral envelope fusion protein comprises a spacer domain between the antibody variable domain and the envelope protein.

19. The VLP-Ab of any one of claims 1 to 15, comprising packaged hepatitis delta (HDV or dAgL).

20. A eukaryotic expression vector encoding the VLP-Ab of any one of claims 1 to 19.

21. A eukaryotic expression vector comprising a recombinant nucleic acid molecule encoding a hepadnavirus envelope-antibody fusion protein comprising an antibody variable domain (EAFP) wherein the variable domain is selected from an antibody heavy chain variable domain and a light chain variable domain.

22. A eukaryotic expression vector comprising a recombinant nucleic acid molecule encoding a second variable domain protein comprising an antibody variable domain wherein the variable domain is selected from an antibody heavy chain variable domain and a light chain variable domain.

23. The expression vector of claim 21 or 22 encoding a single variable domain selected from an antibody heavy chain variable domain and a light chain variable domain.

24. A eukaryotic expression vector operably linked to a polynucleotide encoding a hepadnavirus envelope-antibody fusion protein comprising and an antibody variable domain (EAFP) wherein the antibody variable domain is selected from an antibody

heavy chain variable domain and a light chain variable domain, and further capable of expressing a second polynucleotide encoding a second variable domain protein comprising a complementary antibody variable domain.

25. An isolated eukaryotic cell comprising the expression vector of any one of claim 20 to 24.

26. A composition comprising a VLP-Ab of any one of claims 1 to 19.

27. A composition comprising the VLP-Ab of any one of claim 1 to 19 and a pharmaceutically or physiologically acceptable carrier and/or diluent.

28. A composition comprising a VLP-Ab of any one of claims 1 to 19 for use in medical therapy or imaging

29. Use of a composition comprising a VLP-Ab of any one of claims 1 to 19 in, or in the manufacture of a medicament for, the treatment or prevention of an infection of a subject by a pathogen, or an inflammatory, immunity or cancer-related condition in a subject.

30. A method for treating or preventing an HBV infection in a subject, the method comprising administering to the subject an effective amount of a composition comprising a VLP-Ab according to any one of claims 1 to 14, wherein the EAFP and/or the modified hepadnaviral envelope fusion protein is a hepatitis B envelope protein or a VLP-forming part or variant thereof and the target molecule directs the VLP-Ab to an antigen presenting cell.

31. A method for preparing a VLP-Ab, the method comprising transfecting a eukaryotic cell with an expression vector according to any one of claims 20 to 24, culturing the cell *in vitro* for a time and under conditions sufficient to permit VLP-Ab formation and optionally secretion, and at least partly purifying the VLP-Ab from the cell culture material.

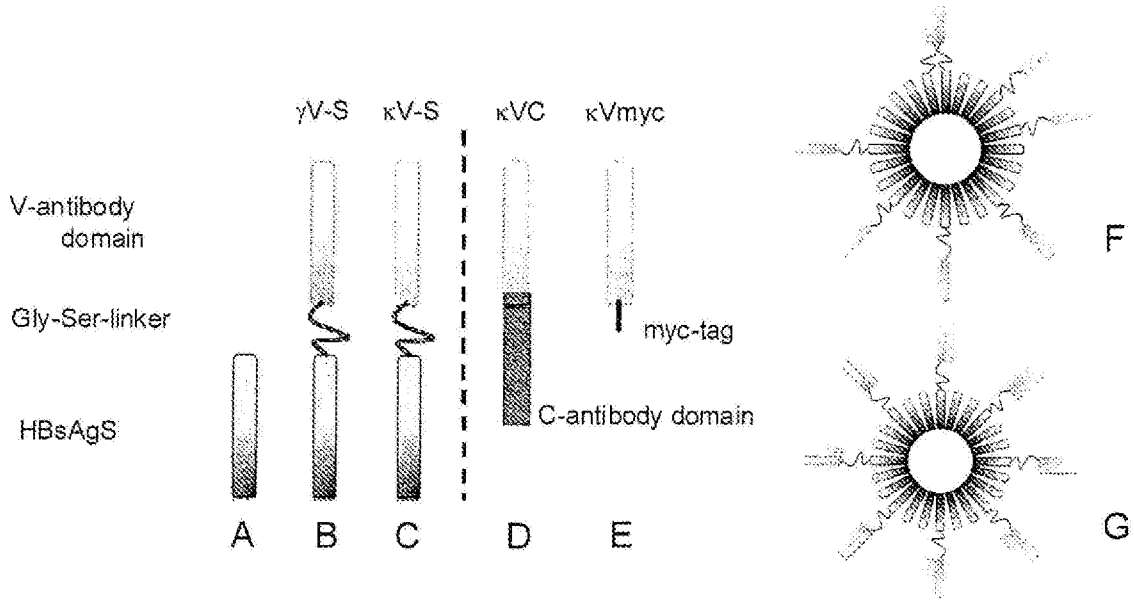

γV-S
κV-S
κVC
κVmyc
V-antibody domain
Gly-Ser-linker
HBsAgS
myc-tag
C-antibody domain
A
B
C
D
E
F
G

FIGURE 1

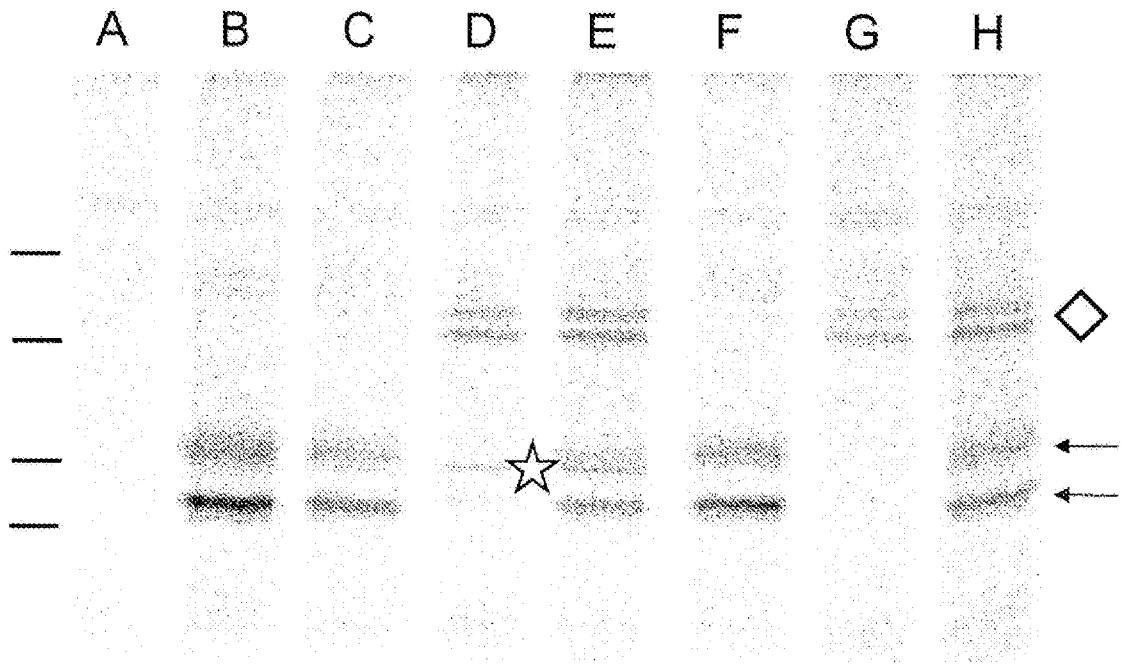


FIGURE 2

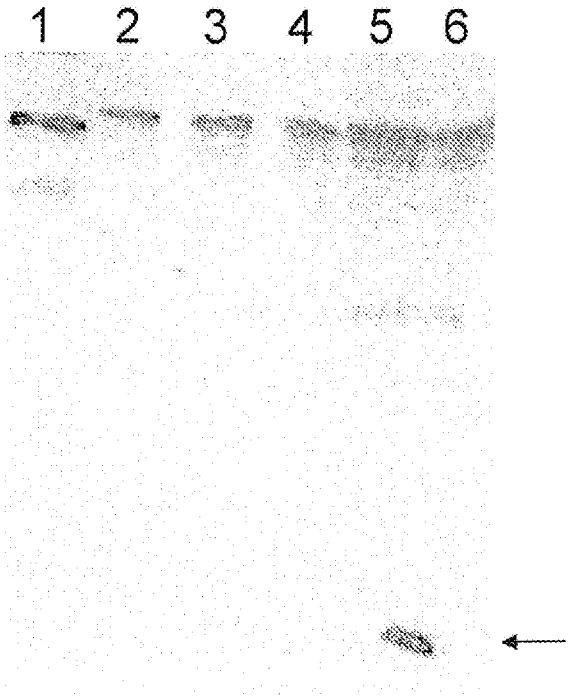


FIGURE 3

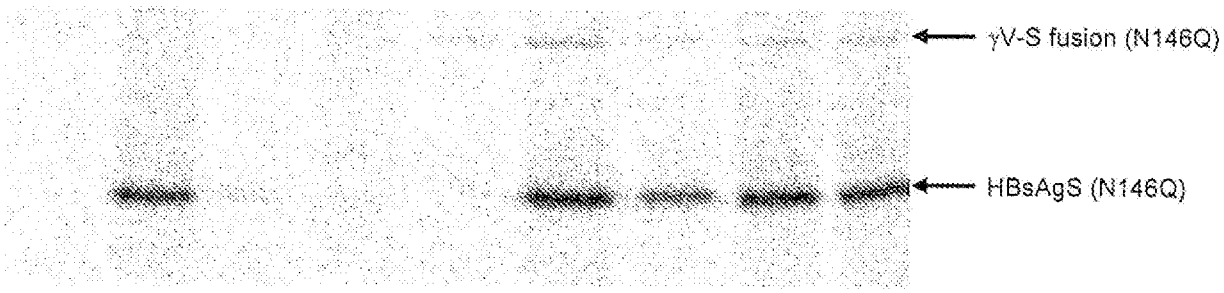
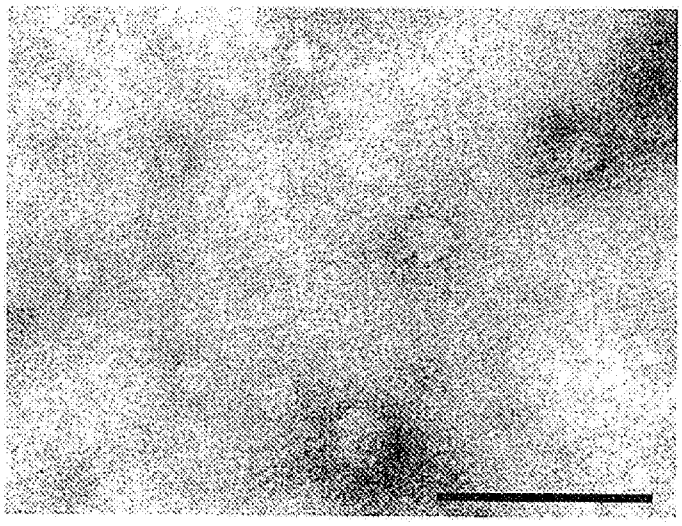
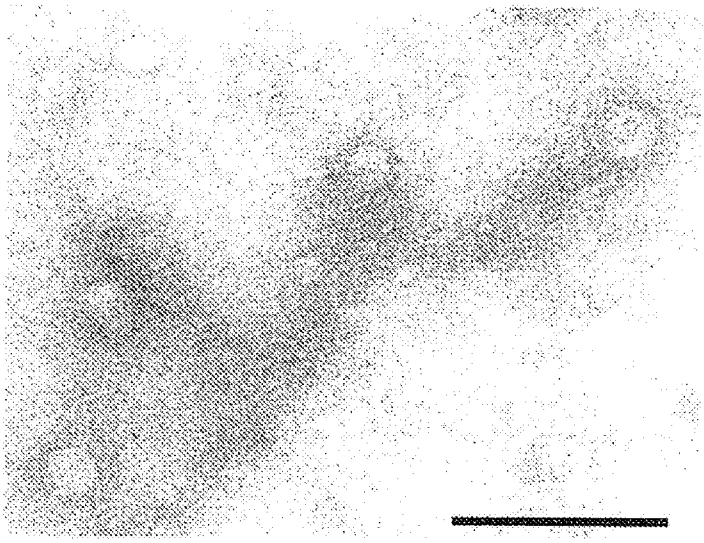


