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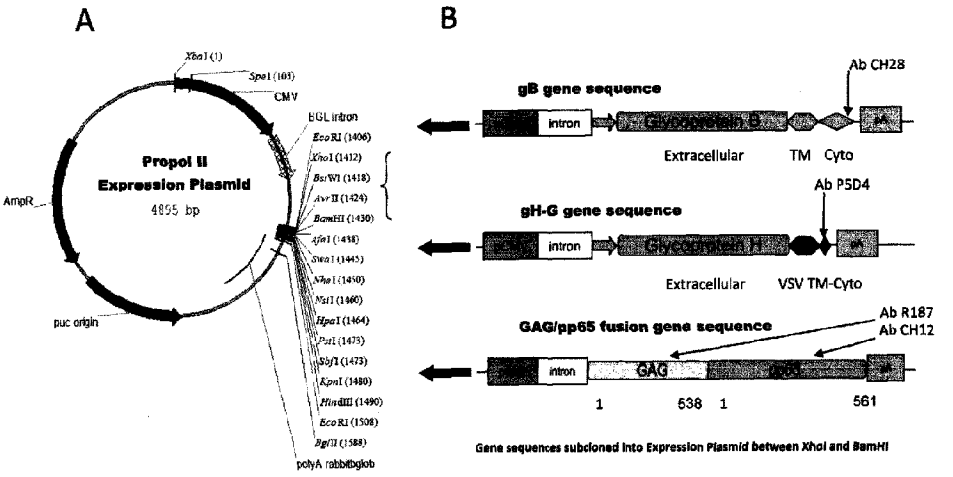

(57) Abrégé/Abstract:
The present disclosure provides compositions and methods useful for treating HCMV infection. As described herein, the compositions and methods are based on development of immunogenic compositions that include virus-like particles (VLPs) which comprise one or more Moloney Murine leukemia virus (MMLV) core proteins and include one or more HCMV epitopes, such as, for example, from HCMV envelope glycoproteins gB and/or gH and/or tegument protein pp65. Among other things, the present invention encompasses the recognition that a combination of antigens (e.g., envelope glycoproteins and structural proteins) can lead to beneficial immune responses, for example that include both a humoral response (e.g., production of neutralizing antibodies) and a cellular response (e.g., T-cell activation).

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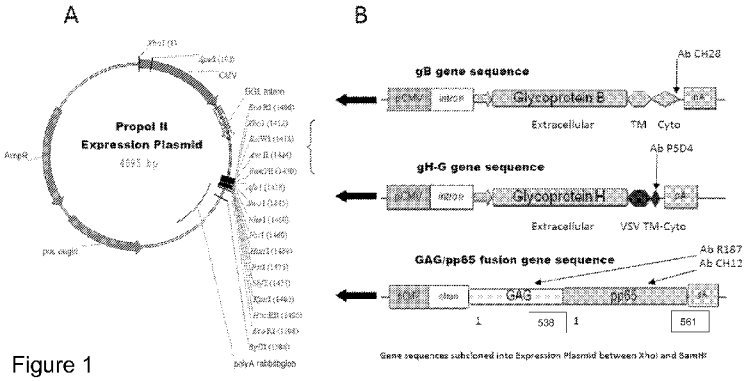

Figure 1

(57) Abstract: The present disclosure provides compositions and methods useful for treating HCMV infection. As described herein, the compositions and methods are based on development of immunogenic compositions that include virus-like particles (VLPs) which comprise one or more Moloney Murine leukemia virus (MMLV) core proteins and include one or more HCMV epitopes, such as, for example, from HCMV envelope glycoproteins gB and/or gH and/or tegument protein pp65. Among other things, the present invention encompasses the recognition that a combination of antigens (e.g., envelope glycoproteins and structural proteins) can lead to beneficial immune responses, for example that include both a humoral response (e.g., production of neutralizing antibodies) and a cellular response (e.g., T-cell activation).

WO 2013/068847 A3

COMPOSITIONS AND METHODS FOR TREATMENT OF CYTOMEGALOVIRUS

[0001]

Background

[0002] Human cytomegalovirus (HCMV), a β-herpesvirus, is a ubiquitously occurring pathogen. In an immunocompetent person, HCMV infection is normally unnoticed, having at most mild and nonspecific symptoms. By contrast, certain risk groups, for example in immunosuppressed patients such as AIDS patients or transplant recipients, and after prenatal infection, HCMV infection has serious manifestations (Staras SA et al., 2006 Clin Infect Dis 43(9):1143-51; Hebart H et al., 2004 Hum Immunol 65(5):432-6; Rowshani AT et al., 2005 Transplantation 79(4):381-6). Existing therapies include the use of immunoglobulins and anti-viral agents such as ganciclovir and its derivatives, which are most effective when used prophylactically or very early during infection in at risk populations. However, existing therapies are characterized by significant toxicity and limited efficacy, especially for late-onset disease (Boeckh M., 2004 Pediatr Transplant 8(Suppl. 5):19-27; Limaye AP., 2004 Transplantation 78(9):1390-6), and they have not had an impact on congenital HCMV disease. Development of an effective vaccine to protect against HCMV disease is recognized as an important public health priority (Arvin AM et al., 2004 Clin Infect Dis 39(2):233-9).

Summary

[0003] Among other things, the present invention provides methods and compositions useful for prophylaxis, treatment, and/or study of human cytomegalovirus (HCMV) infection. In some embodiments, the present invention provides virus-like particles (VLPs) which comprise one or more Moloney Murine leukemia virus (MMLV) core proteins and include one or more

HCMV epitopes, such as, for example, from HCMV envelope glycoproteins gB and/or gH and/or tegument protein pp65. Among other things, the present invention encompasses the recognition that a combination of antigens (e.g., envelope glycoproteins and structural proteins) can lead to improved induction of beneficial immune responses, for example that include both a humoral response (e.g., production of neutralizing antibodies) and a cellular response (e.g., T-cell activation). Provided VLPs may be characterized in that they contain no viral DNA and are non-infectious.

[0004] In some embodiments, provided VLPs are surrounded by a lipid membrane, optionally containing one or more epitopes from viral envelope glycoproteins (e.g., gB and/or gH) which are antigens that play a role in induction of virus-neutralizing antibodies.

[0005] In some embodiments, provided VLPs contain one or more epitopes from viral structural proteins (e.g., pp65) which are antigens that play a role in induction of cellular immune responses (e.g., T-cell response). In some embodiments, utilized viral structural proteins (e.g., pp65) both stimulate formation of T-helper cells and also induce cytotoxic T lymphocytes (CTL) against HCMV.

[0006] In some embodiments, the present invention provides variants of viral envelope glycoproteins (e.g., gB and/or gH). In some embodiments, a variant viral envelope glycoprotein is or comprises a fusion protein. In some embodiments, a variant of a viral glycoprotein comprises a heterologous protein domain (e.g., a transmembrane and/or cytoplasmic domain from a different protein). In some embodiments, a variant of a viral structural protein comprises a heterologous antigen or epitope. In some embodiments, the present invention provides VLPs comprising variants of viral structural proteins. In some embodiments, a variant of a viral structural protein is or comprises a fusion protein.

[0007] As used in this application, the terms "about" and "approximately" are used as equivalents. Any numerals used in this application with or without about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art.

[0008] Other features, objects, and advantages of the present invention are apparent in the detailed description that follows. It should be understood, however, that the detailed

description, while indicating embodiments of the present invention, is given by way of illustration only, not limitation. Various changes and modifications within the scope of the invention will become apparent to those skilled in the art from the detailed description.

Brief Description of the Drawings

[0009] The drawings are for illustration purposes only, not for limitation.

[0010] **Figure 1** shows the DNA expression plasmid map (A) and construction of exemplary recombinant expression plasmids (B).

[0011] **Figure 2** shows FACS analysis of exemplary heterologous surface antigens on HEK 293 packaging cells (A) and western blot analysis of heterologous antigen expression in exemplary VLP compositions (B).

[0012] **Figure 3(A) and (B)** show particle size determination and polydispersity index for two exemplary VLP compositions.

[0013] **Figure 4** shows ELISA titers in mice treated with exemplary gB/pp65 (A), gH-G/pp65 (B), or gB/gH-G/pp65 (C) VLP compositions.

[0014] **Figure 5** shows neutralizing antibody activity in mice treated with exemplary VLP compositions (assayed in human fibroblast cells).

[0015] **Figure 6** shows neutralizing antibody activity in mice treated with exemplary VLP compositions (assayed in human epithelial cells).

[0016] **Figure 7** shows neutralizing antibody activity in mice treated with exemplary VLP compositions versus a recombinant gB protein (assayed in human fibroblast cells).

[0017] **Figure 8** shows antigen-specific CTL responses in mice treated with exemplary VLP compositions expressed in HEK 293 cells, depicted as CTL frequency based on CFSE decay, gating on $CD3^+$ $CD8^+$ T cells (A) or frequency of proliferating pp65-specific CTLs (B).