FIGURE 4

A



B



Bar represents 0.2μm

FIGURE 5

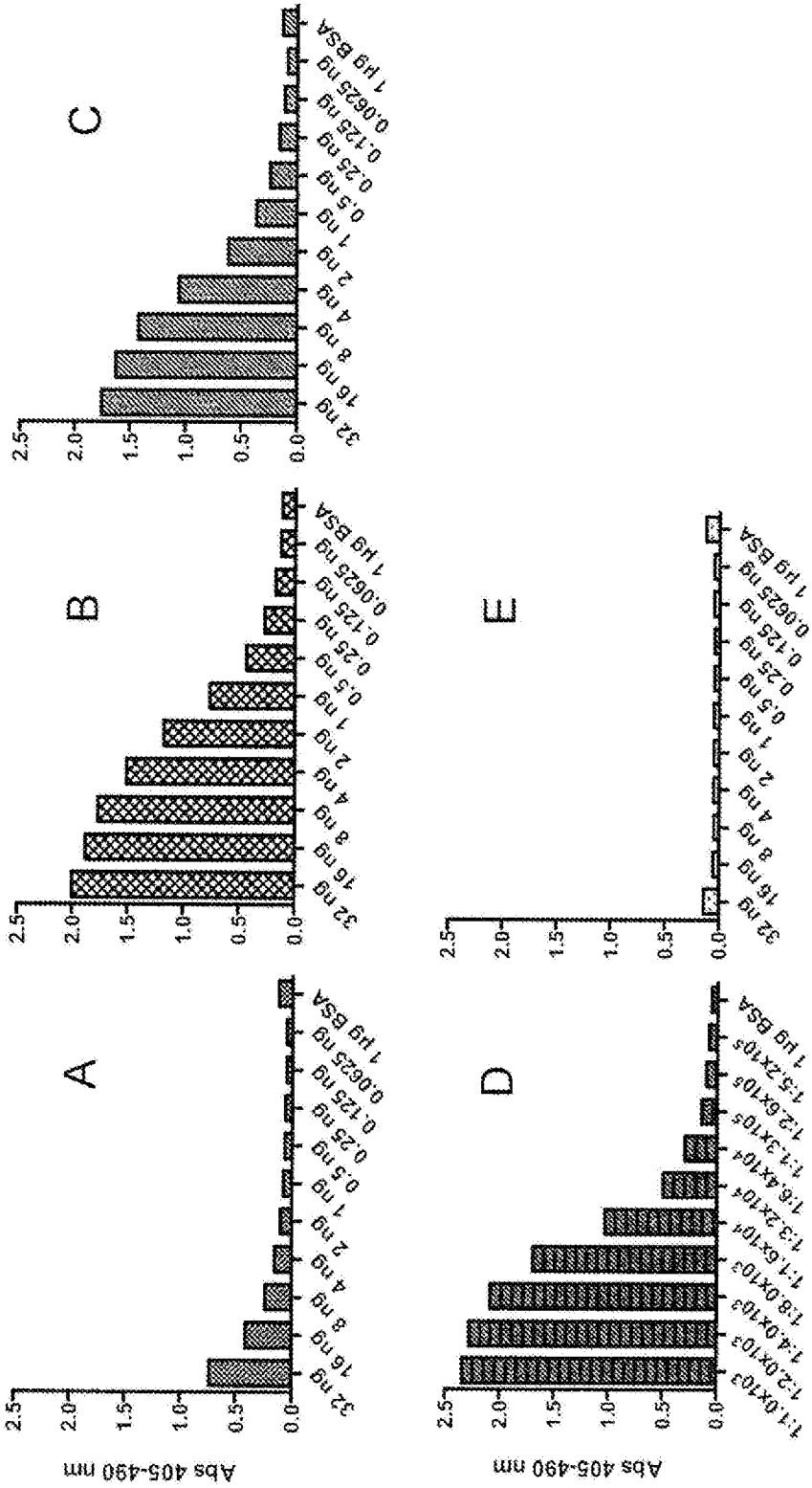

A
B
C
D
E
Abs 405-490 nm
2.5
2.0
1.5
1.0
0.5
0.0
32 ng
16 ng
8 ng
4 ng
2 ng
1 ng
0.5 ng
0.25 ng
0.125 ng
0.0625 ng
1 ng BSA
1:1.0X10^3
1:2.0X10^3
1:4.0X10^3
1:8.0X10^3
1:1.6X10^4
1:3.2X10^4
1:6.4X10^4
1:1.3X10^5
1:2.6X10^5
1:5.2X10^5
1 ug BSA

FIGURE 6

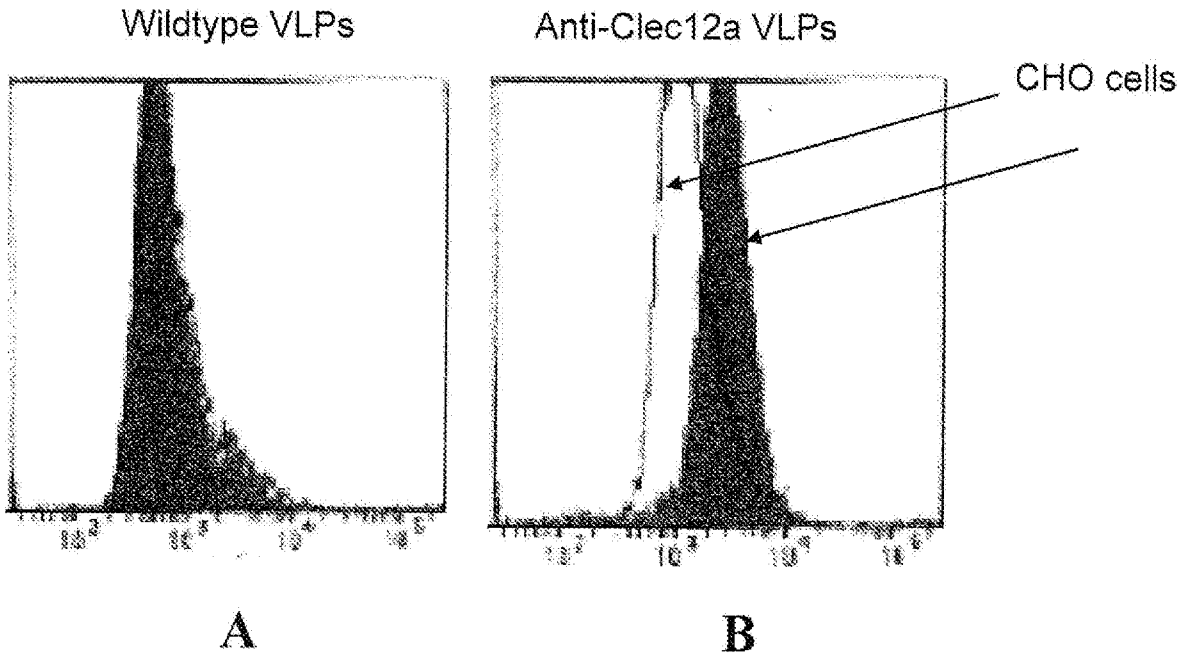


FIGURE 7

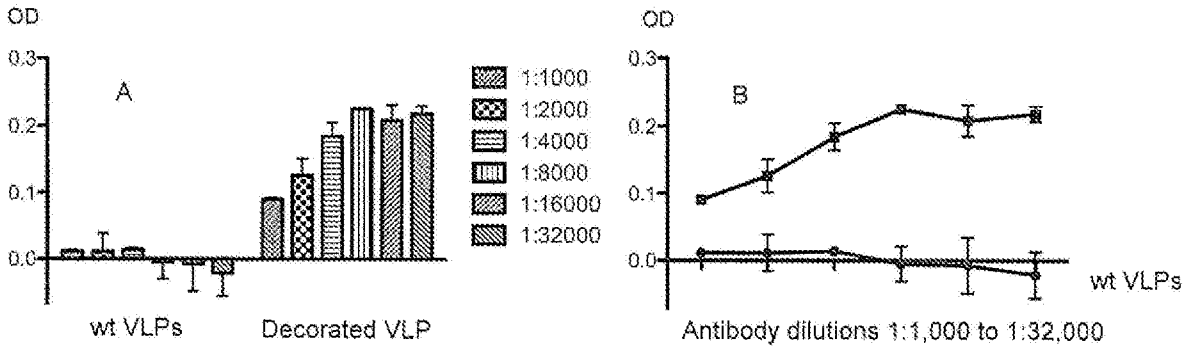

OD
0.3
0.2
0.1
0.0
A
wt VLPs
Decorated VLP
1:1000
1:2000
1:4000
1:8000
1:16000
1:32000
OD
0.3
0.2
0.1
0.0
B
wt VLPs
Antibody dilutions 1:1,000 to 1:32,000

FIGURE 8

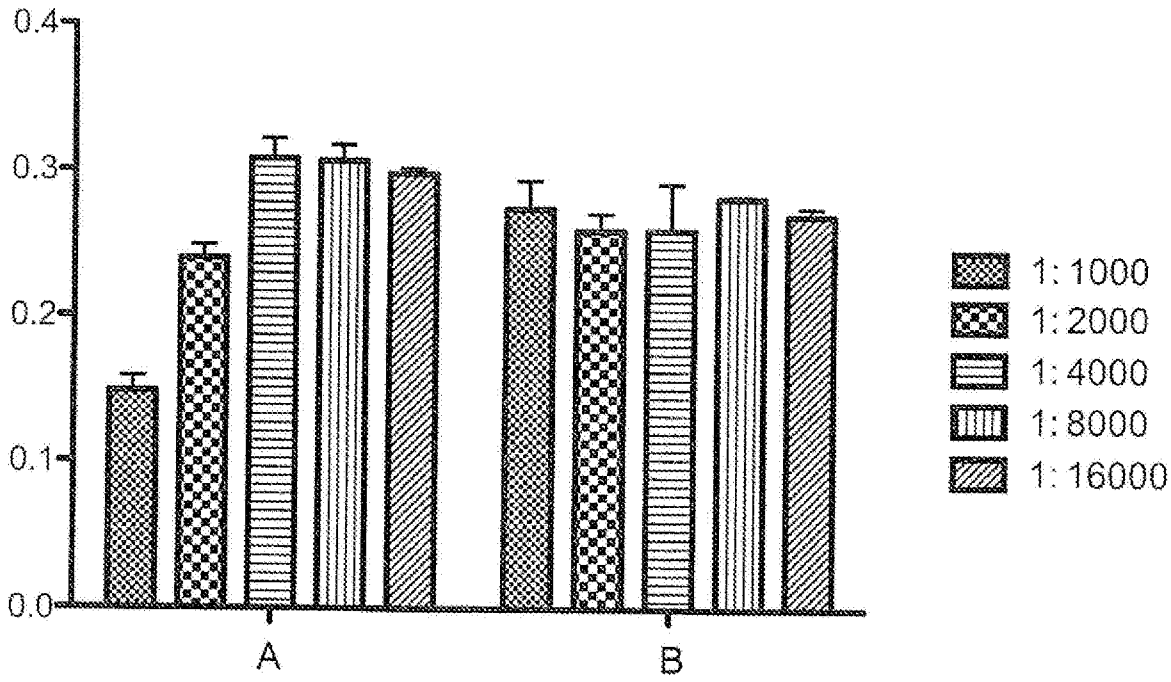


FIGURE 9

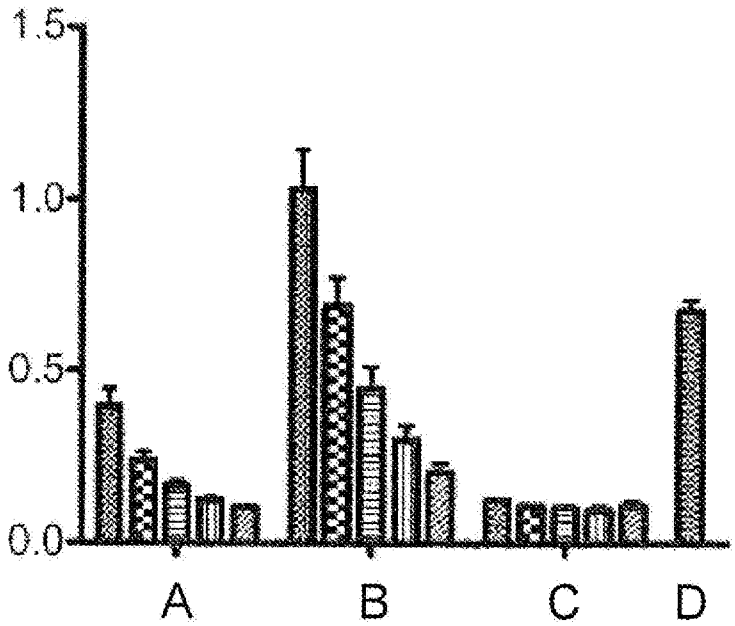


FIGURE 10

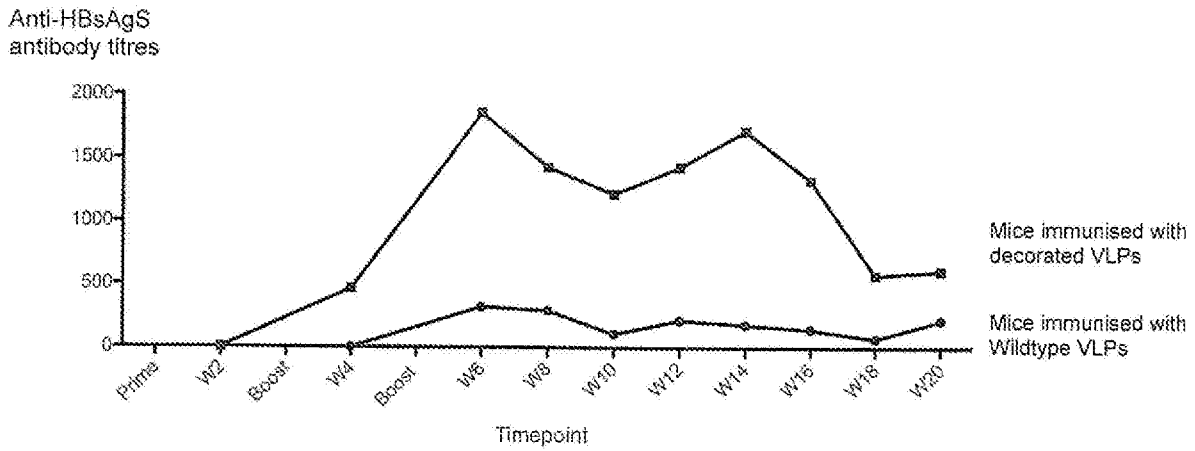

Anti-HBsAgS
antibody titres
2000
1500
1000
500
0
Prime
W2
Boost
W4
Boost
W6
W8
W10
W12
W14
W16
W18
W20
Timepoint
Mice immunised with
decorated VLPs
Mice immunised with
Wildtype VLPs

FIGURE 11

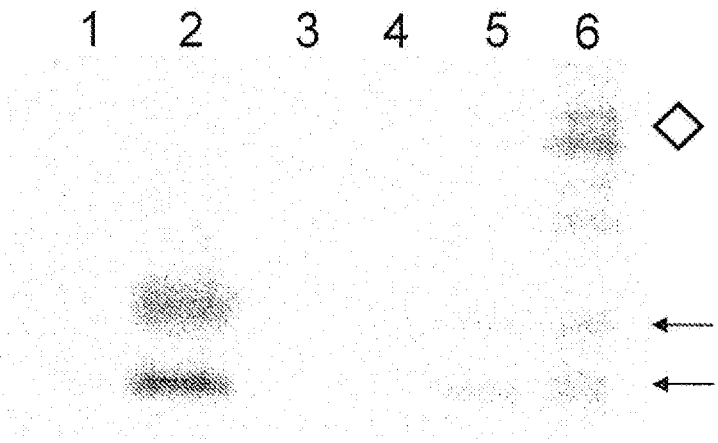


FIGURE 12

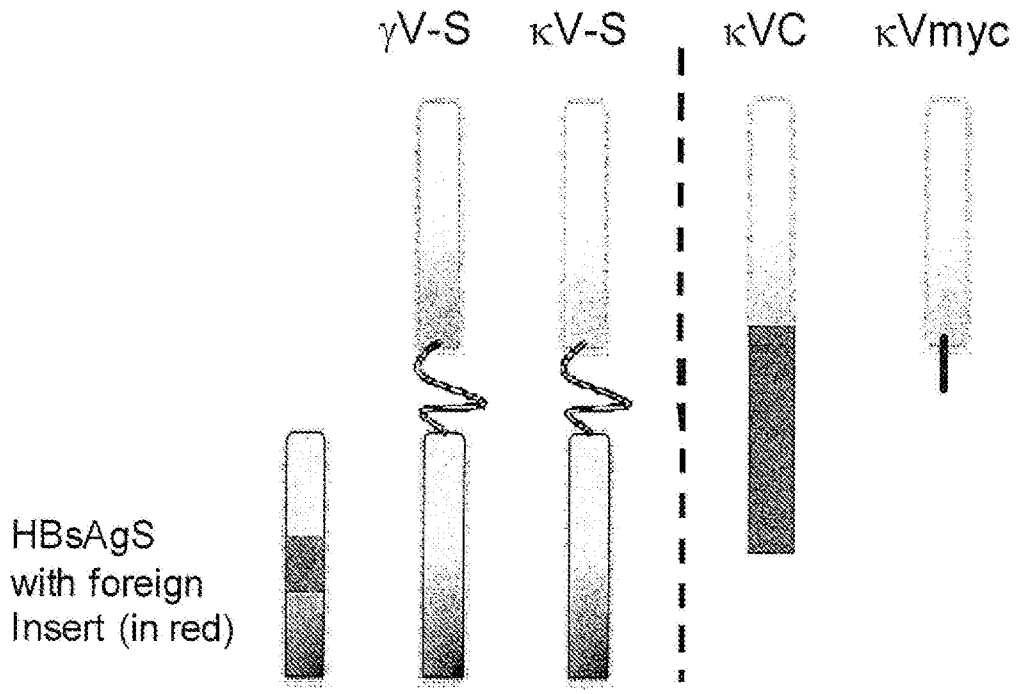


FIGURE 13

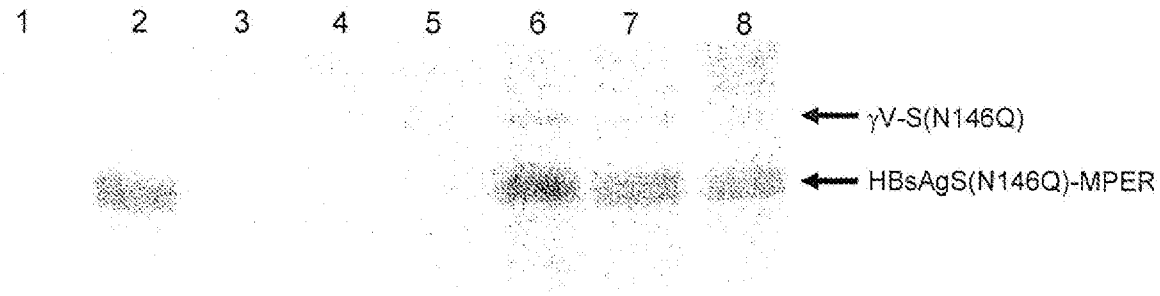


FIGURE 14

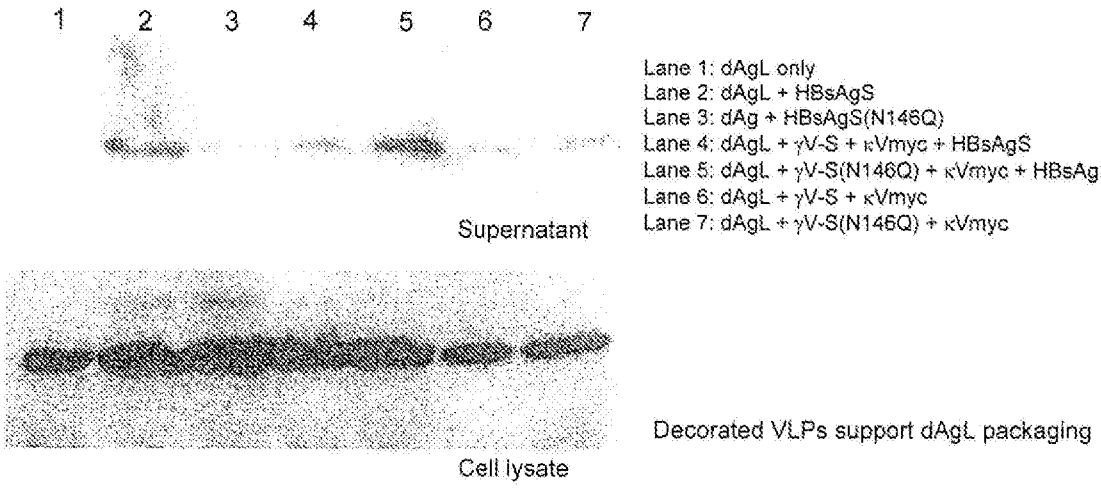

1
2
3
4
5
6
7
Lane 1: dAgL only
Lane 2: dAgL + HBsAgS
Lane 3: dAg + HBsAgS(N146Q)
Lane 4: dAgL + γV-S + κVmyc + HBsAgS
Lane 5: dAgL + γV-S(N146Q) + κVmyc + HBsAgS(N146Q)
Lane 6: dAgL + γV-S + κVmyc
Lane 7: dAgL + γV-S(N146Q) + κVmyc
Supernatant
Cell lysate
Decorated VLPs support dAgL packaging