[0018] **Figure 9** shows anti-gB and neutralizing antibody titers in rabbits treated with exemplary VLP compositions expressed in HEK 293 cells (assayed in human fibroblast cells).

[0019] **Figure 10** shows neutralizing antibody titers in rabbits treated with exemplary VLP compositions expressed in HEK 293 cells (assayed in human epithelial cells).

[0020] **Figure 11** shows negative-staining Electron Microscopy (EM) images of exemplary VLP compositions expressed in CHO cells purified by (A) Tangential Flow Filtration (TFF) or (B) Anion Exchange (AEX) Chromatography.

[0021] **Figure 12** shows neutralizing antibody titers in rabbits treated with exemplary VLP compositions expressed in CHO cells and purified by Tangential Flow Filtration (TFF) and Anion Exchange (AEX) Chromatography (assayed in human fibroblast cells).

[0022] **Figure 13** shows neutralizing antibody titers in rabbits treated with exemplary VLP compositions expressed in CHO cells purified by Tangential Flow Filtration (TFF) and Anion Exchange (AEX) Chromatography (assayed in human epithelial cells).

[0023] **Figure 14** shows the avidity index of antibodies produced in rabbits treated with exemplary VLP compositions expressed in CHO cells and purified by Tangential Flow Filtration (TFF) and Anion Exchange (AEX) Chromatography.

Definitions

[0024] In order for the present invention to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the specification.

[0025] *Amino acid*: As used herein, term "amino acid," in its broadest sense, refers to any compound and/or substance that can be incorporated into a polypeptide chain. In some embodiments, an amino acid has the general structure H_2N–C(H)(R)–COOH. In some embodiments, an amino acid is a naturally occurring amino acid. In some embodiments, an amino acid is a synthetic amino acid; in some embodiments, an amino acid is a d-amino acid; in some embodiments, an amino acid is an l-amino acid. "Standard amino acid" refers to any of the twenty standard l-amino acids commonly found in naturally occurring peptides. "Nonstandard

amino acid" refers to any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or obtained from a natural source. As used herein, "synthetic amino acid" encompasses chemically modified amino acids, including but not limited to salts, amino acid derivatives (such as amides), and/or substitutions. Amino acids, including carboxy- and/or amino-terminal amino acids in peptides, can be modified by methylation, amidation, acetylation, protecting groups, and/or substitution with other chemical groups that can change the peptide's circulating half-life without adversely affecting their activity. Amino acids may participate in a disulfide bond. Amino acids may comprise one or posttranslational modifications, such as association with one or more chemical entities (*e.g.*, methyl groups, acetate groups, acetyl groups, phosphate groups, formyl moieties, isoprenoid groups, sulfate groups, polyethylene glycol moieties, lipid moieties, carbohydrate moieties, biotin moieties, *etc.*). The term "amino acid" is used interchangeably with "amino acid residue," and may refer to a free amino acid and/or to an amino acid residue of a peptide. It will be apparent from the context in which the term is used whether it refers to a free amino acid or a residue of a peptide.

[0026] *Antigen:* As used herein, the term "antigen" refers to a substance containing one or more epitopes (either linear, conformational or both) that are recognized by antibodies. In certain embodiments, an antigen is or comprises a virus or a viral polypeptide. In some embodiments, the term "antigen" refers to a subunit antigen (i.e., an antigen which is separate and discrete from a whole virus with which the antigen is associated in nature; e.g., an antigen which is associated with a virus-like particle). Alternatively or additionally, in some embodiments, the term "antigen" refers to killed, attenuated or inactivated viruses. In certain embodiments, an antigen is an "immunogen."

[0027] *Approximately or about:* As used herein, the term "approximately" or "about," as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0028] *Amelioration*: As used herein, the term "amelioration" is meant the prevention, reduction or palliation of a state, or improvement of the state of a subject. Amelioration includes, but does not require complete recovery or complete prevention of a disease, disorder or condition (e.g., HCMV infection). The term "prevention" refers to a delay of onset of a disease, disorder or condition. Prevention may be considered complete when onset of a disease, disorder or condition has been delayed for a predefined period of time.

[0029] *Characteristic portion*: As used herein, the term a "characteristic portion" of a substance, in the broadest sense, is one that shares a designated degree of structural identity with intact substance. In certain embodiments, a characteristic portion shares at least one functional characteristic with the intact substance. For example, a "characteristic portion" of a protein or polypeptide is one that contains a continuous stretch of amino acids, or a collection of continuous stretches of amino acids, that together are characteristic of a protein or polypeptide. In some embodiments, each such continuous stretch generally contains at least 2, 5, 10, 15, 20, 50, or more amino acids. In general, a characteristic portion of a substance (*e.g.*, of a protein, antibody, *etc.*) is one that, in addition to the sequence and/or structural identity specified above, shares at least one functional characteristic with the relevant intact substance. In some embodiments, a characteristic portion may be biologically active.

[0030] *Characteristic sequence*: A "characteristic sequence" is a sequence that is found in all members of a family of polypeptides or nucleic acids, and therefore can be used by those of ordinary skill in the art to define members of the family.

[0031] *Cytoplasmic domain*: As is known in the art, polypeptides sometimes have transmembrane, cytoplasmic, and/or extracellular domains. In general, a "cytoplasmic domain", as used herein, refers to a domain that has an attribute of being present in the cytoplasm. As will be appreciated, it is not required that every amino acid in a cytoplasmic domain be present in the cytoplasm. For example, in some embodiments, a cytoplasmic domain is characterized in that a designated stretch or portion of a protein is substantially located in the cytoplasm. As is well known in the art, amino acid or nucleic acid sequences may be analyzed using a variety of algorithms to predict protein subcellular localization (e.g., cytoplasmic localization). Exemplary such programs include psort (PSORT.org), Prosite (prosite.expasy.org), among others.

[0032] *Dosage form:* As used herein, the terms "dosage form" and "unit dosage form" refer to a physically discrete unit of a therapeutic agent for the patient to be treated. Each unit contains a predetermined quantity of active material calculated to produce the desired therapeutic effect. It will be understood, however, that the total dosage of the composition will be decided by the attending physician within the scope of sound medical judgment.

[0033] *Dosing regimen:* A "dosing regimen" (or "therapeutic regimen"), as that term is used herein, is a set of unit doses (typically more than one) that are administered individually to a subject, typically separated by periods of time. In some embodiments, a given therapeutic agent has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which are separated from one another by a time period of the same length; in some embodiments, a dosing regimen comprises a plurality of doses and at least two different time periods separating individual doses.

[0034] *Expression*: As used herein, "expression" of a nucleic acid sequence refers to one or more of the following events: (1) production of an RNA template from a DNA sequence (*e.g.*, by transcription); (2) processing of an RNA transcript (*e.g.*, by splicing, editing, 5' cap formation, and/or 3' end formation); (3) translation of an RNA into a polypeptide or protein; and/or (4) post-translational modification of a polypeptide or protein.

[0035] *Extracellular domain:* As is known in the art, polypeptides sometimes have transmembrane, cytoplasmic, and/or extracellular domains. In general, an "extracellular domain", as used herein, refers to a domain that has an attribute of being present outside a cell. As will be appreciated, it is not required that every amino acid in an extracellular domain be present outside the cell. For example, in some embodiments, an extracellular domain is characterized in that a designated stretch or portion of a protein is substantially located outside the cell. As is well known in the art, amino acid or nucleic acid sequences may be analyzed using a variety of algorithms to predict protein subcellular localization (e.g., extracellular localization). Exemplary such programs include psort (PSORT.org), Prosite (prosite.expasy.org), among others.

[0036] *Fusion protein*: As used herein, the term "fusion protein" generally refers to a polypeptide including at least two segments, each of which shows a high degree of amino acid

identity to a peptide moiety that (1) occurs in nature, and/or (2) represents a functional domain of a polypeptide. Typically, a polypeptide containing at least two such segments is considered to be a fusion protein if the two segments are moieties that (1) are not included in nature in the same peptide, and/or (2) have not previously been linked to one another in a single polypeptide, and/or (3) have been linked to one another through action of the hand of man.

[0037] *Gene*: As used herein, the term "gene" has its meaning as understood in the art. It will be appreciated by those of ordinary skill in the art that the term "gene" may include gene regulatory sequences (*e.g.*, promoters, enhancers, *etc.*) and/or intron sequences. It will further be appreciated that definitions of gene include references to nucleic acids that do not encode proteins but rather encode functional RNA molecules such as tRNAs, RNAi-inducing agents, *etc.* For the purpose of clarity we note that, as used in the present application, the term "gene" generally refers to a portion of a nucleic acid that encodes a protein; the term may optionally encompass regulatory sequences, as will be clear from context to those of ordinary skill in the art. This definition is not intended to exclude application of the term "gene" to non-protein–coding expression units but rather to clarify that, in most cases, the term as used in this document refers to a protein-coding nucleic acid.