FIGURE 15

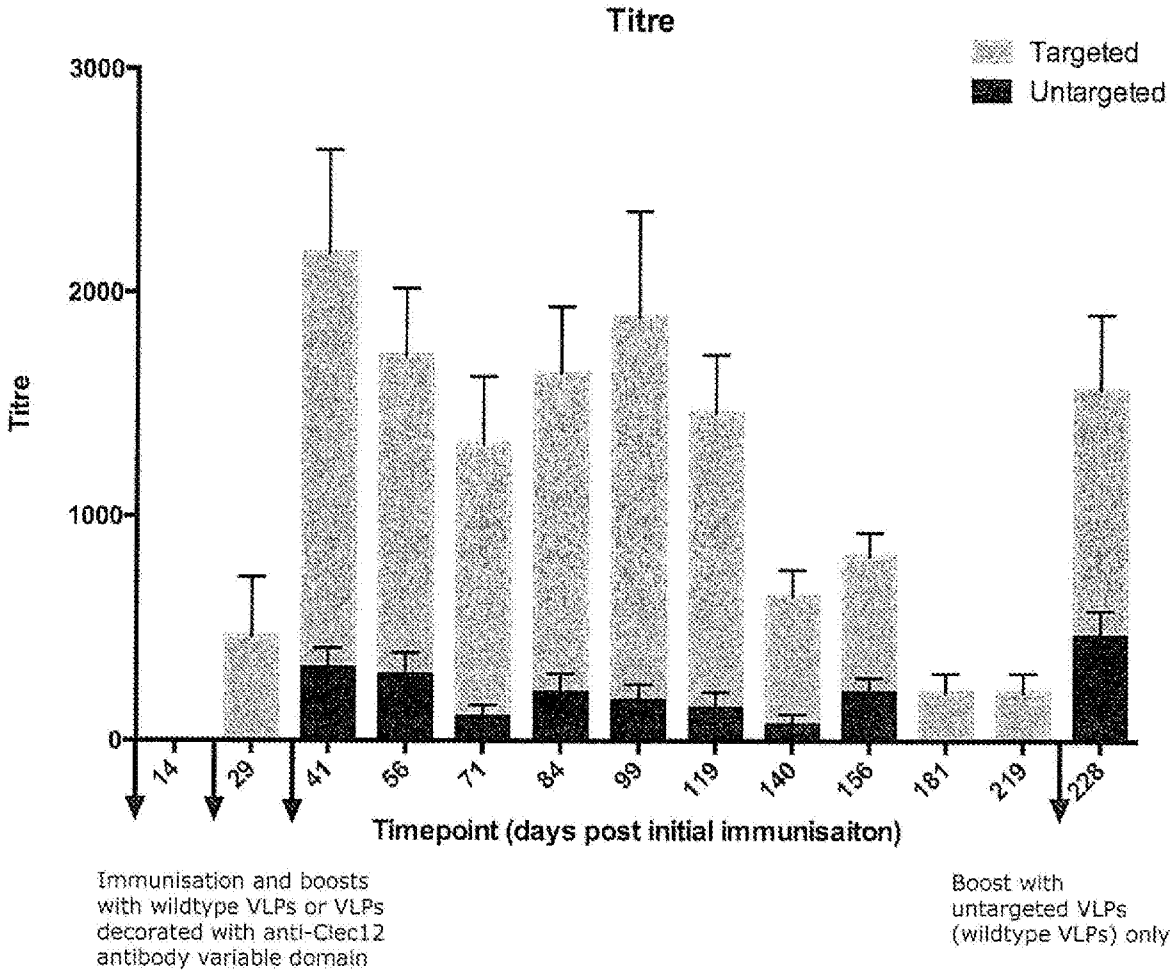

Titre
Targeted
Untargeted
3000
2000
1000
0
Titre
14
29
41
56
71
84
99
119
140
156
181
219
228
Timepoint (days post initial immunisaiton)
Immunisation and boosts with wildtype VLPs or VLPs decorated with anti-Clec12 antibody variable domain
Boost with untargeted VLPs (wildtype VLPs) only

FIGURE 16

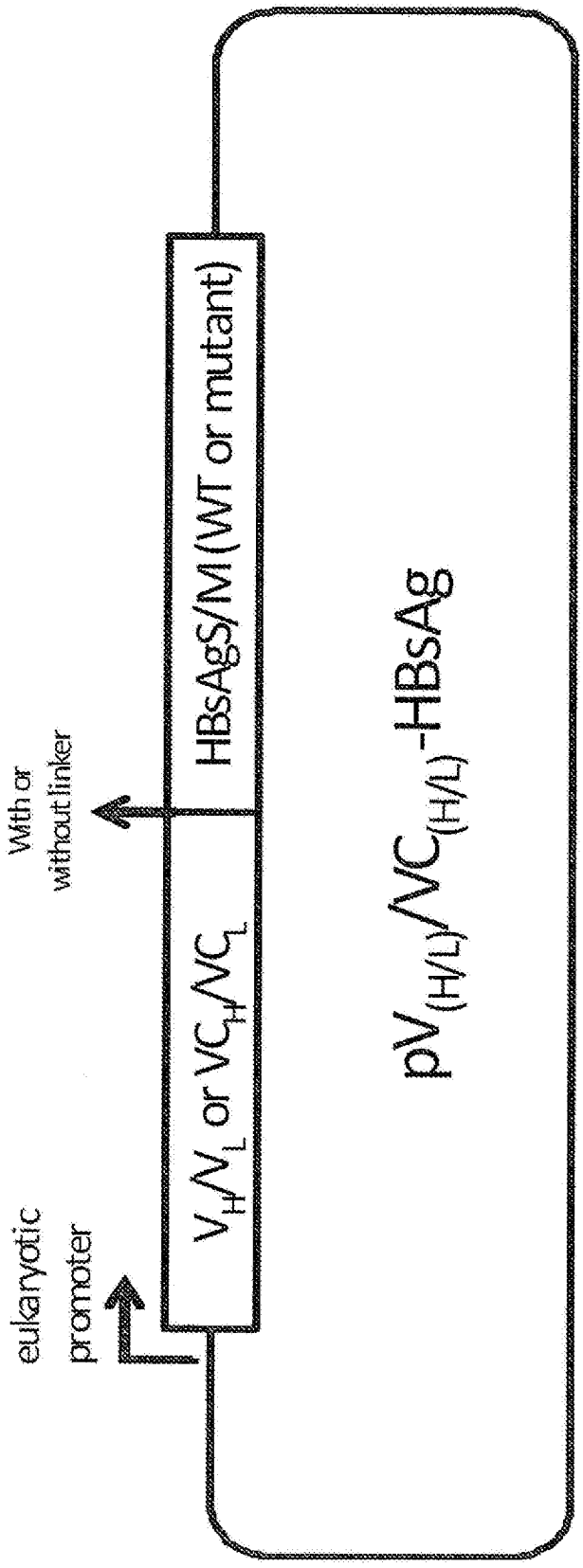

eukaryotic promoter
V_H/V_L or VC_H/VC_L
With or without linker
HBsAgS/M (WT or mutant)
$pV_{(H/L)}/VC_{(H/L)}$-HBsAg

FIGURE 17

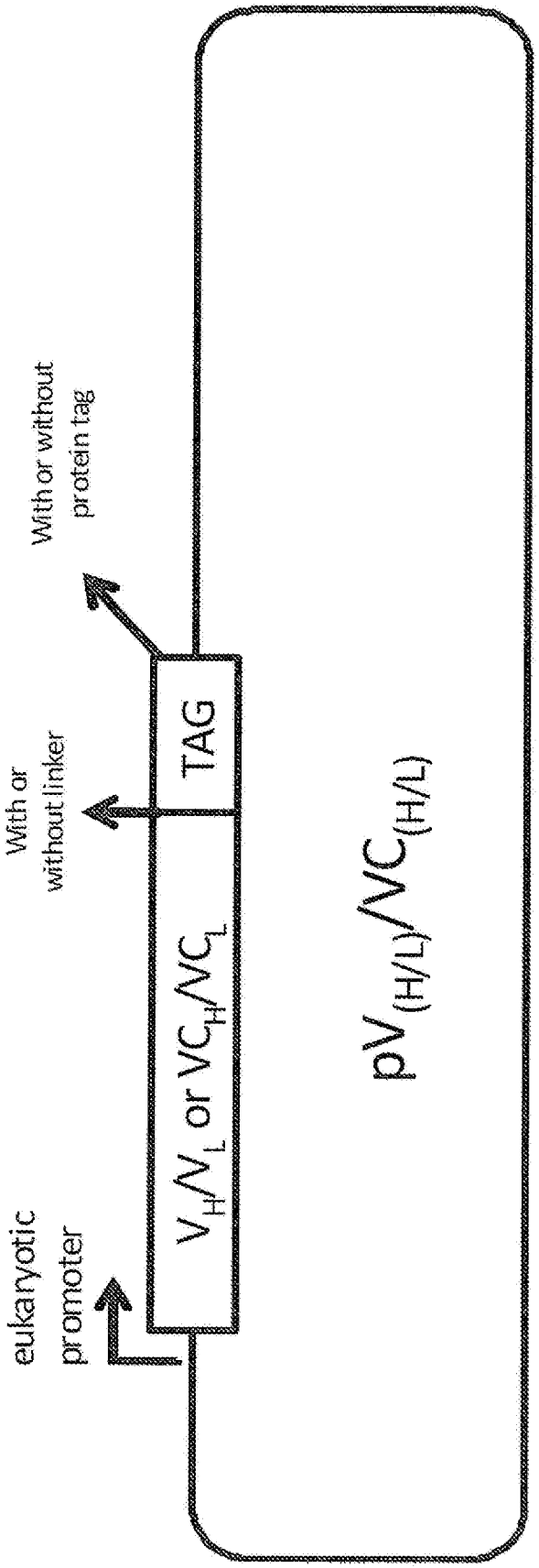

With or without protein tag
With or without linker
TAG
eukaryotic promoter
V_H/V_L or VC_H/VC_L
$pV_{(H/L)}/VC_{(H/L)}$

FIGURE 18

κV myc

```
atgggtgtgcccactcagctcctggggttgttgctgctgtggataacagatggcatatgc
 M  G  V  P  T  Q  L  L  G  L  L  L  L  W  I  T  D  G  I  C
gacatccagatgacacagtctccagcttccctgtctgcatctctgggagaaactgtctcc
 D  I  Q  M  T  Q  S  P  A  S  L  S  A  S  L  G  E  T  V  S
atcgagtgtctagcaagtgagaacatctacagttatttaacatggtatcagcagaagcca
 I  E  C  L  A  S  E  N  I  Y  S  Y  L  T  W  Y  Q  Q  K  P
gggaaatctcctcagctcctgatctatgctgcaaataggttgcaagatggggtcccatca
 G  K  S  P  Q  L  L  I  Y  A  A  N  R  L  Q  D  G  V  P  S
cggttcagtggcagtggatctggcacacagttttctctcaagatcagcggcatgcaacct
 R  F  S  G  S  G  S  G  T  Q  F  S  L  K  I  S  G  M  Q  P
gaagatgaaggggattatttctgtctacaggattccagctttccgtacacgtttggagtt
 E  D  E  G  D  Y  F  C  L  Q  D  S  S  F  P  Y  T  F  G  V
gggaccaagctggaactgaaacgggaacaaaaactaataagtgaagaggacttgtga
 G  T  K  L  E  L  K  R  E  Q  K  L  I  S  E  E  D  L
```

FIGURE 19

κVC

```
atgggtgtgcccactcagctcctggggttgttgctgctgtggataacagatggcatatgc
 M  G  V  P  T  Q  L  L  G  L  L  L  L  W  I  T  D  G  I  C
gacatccagatgacacagtctccagcttccctgtctgcatctctgggagaaactgtctcc
 D  I  Q  M  T  Q  S  P  A  S  L  S  A  S  L  G  E  T  V  S
atcgagtgtctagcaagtgagaacatctacagttatttaacatggtatcagcagaagcca
 I  E  C  L  A  S  E  N  I  Y  S  Y  L  T  W  Y  Q  Q  K  P
gggaaatctcctcagctcctgatctatgctgcaaataggttgcaagatggggtcccatca
 G  K  S  P  Q  L  L  I  Y  A  A  N  R  L  Q  D  G  V  P  S
cggttcagtggcagtggatctggcacacagttttctctcaagatcagcggcatgcaacct
 R  F  S  G  S  G  S  G  T  Q  F  S  L  K  I  S  G  M  Q  P
gaagatgaaggggattatttctgtctacaggattccagctttccgtacacgtttggagtt
 E  D  E  G  D  Y  F  C  L  Q  D  S  S  F  P  Y  T  F  G  V
gggaccaagctggaactgaaacgggctgatgctgcaccaactgtatccatcttcccacca
 G  T  K  L  E  L  K  R  A  D  A  A  P  T  V  S  I  F  P  P
tccatggaacagttaacatctggaggtgccacagtcgtgtgcttcgtgaacaacttctat
 S  M  E  Q  L  T  S  G  G  A  T  V  V  C  F  V  N  N  F  Y
cccagagacatcagtgtcaagtggaagattgatggcagtgaacaacgagatggtgtcctg
 P  R  D  I  S  V  K  W  K  I  D  G  S  E  Q  R  D  G  V  L
gacagtgttactgatcaggacagcaaagacagcacgtacagcatgagcagcaccctctcg
 D  S  V  T  D  Q  D  S  K  D  S  T  Y  S  M  S  S  T  L  S
ttgaccaaggttgaatatgaaaggcataacctctatacctgtgaggttgttcataagaca
 L  T  K  V  E  Y  E  R  H  N  L  Y  T  C  E  V  V  H  K  T
tcatcctcacccgtcgtcaagagcttcaacaggaatgagtgttag
 S  S  S  P  V  V  K  S  F  N  R  N  E  C
```

FIGURE 20

κ12V-HBsAgS- (N146N)

```
atgggtgtgcccactcagctcctggggttgttgctgctgtggataacagatggcatatgc
 M  G  V  P  T  Q  L  L  G  L  L  L  L  W  I  T  D  G  I  C
gacatccagatgacacagtctccagcttccctgtctgcatctctgggagaaactgtctcc
 D  I  Q  M  T  Q  S  P  A  S  L  S  A  S  L  G  E  T  V  S
atcgagtgtctagcaagtgagaacatctacagttatttaacatggtatcagcagaagcca
 I  E  C  L  A  S  E  N  I  Y  S  Y  L  T  W  Y  Q  Q  K  P
gggaaatctcctcagctcctgatctatgctgcaaataggttgcaagatggggtcccatca
 G  K  S  P  Q  L  L  I  Y  A  A  N  R  L  Q  D  G  V  P  S
cggttcagtggcagtggatctggcacacagttttctctcaagatcagcggcatgcaacct
 R  F  S  G  S  G  S  G  T  Q  F  S  L  K  I  S  G  M  Q  P
gaagatgaaggggattatttctgtctacaggattccagctttccgtacacgtttggagtt
 E  D  E  G  D  Y  F  C  L  Q  D  S  S  F  P  Y  T  F  G  V
gggaccaagctggaactgaaacgggggggcgtcgggggcttcaggagctagtggggcgtcg
 G  T  K  L  E  L  K  R  G  A  S  G  A  S  G  A  S  G  A  S
ggctcgaggattggggaccctgcgctgaacatggagaacatcacatcaggattcctagga
 G  S  R  I  G  D  P  A  L  N  M  E  N  I  T  S  G  F  L  G
ccccttctcgtgttacaggcggggttttcttgttgacaagaatcctcacaataccgcag
 P  L  L  V  L  Q  A  G  F  F  L  L  T  R  I  L  T  I  P  Q
agtctagactcgtggtggacttctctcaattttctaggggaactaccgtgtgtcttggc
 S  L  D  S  W  W  T  S  L  N  F  L  G  G  T  T  V  C  L  G
caaaattcgcagtccccaacctccaatcactcaccaacctcttgtcctccaacttgtcct
 Q  N  S  Q  S  P  T  S  N  H  S  P  T  S  C  P  P  T  C  P
ggttatcgctggatgtgtctgcggcgttttatcatcttcctcttcatcctgctgctatgc
 G  Y  R  W  M  C  L  R  R  F  I  I  F  L  F  I  L  L  L  C
ctcatcttcttgttggttcttctggactatcaaggtatgttgcccgtttgtcctctaatt
 L  I  F  L  L  V  L  L  D  Y  Q  G  M  L  P  V  C  P  L  I
ccaggatcctcaacaaccagcacgggaccatgccggacctgcatgactaccggtcaagga
 P  G  S  S  T  T  S  T  G  P  C  R  T  C  M  T  T  G  Q  G
acctctatgtatccctcctgttgctgtaccaaaccttcggacggaaattgcacctgtatt
 T  S  M  Y  P  S  C  C  C  T  K  P  S  D  G  N  C  T  C  I
cccatcccatcatcctgggctttcggaaaattcctatgggagtgggcctcagcccgtttc
 P  I  P  S  S  W  A  F  G  K  F  L  W  E  W  A  S  A  R  F
tcctggctcagtttactagtgccatttgttcagtggttcgtagggctttcccccactgtt
 S  W  L  S  L  L  V  P  F  V  Q  W  F  V  G  L  S  P  T  V
tggctttcagttatatggatgatgtggtattgggggccaagtctgtacagcatcttgagt
 W  L  S  V  I  W  M  M  W  Y  W  G  P  S  L  Y  S  I  L  S
ccctttttaccgctgttaccaattttcttttgtctttgggtatacatttaa
 P  F  L  P  L  L  P  I  F  F  C  L  W  V  Y  I
```

FIGURE 21

κ12V-HBsAgS(N146Q)

```
atgggtgtgcccactcagctcctggggttgttgctgctgtggataacagatggcatatgc
 M  G  V  P  T  Q  L  L  G  L  L  L  L  W  I  T  D  G  I  C
gacatccagatgacacagtctccagcttccctgtctgcatctctgggagaaactgtctcc
 D  I  Q  M  T  Q  S  P  A  S  L  S  A  S  L  G  E  T  V  S
atcgagtgtctagcaagtgagaacatctacagttatttaacatggtatcagcagaagcca
 I  E  C  L  A  S  E  N  I  Y  S  Y  L  T  W  Y  Q  Q  K  P
gggaaatctcctcagctcctgatctatgctgcaaataggttgcaagatggggtcccatca
 G  K  S  P  Q  L  L  I  Y  A  A  N  R  L  Q  D  G  V  P  S
cggttcagtggcagtggatctggcacacagttttctctcaagatcagcggcatgcaacct
 R  F  S  G  S  G  S  G  T  Q  F  S  L  K  I  S  G  M  Q  P
gaagatgaaggggattatttctgtctacaggattccagctttccgtacacgtttggagtt
 E  D  E  G  D  Y  F  C  L  Q  D  S  S  F  P  Y  T  F  G  V
gggaccaagctggaactgaaacgggggggcgtcgggggcttcaggagctagtggggcgtcg
 G  T  K  L  E  L  K  R  G  A  S  G  A  S  G  A  S  G  A  S
ggctcgaggattggggaccctgcgctgaacatggagaacatcacatcaggattcctagga
 G  S  R  I  G  D  P  A  L  N  M  E  N  I  T  S  G  F  L  G
ccccttctcgtgttacaggcgggggttttcttgttgacaagaatcctcacaataccgcag
 P  L  L  V  L  Q  A  G  F  F  L  L  T  R  I  L  T  I  P  Q
agtctagactcgtggtggacttctctcaattttctaggggaactaccgtgtgtcttggc
 S  L  D  S  W  W  T  S  L  N  F  L  G  G  T  T  V  C  L  G
caaaattcgcagtccccaacctccaatcactcaccaacctcttgtcctccaacttgtcct
 Q  N  S  Q  S  P  T  S  N  H  S  P  T  S  C  P  P  T  C  P
ggttatcgctggatgtgtctgcggcgttttatcatcttcctcttcatcctgctgctatgc
 G  Y  R  W  M  C  L  R  R  F  I  I  F  L  F  I  L  L  L  C
ctcatcttcttgttggttcttctggactatcaaggtatgttgcccgtttgtcctctaatt
 L  I  F  L  L  V  L  L  D  Y  Q  G  M  L  P  V  C  P  L  I
ccaggatcctcaacaaccagcacgggaccatgccggacctgcatgactaccggtcaagga
 P  G  S  S  T  T  S  T  G  P  C  R  T  C  M  T  T  G  Q  G
acctctatgtatccctcctgttgctgtaccaaaccttcggacggacagtgcacctgtatt
 T  S  M  Y  P  S  C  C  C  T  K  P  S  D  G  Q  C  T  C  I
cccatcccatcatcctgggctttcggaaaattcctatgggagtgggcctcagcccgtttc
 P  I  P  S  S  W  A  F  G  K  F  L  W  E  W  A  S  A  R  F
tcctggctcagtttactagtgccatttgttcagtggttcgtagggctttccccactgtt
 S  W  L  S  L  L  V  P  F  V  Q  W  F  V  G  L  S  P  T  V
tggctttcagttatatggatgatgtggtattgggggccaagtctgtacagcatcttgagt
 W  L  S  V  I  W  M  M  W  Y  W  G  P  S  L  Y  S  I  L  S
ccctttttaccgctgttaccaatttttcttttgtctttgggtatacatttaa
 P  F  L  P  L  L  P  I  F  F  C  L  W  V  Y  I
```

FIGURE 22

γ12V-HBsAg(N146N)

```
atgggatggatctgtatcatctttcttgtggcaacagctacaggtgtccactcccaggtc
 M  G  W  I  C  I  I  F  L  V  A  T  A  T  G  V  H  S  Q  V
aacctactgcagtctggggctgcactggtgaagcctggggcctctgtgaagttgtcttgc
 N  L  L  Q  S  G  A  A  L  V  K  P  G  A  S  V  K  L  S  C
aaagcttctggttatagattcactgactactatatacattgggtgaagcagagtcatgga
 K  A  S  G  Y  R  F  T  D  Y  Y  I  H  W  V  K  Q  S  H  G
aagagccttgagtggattgggtatattaatcctaacagggattatactaactacaatgaa
 K  S  L  E  W  I  G  Y  I  N  P  N  R  D  Y  T  N  Y  N  E
aagttcaagaggaaggccacattgactgtagacaaatccaccaatacagcctatatggag
 K  F  K  R  K  A  T  L  T  V  D  K  S  T  N  T  A  Y  M  E
cttagtagattgacttctgaggactctgcaacctattactgtacaagactatcgcggatt
 L  S  R  L  T  S  E  D  S  A  T  Y  Y  C  T  R  L  S  R  I
actatgatggtccggggctttgattactggggccaaggagtcatggtcacagtctcctca
 T  M  M  V  R  G  F  D  Y  W  G  Q  G  V  M  V  T  V  S  S
ggggcgtcgggggcttcaggagctagtggggcgtcgggctcgaggattggggaccctgcg
 G  A  S  G  A  S  G  A  S  G  A  S  G  S  R  I  G  D  P  A
ctgaacatggagaacatcacatcaggattcctaggacccttctcgtgttacaggcgggg
 L  N  M  E  N  I  T  S  G  F  L  G  P  L  L  V  L  Q  A  G
tttttcttgttgacaagaatcctcacaataccgcagagtctagactcgtggtggacttct
 F  F  L  L  T  R  I  L  T  I  P  Q  S  L  D  S  W  W  T  S
ctcaattttctaggggaactaccgtgtgtcttggccaaaattcgcagtccccaacctcc
 L  N  F  L  G  G  T  T  V  C  L  G  Q  N  S  Q  S  P  T  S
aatcactcaccaacctcttgtcctccaacttgtcctggttatcgctggatgtgtctgcgg
 N  H  S  P  T  S  C  P  P  T  C  P  G  Y  R  W  M  C  L  R
cgttttatcatcttcctcttcatcctgctgctatgcctcatcttcttgttggttcttctg
 R  F  I  I  F  L  F  I  L  L  L  C  L  I  F  L  L  V  L  L
gactatcaaggtatgttgcccgtttgtcctctaattccaggatcctcaacaaccagcacg
 D  Y  Q  G  M  L  P  V  C  P  L  I  P  G  S  S  T  T  S  T
ggaccatgccggacctgcatgactaccggtcaaggaacctctatgtatccctcctgttgc
 G  P  C  R  T  C  M  T  T  G  Q  G  T  S  M  Y  P  S  C  C
tgtaccaaaccttcggacggaaattgcacctgtattcccatcccatcatcctgggctttc
 C  T  K  P  S  D  G  N  C  T  C  I  P  I  P  S  S  W  A  F
ggaaaattcctatgggagtgggcctcagcccgtttctcctggctcagtttactagtgcca
 G  K  F  L  W  E  W  A  S  A  R  F  S  W  L  S  L  L  V  P
tttgttcagtggttcgtagggctttcccccactgtttggctttcagttatatggatgatg
 F  V  Q  W  F  V  G  L  S  P  T  V  W  L  S  V  I  W  M  M
tggtattgggggccaagtctgtacagcatcttgagtccctttttaccgctgttaccaatt
 W  Y  W  G  P  S  L  Y  S  I  L  S  P  F  L  P  L  L  P  I
ttcttttgtctttgggtatacatttaa
 F  F  C  L  W  V  Y  I
```