[0038] *Gene product* or *expression product*: As used herein, the term "gene product" or "expression product" generally refers to an RNA transcribed from the gene (pre-and/or post-processing) or a polypeptide (pre- and/or post-modification) encoded by an RNA transcribed from the gene.

[0039] *Heterologous:* As used herein, the term "heterologous" with respect to a protein or a polypeptide refers to a protein or polypeptide that is non-naturally occurring in a particular organism, such as a retrovirus or VLP. In some embodiments, a heterologous protein or polypeptide is non-naturally occurring in a particular retrovirus virion. As used herein, the term "heterologous" with respect to a protein domain generally refers to a protein domain that is non-naturally occurring in a particular protein.

[0040] *Immunogenic:* As used herein, the term "immunogenic" means capable of producing an immune response in a host animal against a non-host entity (e.g., an HCMV

antigen). In certain embodiments, this immune response forms the basis of the protective immunity elicited by a vaccine against a specific infectious organism (e.g., an HCMV).

[0041] *Immune response:* As used herein, the term "immune response" refers to a response elicited in an animal. An immune response may refer to cellular immunity, humoral immunity or may involve both. An immune response may also be limited to a part of the immune system. For example, in certain embodiments, an immunogenic composition may induce an increased IFNγ response. In certain embodiments, an immunogenic composition may induce a mucosal IgA response (e.g., as measured in nasal and/or rectal washes). In certain embodiments, an immunogenic composition may induce a systemic IgG response (e.g., as measured in serum). In certain embodiments, an immunogenic composition may induce virus-neutralizing antibodies or a neutralizing antibody response.

[0042] *Improve, increase, or reduce*: As used herein, the terms "improve," "increase" or "reduce," or grammatical equivalents, indicate values that are relative to a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control individual (or multiple control individuals) in the absence of the treatment described herein.

[0043] *Individual, subject, patient*: As used herein, the terms "subject," "individual" or "patient" refer to a human or a non-human mammalian subject. In some embodiments, the individual (also referred to as "patient" or "subject") being treated is an individual (fetus, infant, child, adolescent, or adult) suffering from a disease, for example, HCMV infection. In some embodiments, the subject is at risk for HCMV infection. In some embodiments, the subject is an immunosuppressed subject. For example, in some embodiments, the immunosuppressed subject is selected from the group consisting of an HIV-infected subject, an AIDS patient, a transplant recipient, a pediatric subject, and a pregnant subject. In some embodiments, the subject has been exposed to HCMV infection. In some embodiments, the subject is a human.

[0044] *Isolated*: As used herein, the term "isolated" refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) produced, prepared, and/or manufactured by the hand of man. Isolated substances and/or entities

may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% of the other components with which they were initially associated. In some embodiments, isolated agents are about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is "pure" if it is substantially free of other components. As used herein, calculation of percent purity of isolated substances and/or entities should not include excipients (*e.g.*, buffer, solvent, water, *etc.*).

[0045] *Linker:* As used herein, the term "linker" refers to, e.g., in a fusion protein, an amino acid sequence of an appropriate length other than that appearing at a particular position in the natural protein and is generally designed to be flexible and/or to interpose a structure, such as an α-helix, between two protein moieties. In general, a linker allows two or more domains of a fusion protein to retain 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of the biological activity of each of the domains. A linker may also be referred to as a spacer.

[0046] *Nucleic acid*: As used herein, the term "nucleic acid," in its broadest sense, refers to any compound and/or substance that is or can be incorporated into an oligonucleotide chain. In some embodiments, a nucleic acid is a compound and/or substance that is or can be incorporated into an oligonucleotide chain via a phosphodiester linkage. In some embodiments, "nucleic acid" refers to individual nucleic acid residues (*e.g.*, nucleotides and/or nucleosides). In some embodiments, "nucleic acid" refers to an oligonucleotide chain comprising individual nucleic acid residues. As used herein, the terms "oligonucleotide" and "polynucleotide" can be used interchangeably. In some embodiments, "nucleic acid" encompasses RNA as well as single and/or double-stranded DNA and/or cDNA. Furthermore, the terms "nucleic acid," "DNA," "RNA," and/or similar terms include nucleic acid analogs, *i.e.*, analogs having other than a phosphodiester backbone. For example, the so-called "peptide nucleic acids," which are known in the art and have peptide bonds instead of phosphodiester bonds in the backbone, are considered within the scope of the present invention. The term "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and/or encode the same amino acid sequence. Nucleotide sequences that encode proteins

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

[0047] *Pharmaceutically acceptable*: The term "pharmaceutically acceptable" as used herein, refers to substances that, within the scope of sound medical judgment, are suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0048] *Polypeptide*: As used herein, a "polypeptide", generally speaking, is a string of at least two amino acids attached to one another by a peptide bond. In some embodiments, a polypeptide may include at least 3-5 amino acids, each of which is attached to others by way of at least one peptide bond. Those of ordinary skill in the art will appreciate that polypeptides

sometimes include "non-natural" amino acids or other entities that nonetheless are capable of integrating into a polypeptide chain, optionally.

[0049] *Polyprotein:* As used herein, the term "polyprotein", generally refers to a protein that, after synthesis, may be cleaved to produce several functionally distinct polypeptides. A polyprotein is typically encoded by a single amino acid sequence. In some embodiments, an uncleaved polyprotein retains biological activity of its component parts. Some viruses produce such polyproteins, e.g., a Gag polyprotein, which can be retained as a functional polyprotein or can be processed into several functionally distinct polypeptides. Functionally, the Gag polyprotein is divided into three domains: the membrane binding domain, which targets the Gag polyprotein to the cellular membrane; the interaction domain which promotes Gag polymerization; and the late domain which facilitates release of nascent virions from the host cell. In general, the form of the Gag protein that mediates viral particle assembly is the polyprotein.

[0050] *Self-assembling portion*: In general, a "self-assembling portion", as used herein, refers to a relevant stretch of an entity that adopts a defined arrangement without guidance or management from an outside source. In some embodiments, the entity is a protein. In some embodiments, the entity is a polyprotein. In some such embodiments, the relevant stretch is at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or more residues. Self-assembly may be exhibited, for example, within the context of a cell (e.g., *in vivo*). Alternatively or additionally, self-assembly may be exhibited outside the context of a cell (e.g., *in vitro*). Self-assembly may be intramolecular (e.g., folding) and/or intermolecular. In some embodiments, self-assembly may be macromolecular whereby entities self-assemble into a complex and/or extended macromolecular structure. Self-assembled entities may exhibit a wide range of structural motifs, including, but not limited to particles, fibers, sheets, and ribbons. In some embodiments, self-assembly of an entity is important for a biological function of the entity. For example, in some embodiments, self assembly of a lipid leads to formation of a cell membrane structure. In some embodiments, self assembly of a protein (e.g., a viral structural protein) in a cellular context leads to formation of a particle structure (e.g., a viral particle structure). For example, a viral structural polyprotein may contain a targeting sequence that is capable of

directing its localization to a cellular membrane of its host cell (e.g., plasma membrane, endosome, etc.) from which the viral structural polyprotein may bud out to form a VLP that contains host cellular membranous material surrounding the viral structural polyprotein.

[0051] *Substantial homology*: The phrase "substantial homology" is used herein to refer to a comparison between amino acid or nucleic acid sequences. As will be appreciated by those of ordinary skill in the art, two sequences are generally considered to be "substantially homologous" if they contain homologous residues in corresponding positions. Homologous residues may be identical residues. Alternatively, homologous residues may be non-identical residues with appropriately similar structural and/or functional characteristics. For example, as is well known by those of ordinary skill in the art, certain amino acids are typically classified as "hydrophobic" or "hydrophilic" amino acids, and/or as having "polar" or "non-polar" side chains. Substitution of one amino acid for another of the same type may often be considered a "homologous" substitution.

[0052] As is well known in this art, amino acid or nucleic acid sequences may be compared using any of a variety of algorithms, including those available in commercial computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences. Exemplary such programs are described in Altschul, et al., Basic local alignment search tool, *J. Mol. Biol.*, 215(3): 403-410, 1990; Altschul, et al., *Methods in Enzymology* 266:460-480 (1996); Altschul, et al., "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", *Nucleic Acids Res.* 25:3389-3402, 1997; Baxevanis, et al., *Bioinformatics : A Practical Guide to the Analysis of Genes and Proteins*, Wiley, 1998; and Misener, et al., (eds.), *Bioinformatics Methods and Protocols* (Methods in Molecular Biology, Vol. 132), Humana Press, 1999. In addition to identifying homologous sequences, the programs mentioned above typically provide an indication of the degree of homology. In some embodiments, two sequences are considered to be substantially homologous if at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of their corresponding residues are homologous over a relevant stretch of residues. In some embodiments, the relevant stretch is a complete sequence. In some embodiments, the relevant stretch is at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75,

80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or more residues.

[0053] *Substantial identity*: The phrase "substantial identity" is used herein to refer to a comparison between amino acid or nucleic acid sequences. As will be appreciated by those of ordinary skill in the art, two sequences are generally considered to be "substantially identical" if they contain identical residues in corresponding positions. As is well known in this art, amino acid or nucleic acid sequences may be compared using any of a variety of algorithms, including those available in commercial computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences. Exemplary such programs are described in Altschul, et al., Basic local alignment search tool, *J. Mol. Biol.*, 215(3): 403-410, 1990; Altschul, *et al.*, *Methods in Enzymology* 266:460-480 (1996); Altschul *et al.*, *Nucleic Acids Res.* 25:3389-3402, 1997; Baxevanis *et al.*, *Bioinformatics : A Practical Guide to the Analysis of Genes and Proteins*, Wiley, 1998; and Misener, et al., (eds.), *Bioinformatics Methods and Protocols* (Methods in Molecular Biology, Vol. 132), Humana Press, 1999. In addition to identifying identical sequences, the programs mentioned above typically provide an indication of the degree of identity. In some embodiments, two sequences are considered to be substantially identical if at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of their corresponding residues are identical over a relevant stretch of residues. In some embodiments, the relevant stretch is a complete sequence. In some embodiments, the relevant stretch is at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or more residues.

[0054] *Suffering from*: An individual who is "suffering from" a disease, disorder, or condition (*e.g.*, HCMV infection) has been diagnosed with and/or exhibits one or more symptoms of the disease, disorder, or condition.

[0055] *Susceptible to*: An individual who is "susceptible to" a disease, disorder, or condition (*e.g.*, HCMV infection) is at risk for developing the disease, disorder, or condition. In some embodiments, an individual who is susceptible to a disease, disorder, or condition does not display any symptoms of the disease, disorder, or condition. In some embodiments, an

individual who is susceptible to a disease, disorder, or condition has not been diagnosed with the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, or condition is an individual who has been exposed to conditions associated with development of the disease, disorder, or condition (*e.g.*, the individual has been exposed to HCMV).

[0056] *Symptoms are reduced:* According to the present invention, "symptoms are reduced" when one or more symptoms of a particular disease, disorder or condition is reduced in magnitude (*e.g.*, intensity, severity, *etc.*) or frequency. For purposes of clarity, a delay in the onset of a particular symptom is considered one form of reducing the frequency of that symptom. It is not intended that the present invention be limited only to cases where the symptoms are eliminated. The present invention specifically contemplates treatment such that one or more symptoms is/are reduced (and the condition of the subject is thereby "improved"), albeit not completely eliminated.

[0057] *Therapeutically effective amount:* As used herein, the term "therapeutically effective amount" refers to an amount sufficient to confer a therapeutic effect on the treated subject, at a reasonable benefit/risk ratio applicable to any medical treatment. The therapeutic effect may be objective (i.e., measurable by some test or marker) or subjective (i.e., subject gives an indication of or feels an effect). In particular, the "therapeutically effective amount" refers to an amount of a therapeutic protein or composition effective to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect, such as by ameliorating symptoms associated with the disease, preventing or delaying the onset of the disease, and/or also lessening the severity or frequency of symptoms of the disease. A therapeutically effective amount is commonly administered in a dosing regimen that may comprise multiple unit doses. For any particular immunogenic composition, a therapeutically effective amount (and/or an appropriate unit dose within an effective dosing regimen) may vary, for example, depending on route of administration, on combination with other pharmaceutical agents. Also, the specific therapeutically effective amount (and/or unit dose) for any particular patient may depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific pharmaceutical agent employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time

of administration, route of administration, and/or rate of excretion or metabolism of the specific immunogenic composition employed; the duration of the treatment; and like factors as is well known in the medical arts.

[0058] *Transmembrane domain*: As is known in the art, polypeptides sometimes have transmembrane, cytoplasmic, and/or extracellular domains. In general, a "transmembrane domain", as used herein, refers to a domain having an attribute of being present in the membrane (e.g., spanning a portion or all of a cellular membrane). As will be appreciated, it is not required that every amino acid in a transmembrane domain be present in the membrane. For example, in some embodiments, a transmembrane domain is characterized in that a designated stretch or portion of a protein is substantially located in the membrane. As is well known in the art, amino acid or nucleic acid sequences may be analyzed using a variety of algorithms to predict protein subcellular localization (e.g., transmembrane localization). Exemplary such programs include psort (PSORT.org), Prosite (prosite.expasy.org), among others.

[0059] *Treatment*: As used herein, the term "treatment" (also "treat" or "treating") refers to any administration of an immunogenic composition that partially or completely alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of and/or reduces incidence of one or more symptoms or features of a particular disease, disorder, and/or condition (e.g., HCMV infection) or the predisposition toward the disease. Such treatment may be of a subject who does not exhibit signs of the relevant disease, disorder and/or condition and/or of a subject who exhibits only early signs of the disease, disorder, and/or condition. Alternatively or additionally, such treatment may be of a subject who exhibits one or more established signs of the relevant disease, disorder and/or condition. In certain embodiments, the term "treating" refers to the vaccination of a patient.

[0060] *Vaccination*: As used herein, the term "vaccination" refers to the administration of a composition intended to generate an immune response, for example to a disease-causing agent (e.g., HCMV). For the purposes of the present invention, vaccination can be administered before, during, and/or after exposure to a disease-causing agent, and in certain embodiments, before, during, and/or shortly after exposure to the agent. In some embodiments, vaccination includes multiple administrations, appropriately spaced in time, of a vaccinating composition.

[0061] *Vector*: As used herein, "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it is associated. In some embodiments, vectors are capable of extra-chromosomal replication and/or expression of nucleic acids to which they are linked in a host cell such as a eukaryotic and/or prokaryotic cell. Vectors capable of directing the expression of operatively linked genes are referred to herein as "expression vectors."

Detailed Description of Certain Embodiments

[0062] Among other things, the present invention provides methods and compositions useful for prophylaxis, treatment, and/or study of human cytomegalovirus (HCMV) infection. In some embodiments, the present invention provides virus-like particles (VLPs) which comprise one or more Moloney Murine leukemia virus (MMLV) core proteins and include one or more HCMV epitopes, such as, for example, from HCMV envelope glycoproteins gB and/or gH and/or tegument protein pp65. Among other things, the present invention encompasses the recognition that a combination of antigens (e.g., envelope glycoproteins and structural proteins) can lead to improved induction of beneficial immune responses, for example that include both a humoral response (e.g., production of neutralizing antibodies) and a cellular response (e.g., T-cell activation). Provided VLPs may be characterized in that they contain no viral RNA or DNA and are non-infectious. In some embodiments, provided VLPs do contain viral RNA or DNA and are infectious. In some such embodiments, provided VLPs are useful as a DNA vaccine.

[0063] In some embodiments, the humoral immune response in a subject is sustained for at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about 13 months, at least about 14 months, at least about 15 months, at least about 16 months, at least about 17 months, at least about 18 months, at least about 19 months, at least about 20 months, at least about 21 months, at least about 22 months, at least about 23 months, at least about 24 months, at least about 28 months, at least about 32 months, at least about 36 months, at least about 40 months, at least about 44 months, at least about 48 months, or longer. In some embodiments, the cellular immune response in a subject is sustained for at least

about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, or at least 12 months.

[0064] In some embodiments, provided VLPs are surrounded by a lipid membrane, optionally containing one or more epitopes from viral envelope glycoproteins (e.g., gB and/or gH) which are antigens that play a role in induction of virus-neutralizing antibodies.

[0065] In some embodiments, provided VLPs contain one or more epitopes from viral structural proteins (e.g., pp65) which are antigens that play a role in induction of cellular immune responses (e.g., T-cell response). In some embodiments, utilized viral structural proteins (e.g., pp65) both stimulate formation of T-helper cells (T_H) and also induce cytotoxic T lymphocytes (CTL) against HCMV.

[0066] In some embodiments, the present invention provides variants of viral envelope glycoproteins (e.g., gB and/or gH). In some embodiments, a variant viral envelope glycoprotein is or comprises a fusion protein. In some embodiments, a variant of a viral glycoprotein comprises a heterologous protein domain (e.g., a transmembrane and/or cytoplasmic domain from a different protein). In some embodiments, a variant of a viral structural protein comprises a heterologous antigen or epitope. In some embodiments, the present invention provides VLPs comprising variants of viral structural proteins. In some embodiments, a variant of a viral structural protein is or comprises a fusion protein.