FIGURE 23

γ12V-HBsAgS(N146Q)

```
atgggatggatctgtatcatctttcttgtggcaacagctacaggtgtccactcccaggtc
 M  G  W  I  C  I  I  F  L  V  A  T  A  T  G  V  H  S  Q  V
aacctactgcagtctggggctgcactggtgaagcctggggcctctgtgaagttgtcttgc
 N  L  L  Q  S  G  A  A  L  V  K  P  G  A  S  V  K  L  S  C
aaagcttctggttatagattcactgactactatatacattgggtgaagcagagtcatgga
 K  A  S  G  Y  R  F  T  D  Y  Y  I  H  W  V  K  Q  S  H  G
aagagccttgagtggattgggtatattaatcctaacagggattatactaactacaatgaa
 K  S  L  E  W  I  G  Y  I  N  P  N  R  D  Y  T  N  Y  N  E
aagttcaagaggaaggccacattgactgtagacaaatccaccaatacagcctatatggag
 K  F  K  R  K  A  T  L  T  V  D  K  S  T  N  T  A  Y  M  E
cttagtagattgacttctgaggactctgcaacctattactgtacaagactatcgcggatt
 L  S  R  L  T  S  E  D  S  A  T  Y  Y  C  T  R  L  S  R  I
actatgatggtccggggctttgattactggggccaaggagtcatggtcacagtctcctca
 T  M  M  V  R  G  F  D  Y  W  G  Q  G  V  M  V  T  V  S  S
ggggcgtcgggggcttcaggagctagtggggcgtcgggctcgaggattggggaccctgcg
 G  A  S  G  A  S  G  A  S  G  A  S  G  S  R  I  G  D  P  A
ctgaacatggagaacatcacatcaggattcctaggacccttctcgtgttacaggcgggg
 L  N  M  E  N  I  T  S  G  F  L  G  P  L  L  V  L  Q  A  G
tttttcttgttgacaagaatcctcacaataccgcagagtctagactcgtggtggacttct
 F  F  L  L  T  R  I  L  T  I  P  Q  S  L  D  S  W  W  T  S
ctcaattttctaggggaactaccgtgtgtcttggccaaaattcgcagtccccaacctcc
 L  N  F  L  G  G  T  T  V  C  L  G  Q  N  S  Q  S  P  T  S
aatcactcaccaacctcttgtcctccaacttgtcctggttatcgctggatgtgtctgcgg
 N  H  S  P  T  S  C  P  P  T  C  P  G  Y  R  W  M  C  L  R
cgttttatcatcttcctcttcatcctgctgctatgcctcatcttcttgttggttcttctg
 R  F  I  I  F  L  F  I  L  L  L  C  L  I  F  L  L  V  L  L
gactatcaaggtatgttgcccgtttgtcctctaattccaggatcctcaacaaccagcacg
 D  Y  Q  G  M  L  P  V  C  P  L  I  P  G  S  S  T  T  S  T
ggaccatgccggacctgcatgactaccggtcaaggaacctctatgtatccctcctgttgc
 G  P  C  R  T  C  M  T  T  G  Q  G  T  S  M  Y  P  S  C  C
tgtaccaaaccttcggacggacagtgcacctgtattcccatcccatcatcctgggctttc
 C  T  K  P  S  D  G  Q  C  T  C  I  P  I  P  S  S  W  A  F
ggaaaattcctatgggagtgggcctcagcccgtttctcctggctcagtttactagtgcca
 G  K  F  L  W  E  W  A  S  A  R  F  S  W  L  S  L  L  V  P
tttgttcagtggttcgtagggctttcccccactgtttggctttcagttatatggatgatg
 F  V  Q  W  F  V  G  L  S  P  T  V  W  L  S  V  I  W  M  M
tggtattgggggccaagtctgtacagcatcttgagtccctttttaccgctgttaccaatt
 W  Y  W  G  P  S  L  Y  S  I  L  S  P  F  L  P  L  L  P  I
ttcttttgtctttgggtatacatttaa
 F  F  C  L  W  V  Y  I
```

FIGURE 24

γ12VC-HBsAg (N146N)

```
atgggatggatctgtatcatctttcttgtggcaacagctacaggtgtccactcccaggtc
 M  G  W  I  C  I  I  F  L  V  A  T  A  T  G  V  H  S  Q  V
aacctactgcagtctggggctgcactggtgaagcctggggcctctgtgaagttgtcttgc
 N  L  L  Q  S  G  A  A  L  V  K  P  G  A  S  V  K  L  S  C
aaagcttctggttatagattcactgactactatatacattgggtgaagcagagtcatgga
 K  A  S  G  Y  R  F  T  D  Y  Y  I  H  W  V  K  Q  S  H  G
aagagccttgagtggattgggtatattaatcctaacagggattatactaactacaatgaa
 K  S  L  E  W  I  G  Y  I  N  P  N  R  D  Y  T  N  Y  N  E
aagttcaagaggaaggccacattgactgtagacaaatccaccaatacagcctatatggag
 K  F  K  R  K  A  T  L  T  V  D  K  S  T  N  T  A  Y  M  E
cttagtagattgacttctgaggactctgcaacctattactgtacaagactatcgcggatt
 L  S  R  L  T  S  E  D  S  A  T  Y  Y  C  T  R  L  S  R  I
actatgatggtccggggctttgattactggggccaaggagtcatggtcacagtctcctca
 T  M  M  V  R  G  F  D  Y  W  G  Q  G  V  M  V  T  V  S  S
gctgaaacaacagccccatctgtctatccactggctcctggaactgctctcaaaagtaac
 A  E  T  T  A  P  S  V  Y  P  L  A  P  G  T  A  L  K  S  N
tccatggtgaccctgggatgcctggtcaagggctatttccctgagccagtcaccgtgacc
 S  M  V  T  L  G  C  L  V  K  G  Y  F  P  E  P  V  T  V  T
tggaactctggagccctgtccagcggtgtgcacaccttcccagctgtcctgcagtctgga
 W  N  S  G  A  L  S  S  G  V  H  T  F  P  A  V  L  Q  S  G
ctctacactctcaccagctcagtgactgtaccctccagcacctggtccagccaggccgtc
 L  Y  T  L  T  S  S  V  T  V  P  S  S  T  W  S  S  Q  A  V
acctgcggggcgtcggggcttcaggagctagtggggcgtcgggctcgaggattggggac
 T  C  G  A  S  G  A  S  G  A  S  G  A  S  G  S  R  I  G  D
cctgcgctgaacatggagaacatcacatcaggattcctaggacccctttctcgtgttacag
 P  A  L  N  M  E  N  I  T  S  G  F  L  G  P  L  L  V  L  Q
gcggggtttttcttgttgacaagaatcctcacaataccgcagagtctagactcgtggtgg
 A  G  F  F  L  L  T  R  I  L  T  I  P  Q  S  L  D  S  W  W
acttctctcaattttctaggggaactaccgtgtgtcttggccaaaattcgcagtccccca
 T  S  L  N  F  L  G  G  T  T  V  C  L  G  Q  N  S  Q  S  P
acctccaatcactcaccaacctcttgtcctccaacttgtcctggttatcgctggatgtgt
 T  S  N  H  S  P  T  S  C  P  P  T  C  P  G  Y  R  W  M  C
ctgcggcgttttatcatcttcctcttcatcctgctgctatgcctcatcttcttgttggtt
 L  R  R  F  I  I  F  L  F  I  L  L  L  C  L  I  F  L  L  V
cttctggactatcaaggtatgttgcccgtttgtcctctaattccaggatcctcaacaacc
 L  L  D  Y  Q  G  M  L  P  V  C  P  L  I  P  G  S  S  T  T
agcacgggaccatgccggacctgcatgactaccggtcaaggaacctctatgtatccctcc
 S  T  G  P  C  R  T  C  M  T  T  G  Q  G  T  S  M  Y  P  S
tgttgctgtaccaaaccttcggacggaaattgcacctgtattcccatcccatcatcctgg
 C  C  C  T  K  P  S  D  G  N  C  T  C  I  P  I  P  S  S  W
gctttcggaaaattcctatgggagtgggcctcagcccgtttctcctggctcagtttacta
 A  F  G  K  F  L  W  E  W  A  S  A  R  F  S  W  L  S  L  L
gtgccatttgttcagtggttcgtagggctttccccccactgtttggctttcagttatatgg
 V  P  F  V  Q  W  F  V  G  L  S  P  T  V  W  L  S  V  I  W
atgatgtggtattgggggccaagtctgtacagcatcttgagtccctttttaccgctgtta
 M  M  W  Y  W  G  P  S  L  Y  S  I  L  S  P  F  L  P  L  L
ccaattttcttttgtctttgggtatacatttaa
 P  I  F  F  C  L  W  V  Y  I
```