I. Virus-Like Particles (VLPs)

[0067] Retroviruses are enveloped RNA viruses that belong to the family *Retroviridae.* After infection of a host cell by a retrovirus, RNA is transcribed into DNA via the enzyme reverse transcriptase. DNA is then incorporated into the host cell's genome by an integrase enzyme and thereafter replicates as part of the host cell's DNA. The *Retroviridae* family includes the following genus *Alpharetrovirus, Betaretrovirus, Gammearetrovirus, Deltaretrovirus, Epsilonretrovirus, Lentivirus* and *Spumavirus.* The hosts for this family of retroviruses generally are vertebrates. Retroviruses produce an infectious virion containing a

spherical nucleocapsid (the viral genome in complex with viral structural proteins) surrounded by a lipid bilayer derived from the host cell membrane.

[0068] Retroviral vectors can be used to generate enveloped virions that are infectious and either replication-competent or replication-defective. Replication-competent infectious retroviral vectors contain all of the necessary genes for virion synthesis and continue to propagate themselves once infection of the host cell occurs. Replication-defective infectious retroviral vectors do not spread after the initial infection. This is accomplished by replacement of most of the coding regions of the retrovirus with genes or nucleotide sequences to be transferred; so that the vector is incapable of making proteins required for additional rounds of replication.

[0069] Alternatively or additionally, retroviral vectors can be used to generate virus-like particles (VLPs) that lack a retrovirus-derived genome and are both non-infectious and non-replicating. Because of VLPs advantageous properties, VLPs may be utilized as an antigen delivery system. Furthermore, because VLPs are non-infectious, they can be administered safely as an immunogenic composition (e.g., a vaccine). VLPs are generally structurally similar to enveloped virions described above, but lack a retrovirus-derived genome, making it unlikely that viral replication will occur. Expression of capsid proteins (e.g., Gag) of some viruses (e.g., murine leukemia viruses, such as Moloney Murine leukemia virus (MMLV)) leads to self-assembly into particles similar to the corresponding native virus, which particles are free of viral genetic material.

[0070] A wide variety of VLPs have been prepared. For example, VLPs including single or multiple capsid proteins either with or without envelope proteins and/or surface glycoproteins have been prepared. In some cases, VLPs are non-enveloped and assemble by expression of just one major capsid protein, as shown for VLPs prepared from hepadnaviruses (e.g., Engerix™, GSK and Recombivax HB™, Merck), papillomaviruses (e.g., Cervarix™ , GSK and Gardasil™, Merck), paroviruses, or polyomaviruses. In some embodiments, VLPs are enveloped and can comprise multiple antigenic proteins found in the corresponding native virus. VLPs typically resemble their corresponding native virus and can be multivalent particulate structures. In some embodiments, antigenic proteins may be presented internally within the VLP, as a component of

the VLP structure, and/or on the surface of the VLP. The present invention encompasses the recognition that presentation of an antigen in the context of a VLP is advantageous for induction of neutralizing antibodies against the antigen as compared to other forms of antigen presentation, e.g., soluble antigens not associated with a VLP. Neutralizing antibodies most often recognize tertiary or quarternary structures; this often requires presenting antigenic proteins, like envelope glycoproteins, in their native viral conformation. Alternatively or additionally, VLPs may be useful for presenting antigens in a context which induces cellular immunity (e.g., T cell response). The present invention further encompasses the insight that use of antigen combinations in VLP systems can generate improved immune response.

A. Structural Proteins

[0071] In some embodiments, the present invention utilizes VLPs comprised of one or more retroviral structural proteins (e.g., Gag). In some embodiments, a structural protein for use in accordance with the present invention is Alpharetrovirus (e.g., Avian Leukosis Virus), Betaretrovirus (Mouse Mammary Tumor Virus), Gammearetrovirus (Murine Leukemia Virus), Deltaretrovirus (Bovine Leukemia Virus), Epsilonretrovirus (Walley Dermal Sarcoma Virus), Lentivirus (Human Immunodeficiency Virus 1) or Spumavirus (Chimpanzee Foamy Virus) structural protein. In certain embodiments, a structural polyprotein is a Murine Leukemia Virus (MLV) structural protein. Genomes of these retroviruses are readily available in databases. The Gag genes of all these retroviruses have an overall structural similarity and within each group of retroviruses are conserved at the amino acid level. Retroviral Gag proteins primarily function in viral assembly. The Gag gene in the form of a polyprotein gives rise to the core structural proteins of the VLP. The MLV Gag gene encodes a 65kDa polyprotein precursor which is proteolytically cleaved into 4 structural proteins (Matrix (MA); p12; Capsid (CA); and Nucleocapsid (NC)), by MLV protease, in the mature virion. Retroviruses assemble immature capsid composed of the Gag polyprotein formed from the Gag polypeptide but devoid of other viral elements like viral protease with Gag as the structural protein of the immature virus particle. Functionally, the Gag polyprotein is divided into three domains: the membrane binding domain, which targets the Gag polyprotein to the cellular membrane; the interaction domain which promotes Gag polymerization; and the late domain which facilitates release of nascent

virions from the host cell. The form of the Gag protein that mediates viral particle assembly is the polyprotein.

[0072] In some embodiments, a retroviral structural protein for use in accordance with the present invention is a Gag polypeptide. As used herein, the term "Gag polypeptide" is the retrovirus derived structural polypeptide that is responsible for formation of the VLPs described herein and refers to a polypeptide sequence whose amino acid sequence includes at least one characteristic sequence of Gag. A wide variety of Gag sequences from various retroviruses are known in the art and those of ordinary skill in the art, referring to such sequences, can readily identify sequences that are characteristic of Gag proteins generally, and/or of particular Gag polypeptides.

[0073] An exemplary Gag polypeptide for use in accordance with the present invention is shown as SEQ ID NO:1 below. In some embodiments, a suitable Gag polypeptide is substantially homologous to a known retroviral Gag polypeptide. For example, a Gag polypeptide may be a modified retroviral Gag polypeptide containing one or more amino acid substitutions, deletions, and/or insertions as compared to a wild-type or naturally-occurring Gag polypeptide (e.g., SEQ ID NO:1), while retaining substantial self-assembly activity. Thus, in some embodiments, a Gag polypeptide suitable for the present invention is substantially homologous to an MMLV Gag polypeptide (SEQ ID NO:1). In some embodiments, a Gag polypeptide suitable for the present invention has an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous to SEQ ID NO:1. In some embodiments, a Gag polypeptide suitable for the present invention is substantially identical to an MMLV Gag polypeptide (SEQ ID NO:1). In some embodiments, a Gag polypeptide suitable for the present invention has an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:1.

MMLV Gag Amino Acid Sequence (SEQ ID NO:1)

MGQTVTTPLSLTLGHWKDVERIAHNQSVDVKKRRWVTFCSAEWPTFNVGWPRDGTFN
RDLITQVKIKVFSPGPHGHPDQVPYIVTWEALAFDPPPWVKPFVHPKPPPPLPPSAPSLPL
EPPRSTPPRSSLYPALTPSLGAKPKPQVLSDSGGPLIDLLTEDPPPYRDPRPPPSDRDGNGG
EATPAGEAPDPSPMASRLRGRREPPVADSTTSQAFPLRAGGNGQLQYWPFSSSDLYNW
KNNNPSFSEDPGKLTALIESVLITHQPTWDDCQQLLGTLLTGEEKQRVLLEARKAVRGD
DGRPTQLPNEVDAAFPLERPDWDYTTQAGRNHLVHYRQLLLAGLQNAGRSPTNLAKV

KGITQGPNESPSAFLERLKEAYRRYTPYDPEDPGQETNVSMSFIWQSAPDIGRKLERLED
LKNKTLGDLVREAEKIFNKRETPEEREERIRRETEEKEERRRTEDEQKEKERDRRRHREM
SKLLATVVSGQKQDRQGGERRRSQLDRDQCAYCKEKGHWAKDCPKKPRGPRGPRPQT
SLLTLDD (SEQ ID NO:1)

MMLV Gag Nucleotide Sequence (SEQ ID NO:2)

ATGGGCCAGACTGTTACCACTCCCTTAAGTTTGACCTTAGGTCACTGGAAAGATGTC
GAGCGGATCGCTCACAACCAGTCGGTAGATGTCAAGAAGAGACGTTGGGTTACCTT
CTGCTCTGCAGAATGGCCAACCTTTAACGTCGGATGGCCGCGAGACGGCACCTTTAA
CCGAGACCTCATCACCCAGGTTAAGATCAAGGTCTTTTCACCTGGCCCGCATGGACA
CCCAGACCAGGTCCCCTACATCGTGACCTGGGAAGCCTTGGCTTTTGACCCCCCTCC
CTGGGTCAAGCCCTTTGTACACCCTAAGCCTCCGCCTCCTCTTCCTCCATCCGCCCCG
TCTCTCCCCCTTGAACCTCCTCGTTCGACCCCGCCTCGATCCTCCCTTTATCCAGCCC
TCACTCCTTCTCTAGGCGCCAAACCTAAACCTCAAGTTCTTTCTGACAGTGGGGGGC
CGCTCATCGACCTACTTACAGAAGACCCCCCGCCTTATAGGGACCCAAGACCACCCC
CTTCCGACAGGGACGGAAATGGTGGAGAAGCGACCCCTGCGGGAGAGGCACCGGA
CCCCTCCCCAATGGCATCTCGCCTACGTGGGAGACGGGAGCCCCCTGTGGCCGACTC
CACTACCTCGCAGGCATTCCCCCTCCGCGCAGGAGGAAACGGACAGCTTCAATACT
GGCCGTTCTCCTCTTCTGACCTTTACAACTGGAAAAATAATAACCCTTCTTTTTCTGA
AGATCCAGGTAAACTGACAGCTCTGATCGAGTCTGTTCTCATCACCCATCAGCCCAC
CTGGGACGACTGTCAGCAGCTGTTGGGGACTCTGCTGACCGGAGAAGAAAAACAAC
GGGTGCTCTTAGAGGCTAGAAAGGCGGTGCGGGGCGATGATGGGCGCCCCACTCAA
CTGCCCAATGAAGTCGATGCCGCTTTTCCCCTCGAGCGCCCAGACTGGGATTACACC
ACCCAGGCAGGTAGGAACCACCTAGTCCACTATCGCCAGTTGCTCCTAGCGGGTCTC
CAAAACGCGGGCAGAAGCCCCACCAATTTGGCCAAGGTAAAAGGAATAACACAAG
GGCCCAATGAGTCTCCCTCGGCCTTCCTAGAGAGACTTAAGGAAGCCTATCGCAGGT
ACACTCCTTATGACCCTGAGGACCCAGGGCAAGAAACTAATGTGTCTATGTCTTTCA
TTTGGCAGTCTGCCCCAGACATTGGGAGAAAGTTAGAGAGGTTAGAAGATTTAAAA
AACAAGACGCTTGGAGATTTGGTTAGAGAGGCAGAAAAGATCTTTAATAAACGAGA
AACCCCGGAAGAAAGAGAGGAACGTATCAGGAGAGAAACAGAGGAAAAAGAAGA
ACGCCGTAGGACAGAGGATGAGCAGAAAGAGAAAGAAAGAGATCGTAGGAGACAT
AGAGAGATGAGCAAGCTATTGGCCACTGTCGTTAGTGGACAGAAACAGGATAGACA
GGGAGGAGAACGAAGGAGGTCCCAACTCGATCGCGACCAGTGTGCCTACTGCAAAG
AAAAGGGGCACTGGGCTAAAGATTGTCCCAAGAAACCACGAGGACCTCGGGGACC
AAGACCCCAGACCTCCCTCCTGACCCTAGATGAC (SEQ ID NO:2)

Codon Optimized MMLV Gag Nucleotide Sequence (SEQ ID NO:3)

ATGGGACAGACAGTCACTACACCCCTGAGCCTGACACTGGGACATTGGAAAGACGT
GGAGAGGATTGCACATAACCAGAGCGTGGACGTGAAGAAACGGAGATGGGTCACCT
TTTGCTCCGCCGAGTGGCCAACATTCAATGTGGGATGGCCCCGAGATGGCACCTTCA
ACCGGGACCTGATCACTCAGGTGAAGATCAAGGTCTTCTCTCCAGGACCCCACGGCC
ATCCAGATCAGGTGCCCTACATCGTCACCTGGGAGGCTCTGGCATTTGACCCCCCTC
CATGGGTGAAGCCTTTCGTCCACCCAAAACCACCTCCACCACTGCCTCCATCTGCCC
CTAGTCTGCCACTGGAACCCCCTCGGTCAACCCCACCCAGAAGCTCCCTGTATCCCG
CACTGACACCTAGCCTGGGGGCCAAGCCTAAACCACAGGTGCTGTCTGATAGTGGC
GGGCCTCTGATCGATCTGCTGACCGAGGACCCTCCACCATACCGCGACCCACGACCT

CCACCAAGCGACCGGGACGGAAACGGAGGAGAGGCTACACCCGCAGGCGAAGCCC
CCGATCCTAGTCCAATGGCATCAAGGCTGCGCGGGAGGCGCGAACCTCCAGTGGCC
GACTCAACCACAAGCCAGGCATTTCCACTGAGGGCCGGGGGAAATGGACAGCTCCA
GTATTGGCCCTTCTCTAGTTCAGATCTGTACAACTGGAAGAACAATAACCCTAGCTT
CAGCGAGGACCCAGGCAAACTGACCGCCCTGATCGAATCCGTGCTGATTACCCACC
AGCCCACATGGGACGATTGTCAGCAGCTCCTGGGCACCCTGCTGACCGGAGAGGAA
AAGCAGAGAGTGCTGCTGGAGGCTAGGAAAGCAGTCCGCGGGGACGATGGAAGGC
CAACACAGCTCCCCAATGAGGTGGATGCCGCTTTCCCTCTGGAACGGCCAGATTGGG
ACTATACTACCCAGGCTGGACGCAACCACCTGGTGCATTACCGGCAGCTCCTGCTGG
CTGGACTGCAGAATGCAGGGCGCAGCCCCACTAACCTGGCCAAGGTGAAAGGAATC
ACCCAGGGCCCCAATGAGTCCCCTTCTGCATTCCTGGAGCGGCTGAAGGAAGCCTAC
CGACGGTATACTCCCTACGATCCTGAGGACCCAGGCCAGGAAACCAACGTGAGTAT
GAGCTTCATCTGGCAGTCCGCTCCTGACATTGGCCGAAAACTGGAGCGGCTGGAAG
ATCTGAAGAACAAGACCCTGGGCGACCTGGTGCGGGAGGCAGAAAAGATCTTCAAC
AAAAGGGAGACTCCAGAGGAACGGGAGGAAAGAATTAGAAGGGAAACAGAGGAA
AAGGAGGAACGCCGACGGACTGAGGATGAACAGAAGGAGAAAGAAAGAGACCGGC
GGCGGCACCGGGAGATGTCTAAGCTGCTGGCCACCGTGGTCAGTGGCCAGAAACAG
GATCGACAGGGAGGAGAGCGACGGAGAAGCCAGCTCGATCGGGACCAGTGCGCCT
ATTGTAAGGAAAAAGGGCATTGGGCTAAGGACTGCCCCAAGAAACCCAGAGGCCCA
CGCGGGCCCCGACCTCAGACTTCCCTGCTGACCCTGGACGAT (SEQ ID NO:3)

Codon Optimized MMLV Gag Nucleotide Sequence (SEQ ID NO:21)

ATGGGACAGACCGTCACAACACCCCTGAGCCTGACCCTGGGACATTGGAAAGACGT
GGAGAGGATCGCACATAACCAGAGCGTGGACGTGAAGAAACGGAGATGGGTCACA
TTCTGCAGTGCTGAGTGGCCAACTTTTAATGTGGGATGGCCCCGAGACGGCACTTTC
AACAGGGATCTGATCACCCAGGTGAAGATCAAGGTCTTTAGCCCAGGACCTCACGG
ACATCCAGACCAGGTGCCTTATATCGTCACCTGGGAGGCACTGGCCTTCGATCCCCC
TCCATGGGTGAAGCCATTTGTCCACCCAAAACCACCTCCACCACTGCCTCCAAGTGC
CCCTTCACTGCCACTGGAACCACCCCGGAGCACACCACCCCGCAGCTCCCTGTATCC
TGCTCTGACTCCATCTCTGGGCGCAAAGCCAAAACCACAGGTGCTGAGCGACTCCG
GAGGACCACTGATTGACCTGCTGACAGAGGACCCCCCACCATACCGAGATCCTCGG
CCTCCACCAAGCGACCGCGATGGAAATGGAGGAGAGGCTACTCCTGCCGGCGAAGC
CCCTGACCCATCTCCAATGGCTAGTAGGCTGCGCGGCAGGCGCGAGCCTCCAGTGG
CAGATAGCACCACATCCCAGGCCTTCCCTCTGAGGGCTGGGGGAAATGGGCAGCTC
CAGTATTGGCCATTTTCTAGTTCAGACCTGTACAACTGGAAGAACAATAACCCCTCT
TTCAGTGAGGACCCCGGCAAACTGACCGCCCTGATCGAATCCGTGCTGATTACCCAT
CAGCCCACATGGGACGATTGTCAGCAGCTCCTGGGCACCCTGCTGACCGGAGAGGA
AAAGCAGCGCGTGCTGCTGGAGGCTCGCAAAGCAGTCCGAGGGGACGATGGACGG
CCCACACAGCTCCCTAATGAGGTGGACGCCGCTTTTCCACTGGAAAGACCCGACTGG
GATTATACTACCCAGGCAGGGAGAAACCACCTGGTCCATTACAGGCAGCTCCTGCT
GGCAGGCCTGCAGAATGCCGGGAGATCCCCCACCAACCTGGCCAAGGTGAAAGGCA
TCACACAGGGGCCTAATGAGTCACCAAGCGCCTTTCTGGAGAGGCTGAAGGAAGCT
TACCGACGGTATACCCCATACGACCCTGAGGACCCCGGACAGGAAACAAACGTCTC
CATGTCTTTCATCTGGCAGTCTGCCCCAGACATTGGGCGGAAGCTGGAGAGACTGGA
AGACCTGAAGAACAAGACCCTGGGCGACCTGGTGCGGGAGGCTGAAAAGATCTTCA