FIGURE 25

NIH45-46 Anti-HIV-1 Heavy chain with signal sequence:

```
atgggttggatctgtataatcttcctagtcgctactgctacaggagtccactcgcaagtg
 M  G  W  I  C  I  I  F  L  V  A  T  A  T  G  V  H  S  Q  V
cgactgtcgcagtctggaggtcagatgaagaagcctggcgagtcgatgagactttcctgt
 R  L  S  Q  S  G  G  Q  M  K  K  P  G  E  S  M  R  L  S  C
cgggcttccggatatgaatttctgaattgtccaataaattggattcgcctggcccccgga
 R  A  S  G  Y  E  F  L  N  C  P  I  N  W  I  R  L  A  P  G
agacggcctgagtggatgggatggctgaagcctaggtgggggccgtcaattacgcacgt
 R  R  P  E  W  M  G  W  L  K  P  R  W  G  A  V  N  Y  A  R
aaatttcagggcagagtgaccatgacgcgagacgtgtattccgacacagccttttggag
 K  F  Q  G  R  V  T  M  T  R  D  V  Y  S  D  T  A  F  L  E
ttgcgctccttgacatcagacgacacggccgtctatttttgtactaggggaaaatattgt
 L  R  S  L  T  S  D  D  T  A  V  Y  F  C  T  R  G  K  Y  C
actgcgcgcgactattataattgggacttcgaacactggggccggggtgccccggtcacc
 T  A  R  D  Y  Y  N  W  D  F  E  H  W  G  R  G  A  P  V  T
gtctcatcagcgtcgaccaagggcccatcggtcggggcgtcgggggcttcaggagctagt
 V  S  S  A  S  T  K  G  P  S  V  G  A  S  G  A  S  G  A  S
ggggcgtcgggctcgaggattatggagaacatcacatcaggattcctaggacccttctc
 G  A  S  G  S  R  I  M  E  N  I  T  S  G  F  L  G  P  L  L
gtgttacaggcggggttttttcttgttgacaagaatcctcacaataccgcagagtctagac
 V  L  Q  A  G  F  F  L  L  T  R  I  L  T  I  P  Q  S  L  D
tcgtggtggacttctctcaattttctaggggaactaccgtgtgtcttggccaaaattcg
 S  W  W  T  S  L  N  F  L  G  G  T  T  V  C  L  G  Q  N  S
cagtccccaacctccaatcactcaccaacctcttgtcctccaacttgtcctggttatcgc
 Q  S  P  T  S  N  H  S  P  T  S  C  P  P  T  C  P  G  Y  R
tggatgtgtctgcggcgttttatcatcttcctcttcatcctgctgctatgcctcatcttc
 W  M  C  L  R  R  F  I  I  F  L  F  I  L  L  L  C  L  I  F
ttgttggttcttctggactatcaaggtatgttgcccgtttgtcctctaattccaggatcc
 L  L  V  L  L  D  Y  Q  G  M  L  P  V  C  P  L  I  P  G  S
tcaacaaccagcacgggaccatgccggacctgcatgactaccggtcaaggaacctctatg
 S  T  T  S  T  G  P  C  R  T  C  M  T  T  G  Q  G  T  S  M
tatccctcctgttgctgtaccaaaccttcggacggaaattgcacctgtattcccatccca
 Y  P  S  C  C  C  T  K  P  S  D  G  N  C  T  C  I  P  I  P
tcatcctgggctttcggaaaattcctatgggagtgggcctcagcccgtttctcctggctc
 S  S  W  A  F  G  K  F  L  W  E  W  A  S  A  R  F  S  W  L
agtttactagtgccatttgttcagtggttcgtagggctttcccccactgtttggctttca
 S  L  L  V  P  F  V  Q  W  F  V  G  L  S  P  T  V  W  L  S
gttatatggatgatgtggtattgggggccaagtctgtacagcatcttgagtccctttttta
 V  I  W  M  M  W  Y  W  G  P  S  L  Y  S  I  L  S  P  F  L
ccgctgttaccaatttttcttttgtctttgggtatacatttaa
 P  L  L  P  I  F  F  C  L  W  V  Y  I
```

FIGURE 26

NIH45-46 Anti-HIV-1 Light chain with signal sequence:

```
atggagacaccagcaggcctcctattcttactcctgctatggctgccagataccacagga
 M  E  T  P  A  G  L  L  F  L  L  L  L  W  L  P  D  T  T  G
gaaattgtgttgacacagtctccagccaccctgtctttgtctccaggggaaacagccatc
 E  I  V  L  T  Q  S  P  A  T  L  S  L  S  P  G  E  T  A  I
atctcttgtcggaccagtcagtctggttccttagcctggtatcaacagaggcccggccag
 I  S  C  R  T  S  Q  S  G  S  L  A  W  Y  Q  Q  R  P  G  Q
gccccaggctcgtcatctattcgggttctactcgggccgctggcatcccagacaggttc
 A  P  R  L  V  I  Y  S  G  S  T  R  A  A  G  I  P  D  R  F
agcggcagtcggtgggggcagactacaatctcagcatcagcaacctggagtcgggagat
 S  G  S  R  W  G  A  D  Y  N  L  S  I  S  N  L  E  S  G  D
tttggtgtttattattgtcagcagtatgaatttttggccaggggaccaaggtccaggtc
 F  G  V  Y  Y  C  Q  Q  Y  E  F  F  G  Q  G  T  K  V  Q  V
gacatcaaacgtacggtggctgcaccaggggcgtcgggggcttcaggagctagtggggcg
 D  I  K  R  T  V  A  A  P  G  A  S  G  A  S  G  A  S  G  A
tcgggctcgaggattatggagaacatcacatcaggattcctaggacccctttctcgtgtta
 S  G  S  R  I  M  E  N  I  T  S  G  F  L  G  P  L  L  V  L
caggcggggtttttcttgttgacaagaatcctcacaataccgcagagtctagactcgtgg
 Q  A  G  F  F  L  L  T  R  I  L  T  I  P  Q  S  L  D  S  W
tggacttctctcaattttctaggggaactaccgtgtgtcttggccaaaattcgcagtcc
 W  T  S  L  N  F  L  G  G  T  T  V  C  L  G  Q  N  S  Q  S
ccaacctccaatcactcaccaacctcttgtcctccaacttgtcctggttatcgctggatg
 P  T  S  N  H  S  P  T  S  C  P  P  T  C  P  G  Y  R  W  M
tgtctgcggcgttttatcatcttcctcttcatcctgctgctatgcctcatcttcttgttg
 C  L  R  R  F  I  I  F  L  F  I  L  L  L  C  L  I  F  L  L
gttcttctggactatcaaggtatgttgcccgtttgtcctctaattccaggatcctcaaca
 V  L  L  D  Y  Q  G  M  L  P  V  C  P  L  I  P  G  S  S  T
accagcacgggaccatgccggacctgcatgactaccggtcaaggaacctctatgtatccc
 T  S  T  G  P  C  R  T  C  M  T  T  G  Q  G  T  S  M  Y  P
tcctgttgctgtaccaaaccttcggacggaaattgcacctgtattcccatcccatcatcc
 S  C  C  C  T  K  P  S  D  G  N  C  T  C  I  P  I  P  S  S
tgggctttcggaaaattcctatgggagtgggcctcagcccgtttctcctggctcagttta
 W  A  F  G  K  F  L  W  E  W  A  S  A  R  F  S  W  L  S  L
ctagtgccatttgttcagtggttcgtagggctttcccccactgtttggctttcagttata
 L  V  P  F  V  Q  W  F  V  G  L  S  P  T  V  W  L  S  V  I
tggatgatgtggtattgggggccaagtctgtacagcatcttgagtccctttttaccgctg
 W  M  M  W  Y  W  G  P  S  L  Y  S  I  L  S  P  F  L  P  L
ttaccaattttcttttgtctttgggtatacatttaa
 L  P  I  F  F  C  L  W  V  Y  I
```