ACAAACGGGAGACCCCCGAGGAAAGAGAGGAAAGGATTAGAAGGGAAACTGAGGA
AAAGGAGGAACGCCGACGGACCGAGGACGAACAGAAGGAGAAAGAACGAGATCG
GCGGCGGCACCGGGAGATGTCAAAGCTGCTGGCCACCGTGGTCAGCGGACAGAAAC
AGGACAGACAGGGAGGAGAGCGACGGAGAAGCCAGCTCGACAGGGATCAGTGCGC
ATACTGTAAGGAAAAAGGCCATTGGGCCAAGGATTGCCCCAAAAAGCCAAGAGGAC
CAAGAGGACCAAGACCACAGACATCACTGCTGACCCTGGACGAC (SEQ ID NO:21)

[0074] Typically in nature, a Gag protein includes a large C-terminal extension which may contain retroviral protease, reverse transcriptase, and integrase enzymatic activity. Assembly of VLPs, however, generally does not require the presence of such components. In some cases, a retroviral Gag protein alone (e.g., lacking a C-terminal extension, lacking one or more of genomic RNA, reverse transcriptase, viral protease, or envelope protein) can self-assemble to form VLPs both *in vitro* and *in vivo* (Sharma S et al., 1997 Proc. Natl. Acad. Sci. USA 94: 10803-8). Retroviral Gag polyprotein alone can oligomerize and assemble into VLPs.

[0075] In some embodiments, a Gag polypeptide for use in accordance with the present invention lacks a C-terminal extension and/or contains a modified C-terminal extension. A Gag polypeptide may optionally include one or more additional polypeptides (e.g., a heterologous antigen). In some embodiments, a Gag polypeptide is co-expressed with a heterologous antigen (e.g., under separate promoters and/or as separate proteins). In some embodiments, a Gag polypeptide is expressed as a fusion protein with a heterologous antigen. The Gag polypeptide can be linked to a heterologous antigen to create a fusion protein without altering Gag function. For example, a coding sequence for a heterologous antigen may be spliced into the Gag polypeptide coding sequence, e.g., at the 3' end of the Gag polypeptide coding sequence. In some embodiments, a coding sequence for a heterologous antigen may be spliced in frame into the Gag polypeptide coding sequence. In some embodiments, a Gag polypeptide-coding sequence and heterologous antigen may be expressed by a single promoter. In some embodiments, a heterologous antigen is inserted at (e.g., fused to) the C-terminus of a Gag polypeptide. Without wishing to be bound by any theory, it is thought that fusion of a self-assembling Gag polypeptide to a heterologous antigen creates a fusion protein that acts as unmodified Gag and as a result will allow the antigen to be incorporated into the structural components of a resulting VLP. In some embodiments, VLP structural components serve as effective immunogens (e.g., for induction of cellular immune response). For example, provided VLPs may comprise a retroviral Gag polypeptide (e.g., MMLV Gag) and a structural component

of HCMV (e.g., pp65). In some such embodiments, pp65 is incorporated into the VLP and serves as an antigen for eliciting an immune response against HCMV.

[0076] An exemplary Gag-pp65 fusion polypeptide for use in accordance with the present invention is shown as SEQ ID NO:4 below. In some embodiments, a suitable Gag polypeptide fusion protein includes all or a portion of a Gag polypeptide that is substantially homologous to a known retroviral Gag polypeptide and all or a portion of a pp65 polypeptide that is substantially homologous to a known pp65 polypeptide. For example, a Gag-pp65 polypeptide fusion protein may contain one or more amino acid substitutions, deletions, and/or insertions as compared to a wild-type or naturally-occurring Gag polypeptide and/or pp65 polypeptide, while retaining substantial self-assembly activity. Thus, in some embodiments, a Gag-pp65 polypeptide fusion protein suitable for the present invention is substantially homologous to SEQ ID NO:4. In some embodiments, a Gag-pp65 polypeptide fusion protein suitable for the present invention has an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous to SEQ ID NO:4. In some embodiments, a Gag-pp65 polypeptide fusion protein suitable for the present invention is substantially identical to SEQ ID NO:4. In some embodiments, a Gag-pp65 polypeptide fusion protein suitable for the present invention has an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:4.

MMLV Gag – CMV pp65 Amino Acid Sequence (SEQ ID NO:4)

MGQTVTTPLSLTLGHWKDVERIAHNQSVDVKKRRWVTFCSAEWPTFNVGWPRDG
TFNRDLITQVKIKVFSPGPHGHPDQVPYIVTWEALAFDPPPWVKPFVHPKPPPPLPP
SAPSLPLEPPRSTPPRSSLYPALTPSLGAKPKPQVLSDSGGPLIDLLTEDPPPYRDPRP
PPSDRDGNGGEATPAGEAPDPSPMASRLRGRREPPVADSTTSQAFPLRAGGNGQLQ
YWPFSSSDLYNWKNNNPSFSEDPGKLTALIESVLITHQPTWDDCQQLLGTLLTGEE
KQRVLLEARKAVRGDDGRPTQLPNEVDAAFPLERPDWDYTTQAGRNHLVHYRQL
LLAGLQNAGRSPTNLAKVKGITQGPNESPSAFLERLKEAYRRYTPYDPEDPGQETN
VSMSFIWQSAPDIGRKLERLEDLKNKTLGDLVREAEKIFNKRETPEEREERIRRETE
EKEERRRTEDEQKEKERDRRRHREMSKLLATVVSGQKQDRQGGERRRSQLDRDQ
CAYCKEKGHWAKDCPKKPRGPRGPRPQTSLLTLDDCESRGRRCPEMISVLGPISGHV
LKAVFSRGDTPVLPHETRLLQTGIHVRVSQPSLILVSQYTPDSTPCHRGDNQLQVQHTYF
TGSEVENVSVNVHNPTGRSICPSQEPMSIYVYALPLKMLNIPSINVHHYPSAAERKHRHL
PVADAVIHASGKQMWQARLTVSGLAWTRQQNQWKEPDVYYTSAFVFPTKDVALRHV
VCAHELVCSMENTRATKMQVIGDQYVKVYLESFCEDVPSGKLFMHVTLGSDVEEDLT
MTRNPQPFMRPHERNGFTVLCPKNMIIKPGKISHIMLDVAFTSHEHFGLLCPKSIPGLSIS
GNLLMNGQQIFLEVQAIRETVELRQYDPVAALFFFDIDLLLQRGPQYSEHPTFTSQYRIQ

GKLEYRHTWDRHDEGAAQGDDDVWTSGSDSDEELVTTERKTPRVTGGGAMAGASTSA
GRKRKSASSATACTAGVMTRGRLKAESTVAPEEDTDEDSDNEIHNPAVFTWPPWQAGI
LARNLVPMVATVQGQNLKYQEFFWDANDIYRIFAELEGVWQPAAQPKRRRHRQDALP
GPCIASTPKKHRG* (SEQ ID NO:4) (MMLV Gag amino acid sequence bolded)

MMLV Gag – CMV pp65 Nucleotide Sequence (SEQ ID NO:5)