FIGURE 27

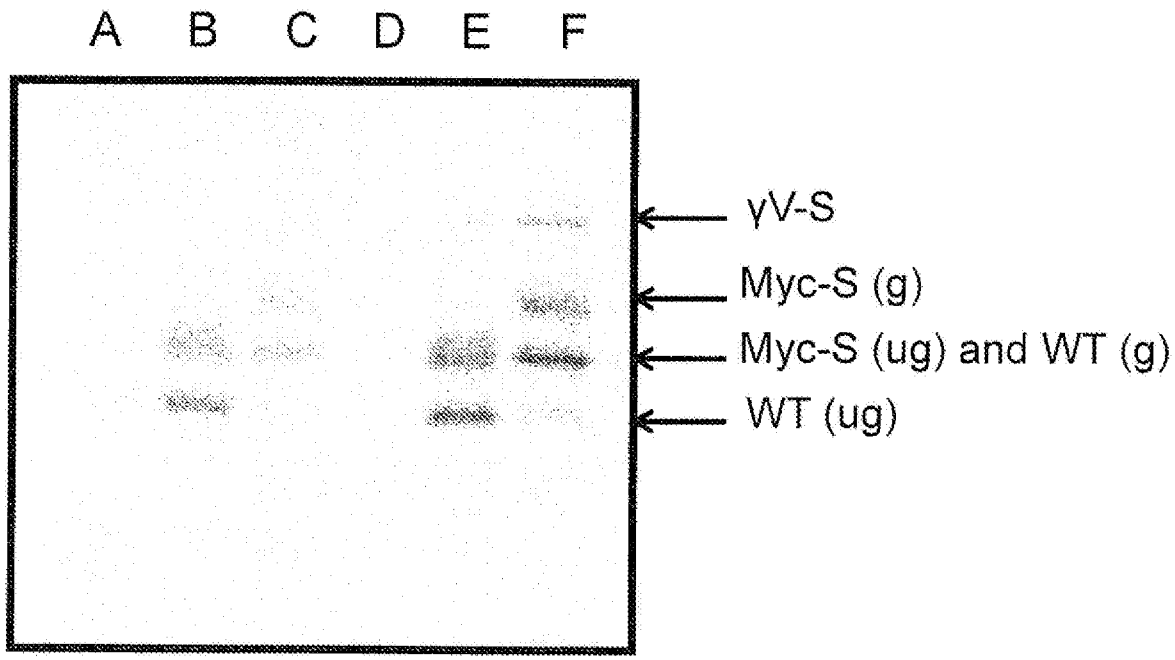


FIGURE 28

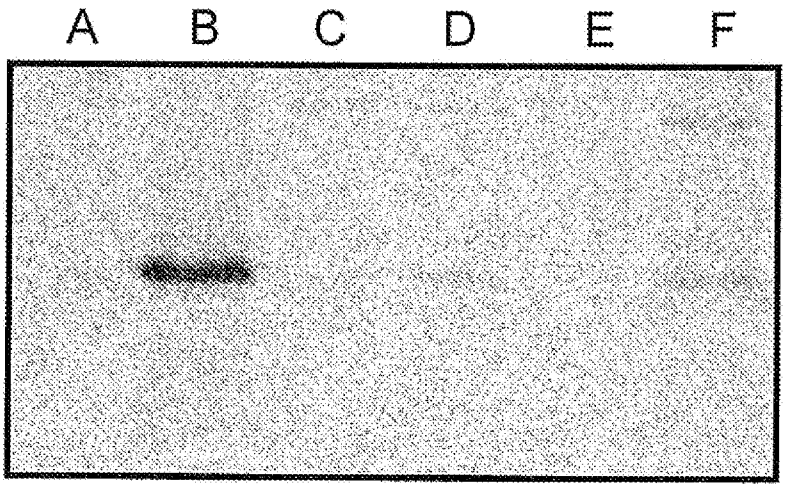


FIGURE 29

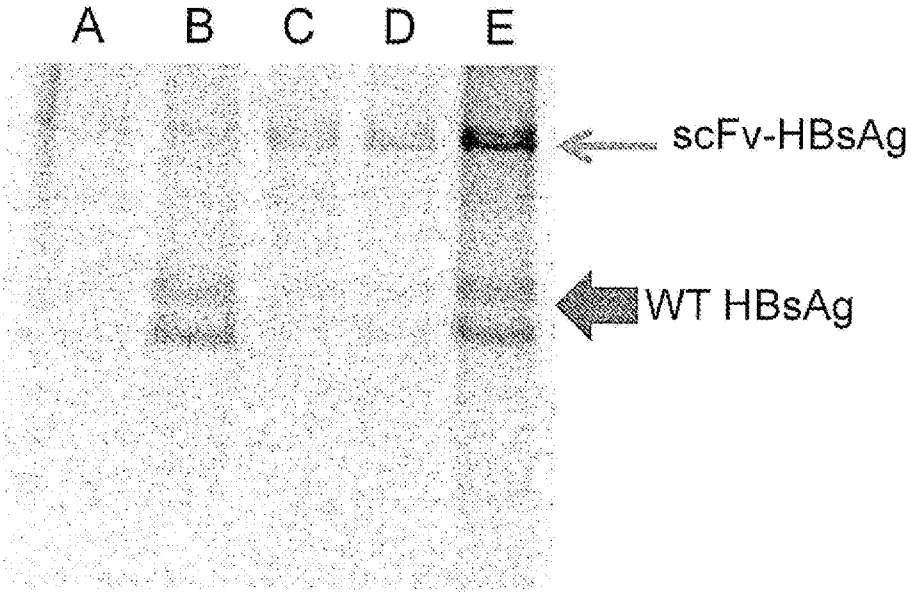


FIGURE 30

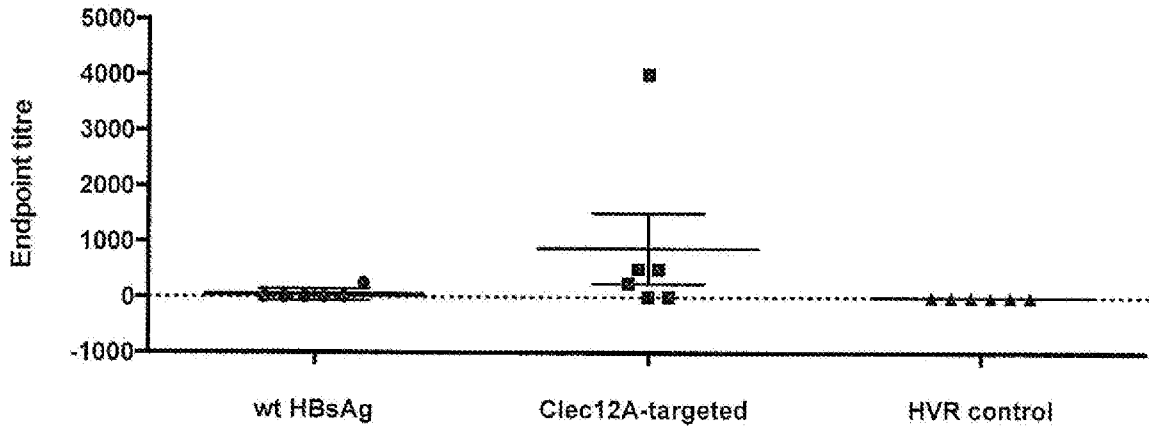
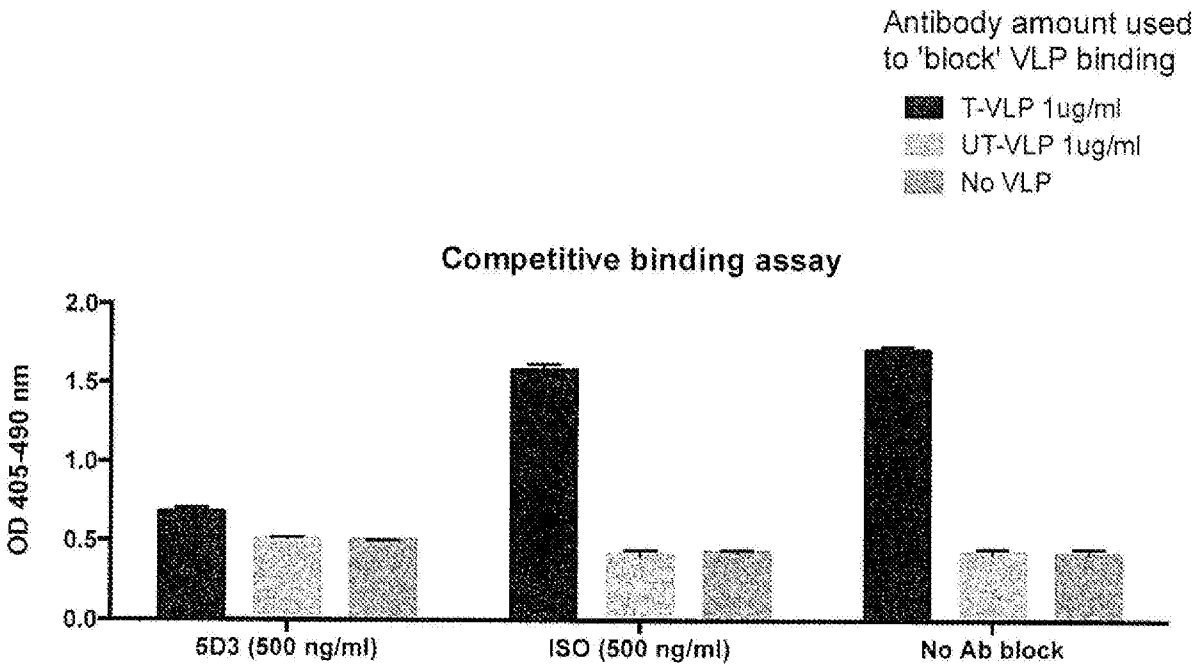

5000
4000
3000
2000
1000
0
-1000
Endpoint titre
wt HBsAg
Clec12A-targeted
HVR control

FIGURE 31



T-VLP = targeted VLPs
UT-VLP = untargeted VLPs

FIGURE 32

Dec 205 scFv-HBsAg sequence:

```
atggctggagctgcataatactgttcctcgtggcgacagcgaccgggtgttcatagcgag
M  G  W  S  C  I  I  L  F  L  V  A  T  A  T  G  V  H  S  E
gtgcagctgctcgaatctggaggaggcctcgtccagccgggggtggttcactgcggttgagt
V  K  L  L  E  S  G  G  G  L  V  Q  P  G  G  S  L  R  L  S
tgtgctgccagcggatttacttttaatgactttatatgaattggatcaggcagccaccca
C  A  A  S  G  F  T  F  N  D  F  Y  M  N  W  I  R  Q  P  P
ggccaggccccagaatggctcggcgtgatcagaaacaaaggcaaatggatacacaactgaa
G  Q  A  P  E  W  L  G  V  I  R  N  K  G  N  G  Y  T  T  E
gtgaatacttcagtaaaaggccggtttacgatctcaagagataatacccagaaacatttctg
V  N  T  S  V  K  G  R  F  T  I  S  R  D  N  T  Q  N  I  L
tacctccagatgaactcactgcgcgctgaagataccgctatatatttattgtgctcgcgga
Y  L  Q  M  N  S  L  R  A  E  D  T  A  I  Y  Y  C  A  R  G
ggcccatactactactctggagacgatgcccctattggggccaggcgctgatggtcacc
G  P  Y  Y  Y  S  G  D  D  A  F  Y  W  G  Q  G  V  M  V  T
gttagttctgggggcgggggatctggggggtggcggctcaggcgggggggggaagcgatatt
V  S  S  G  G  G  G  S  G  G  G  G  S  G  G  G  G  S  D  I
cagatgacccagtcaccttcatttctgagcaccagcctgggggaatagcatcactatcacc
Q  M  T  Q  S  P  S  F  L  S  T  S  L  G  N  S  I  T  I  T
tgccacgcttcacaaaatatcaagggctggctggcctggtaccagcaaaaaagtggaaaac
C  H  A  S  Q  N  I  K  G  W  L  A  W  Y  Q  Q  K  S  G  N
gccccacagctttctgatttacaaagcatcaagcctccagagcggtgtaccctcacgcttt
A  P  Q  L  L  I  Y  K  A  S  S  L  Q  S  G  V  P  S  R  F
tccggctcaggatctggcactgattacatctttactatttctaacttgcagccccgaagac
S  G  S  G  S  G  T  D  Y  I  F  T  I  S  N  L  Q  P  E  D
atagccacctattactgccaacactatcagagcttccccctggacttttgggggcggcaca
I  A  T  Y  Y  C  Q  H  Y  Q  S  F  P  W  T  F  G  G  G  T
aagctggagctgggcggctctggtagccaccaccatcaccatcaccaccatcaccatggga
K  L  E  L  G  A  S  G  S  H  H  H  H  H  H  H  H  H  G  G
gcatccggctcgAggattggggaccctgcgctgaacatggagaacatcacatcaggattc
A  S  G  S  R  I  G  D  P  A  L  N  M  E  N  I  T  S  G  F
ctaggaccccttctcgtgttacaggcgggggttttttcttgttgacaagaatcctcacaata
L  G  P  L  L  V  L  Q  A  G  F  F  L  L  T  R  I  L  T  I
ccgcagagtctagactcgtggtggacttctctcaattttctagggggaactaccgtgtgt
P  Q  S  L  D  S  W  W  T  S  L  N  F  L  G  G  T  T  V  C
cttggccaaaattgcagtccccaacctccaatcactcaccaacctcttgtcctccaact
L  G  Q  N  S  Q  S  P  T  S  N  H  S  P  T  S  C  P  P  T
tgtcctggttatcgctggatgtgtctgcggcgtttatcatcttcctcttcatcctgctg
C  P  G  Y  R  W  M  C  L  R  R  F  I  I  F  L  F  I  L  L
ctatgcctcatcttcttgttggttcttctggactatcaaggtatgttgcccgtttgtcct
L  C  L  I  F  L  L  V  L  L  D  Y  Q  G  M  L  P  V  C  P
ctaatccaggatcctcaacaaccagcaccgggaccatgccggacctgcatgactaccggt
L  I  P  G  S  S  T  T  S  T  G  P  C  R  T  C  M  T  T  G
cgaggaacctctatgtatcctcctgttgctgtaccaaaccttcggacggaaattgcacc
Q  G  T  S  M  Y  P  S  C  C  C  T  K  P  S  D  G  N  C  T
tgtattcccatcccatcatcctgggcttccggaaaattcctatgggagtgggcctcagcc
C  I  P  I  P  S  S  W  A  F  G  K  F  L  W  E  W  A  S  A
cgtttctcctggctcagtttactagtgccatttgttcagtggttcgtagggctttccccc
R  F  S  W  L  S  L  L  V  P  F  V  Q  W  F  V  G  L  S  P
actgttggctttcagttatatggatgatgtgggtattgggggccaagtcgtacagccatc
T  V  W  L  S  V  I  W  M  M  W  Y  W  G  P  S  L  Y  S  I
ttgagtccctttttaccgctgttaccaatttctttttgtcttttgggtatacatttaa
L  S  P  F  L  P  L  L  P  I  F  F  C  L  W  V  Y  I  -
```

FIGURE 33

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2014/000662

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/44 (2006.01) A61K 35/76 (2006.01) A61P 31/20 (2006.01) C07K 19/00 (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

MEDLINE, EPODOC, WPI, CAPLUS, BIOSIS. Keywords: VLP, virus like particle, Antibody, immunoglobulin, Hepadnavirus, orthohepadnavirae, hepatitis B, avihepadnavirae, envelope, HBsAg, fusion, linked, chimeric, conjugated, VLP antibody, VLP-Ab OR HBsAgS-VLP and synonyms and similar terms. Inventor/Applicant also searched on Pubmed and Patentscope websites.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

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Date of the actual completion of the international search	Date of mailing of the international search report
12 August 2014	12 August 2014
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Monica Graham AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262833179

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation).	DOCUMENTS CONSIDERED TO BE RELEVANT	**PCT/AU2014/000662**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2002/010416 A1 (THE CROWN IN THE RIGHT OF THE QUEENSLAND DEPARTMENT OF HEALTH) 07 February 2002 See abstract and claims	1-31
A	WO 2006/108226 A1 (THE UNIVERSITY OF QUEENSLAND & THE STATE OF QUEENSLAND ACTING THROUGH ITS DEPARTMENT OF HEALTH) 19 October 2006 See abstract and claims	1-31
A	WOO, W.P. et al. "Hepatitis B surface antigen vector delivers protective cytotoxic T-lymphocyte responses to disease-relevant foreign epitopes." J Virol. (April 2006); vol. 80, no.8, pages 3975-3984. See whole document	1-31
P,X	WO 2013/147232 A1 (NATIONAL UNIVERSITY CORPORATION NAGOYA UNIVERSITY)) 03 October 2013 (Derwent abstract retrieved online from WPI database)	1-31

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2014/000662**

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Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2002/010416 A1	07 February 2002	AU 7618201 A	13 Feb 2002
		AU 2001276182 B2	04 May 2006
		CA 2417719 A1	07 Feb 2002
		EP 1305438 A1	02 May 2003
		EP 1780285 A1	02 May 2007
		JP 2004504067 A	12 Feb 2004
		US 2003211996 A1	13 Nov 2003
		US 2009239265 A1	24 Sep 2009
WO 2006/108226 A1	19 October 2006	US 2009220537 A1	03 Sep 2009
WO 2013/147232 A1	03 October 2013	None	

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.