ATGGGCCAGACTGTTACCACTCCCTTAAGTTTGACCTTAGGTCACTGGAAAGAT
GTCGAGCGGATCGCTCACAACCAGTCGGTAGATGTCAAGAAGAGACGTTGGGT
TACCTTCTGCTCTGCAGAATGGCCAACCTTTAACGTCGGATGGCCGCGAGACG
GCACCTTTAACCGAGACCTCATCACCCAGGTTAAGATCAAGGTCTTTTCACCTG
GCCCGCATGGACACCCAGACCAGGTCCCCTACATCGTGACCTGGGAAGCCTTG
GCTTTTGACCCCCCTCCCTGGGTCAAGCCCTTTGTACACCCTAAGCCTCCGCCT
CCTCTTCCTCCATCCGCCCCGTCTCTCCCCCTTGAACCTCCTCGTTCGACCCCG
CCTCGATCCTCCCTTTATCCAGCCCTCACTCCTTCTCTAGGCGCCAAACCTAAA
CCTCAAGTTCTTTCTGACAGTGGGGGGCCGCTCATCGACCTACTTACAGAAGA
CCCCCCGCCTTATAGGGACCCAAGACCACCCCCTTCCGACAGGGACGGAAATG
GTGGAGAAGCGACCCCTGCGGGAGAGGCACCGGACCCCTCCCCAATGGCATCT
CGCCTACGTGGGAGACGGGAGCCCCCTGTGGCCGACTCCACTACCTCGCAGGC
ATTCCCCCTCCGCGCAGGAGGAAACGGACAGCTTCAATACTGGCCGTTCTCCT
CTTCTGACCTTTACAACTGGAAAAATAATAACCCTTCTTTTTCTGAAGATCCAG
GTAAACTGACAGCTCTGATCGAGTCTGTTCTCATCACCCATCAGCCCACCTGGG
ACGACTGTCAGCAGCTGTTGGGGACTCTGCTGACCGGAGAAGAAAAACAACGG
GTGCTCTTAGAGGCTAGAAAGGCGGTGCGGGGCGATGATGGGCGCCCCACTCA
ACTGCCCAATGAAGTCGATGCCGCTTTTCCCCTCGAGCGCCCAGACTGGGATT
ACACCACCCAGGCAGGTAGGAACCACCTAGTCCACTATCGCCAGTTGCTCCTA
GCGGGTCTCCAAAACGCGGGCAGAAGCCCCACCAATTTGGCCAAGGTAAAAGG
AATAACACAAGGGCCCAATGAGTCTCCCTCGGCCTTCCTAGAGAGACTTAAGG
AAGCCTATCGCAGGTACACTCCTTATGACCCTGAGGACCCAGGGCAAGAAACT
AATGTGTCTATGTCTTTCATTTGGCAGTCTGCCCCAGACATTGGGAGAAAGTTA
GAGAGGTTAGAAGATTTAAAAAACAAGACGCTTGGAGATTTGGTTAGAGAGGC
AGAAAAGATCTTTAATAAACGAGAAACCCCGGAAGAAAGAGAGGAACGTATCA
GGAGAGAAACAGAGGAAAAAGAAGAACGCCGTAGGACAGAGGATGAGCAGAA
AGAGAAAGAAAGAGATCGTAGGAGACATAGAGAGATGAGCAAGCTATTGGCCA
CTGTCGTTAGTGGACAGAAACAGGATAGACAGGGAGGAGAACGAAGGAGGTC
CCAACTCGATCGCGACCAGTGTGCCTACTGCAAAGAAAAGGGGCACTGGGCTA
AAGATTGTCCCAAGAAACCACGAGGACCTCGGGGACCAAGACCCCAGACCTCC
CTCCTGACCCTAGATGACTGTGAGTCGCGCGGTCGCCGTTGTCCCGAAATGATATC
CGTACTGGGTCCCATTTCGGGGCACGTGCTGAAAGCCGTGTTTAGTCGCGGCGACAC
GCCGGTGCTGCCGCACGAGACGCGACTCCTGCAGACGGGTATCCACGTGCGCGTGA
GCCAGCCCTCGCTGATCCTGGTGTCGCAGTACACGCCCGACTCGACGCCATGCCACC
GCGGCGACAATCAGCTGCAGGTGCAGCACACGTACTTTACGGGCAGCGAGGTGGAG
AACGTGTCGGTCAACGTGCACAACCCCACGGGCCGGAGCATCTGCCCCAGCCAAGA
GCCCATGTCGATCTATGTGTACGCGCTGCCGCTCAAGATGCTGAACATCCCCAGCAT
CAACGTGCACCACTACCCGTCGGCGGCCGAGCGCAAACACCGACACCTGCCCGTAG
CTGACGCTGTGATTCACGCGTCGGGCAAGCAGATGTGGCAGGCGCGTCTCACGGTCT
CGGGACTGGCCTGGACGCGTCAGCAGAACCAGTGGAAAGAGCCCGACGTCTACTAC

ACGTCAGCGTTCGTGTTTCCCACCAAGGACGTGGCACTGCGGCACGTGGTGTGCGCG
CACGAGCTGGTTTGCTCCATGGAGAACACGCGCGCAACCAAGATGCAGGTGATAGG
TGACCAGTACGTCAAGGTGTACCTGGAGTCCTTCTGCGAGGACGTGCCCTCCGGCAA
GCTCTTTATGCACGTCACGCTGGGCTCTGACGTGGAAGAGGACCTGACGATGACCCG
CAACCCGCAACCCTTCATGCGCCCCCACGAGCGCAACGGCTTTACGGTGTTGTGTCC
CAAAAATATGATAATCAAACCGGGCAAGATCTCGCACATCATGCTGGATGTGGCTTT
TACCTCACACGAGCATTTTGGGCTGCTGTGTCCCAAGAGCATCCCGGGCCTGAGCAT
CTCAGGTAACCTATTGATGAACGGGCAGCAGATCTTCCTGGAGGTGCAAGCGATAC
GCGAGACCGTGGAACTGCGTCAGTACGATCCCGTGGCTGCGCTCTTCTTTTTCGATA
TCGACTTGCTGCTGCAGCGCGGGCCTCAGTACAGCGAACACCCCACCTTCACCAGCC
AGTATCGCATCCAGGGCAAGCTTGAGTACCGACACACCTGGGACCGGCACGACGAG
GGTGCCGCCCAGGGCGACGACGACGTCTGGACCAGCGGATCGGACTCCGACGAGGA
ACTCGTAACCACCGAGCGCAAGACGCCCCGCGTTACCGGCGGCGGCGCCATGGCGG
GCGCCTCCACTTCCGCGGGCCGCAAACGCAAATCAGCATCCTCGGCGACGGCGTGC
ACGGCGGGCGTTATGACACGCGGCCGCCTTAAGGCCGAGTCCACCGTCGCGCCCGA
AGAGGACACCGACGAGGATTCCGACAACGAAATCCACAATCCGGCCGTGTTCACCT
GGCCGCCCTGGCAGGCCGGCATCCTGGCCCGCAACCTGGTGCCCATGGTGGCTACG
GTTCAGGGTCAGAATCTGAAGTACCAGGAGTTCTTCTGGGACGCCAACGACATCTAC
CGCATCTTCGCCGAATTGGAAGGCGTATGGCAGCCCGCTGCGCAACCCAAACGTCG
CCGCCACCGGCAAGACGCCTTGCCCGGGCCATGCATCGCCTCGACGCCCAAAAAGC
ACCGAGGTTAG (SEQ ID NO:5) (MMLV Gag nucleotide sequence bolded)

Codon Optimized MMLV Gag – CMV pp65 Nucleotide Sequence (SEQ ID NO:6)

ATGGGACAGACAGTCACTACACCCCTGAGCCTGACACTGGGACATTGGAAAGA
CGTGGAGAGGATTGCACATAACCAGAGCGTGGACGTGAAGAAACGGAGATGG
GTCACCTTTTGCTCCGCCGAGTGGCCAACATTCAATGTGGGATGGCCCCGAGA
TGGCACCTTCAACCGGGACCTGATCACTCAGGTGAAGATCAAGGTCTTCTCTCC
AGGACCCCACGGCCATCCAGATCAGGTGCCCTACATCGTCACCTGGGAGGCTC
TGGCATTTGACCCCCCTCCATGGGTGAAGCCTTTCGTCCACCCAAAACCACCTC
CACCACTGCCTCCATCTGCCCCTAGTCTGCCACTGGAACCCCCTCGGTCAACCC
CACCCAGAAGCTCCCTGTATCCCGCACTGACACCTAGCCTGGGGGCCAAGCCT
AAACCACAGGTGCTGTCTGATAGTGGCGGGCCTCTGATCGATCTGCTGACCGA
GGACCCTCCACCATACCGCGACCCACGACCTCCACCAAGCGACCGGGACGGAA
ACGGAGGAGAGGCTACACCCGCAGGCGAAGCCCCCGATCCTAGTCCAATGGCA
TCAAGGCTGCGCGGGAGGCGCGAACCTCCAGTGGCCGACTCAACCACAAGCCA
GGCATTTCCACTGAGGGCCGGGGGAAATGGACAGCTCCAGTATTGGCCCTTCT
CTAGTTCAGATCTGTACAACTGGAAGAACAATAACCCTAGCTTCAGCGAGGAC
CCAGGCAAACTGACCGCCCTGATCGAATCCGTGCTGATTACCCACCAGCCCAC
ATGGGACGATTGTCAGCAGCTCCTGGGCACCCTGCTGACCGGAGAGGAAAAGC
AGAGAGTGCTGCTGGAGGCTAGGAAAGCAGTCCGCGGGGACGATGGAAGGCC
AACACAGCTCCCCAATGAGGTGGATGCCGCTTTCCCTCTGGAACGGCCAGATT
GGGACTATACTACCCAGGCTGGACGCAACCACCTGGTGCATTACCGGCAGCTC
CTGCTGGCTGGACTGCAGAATGCAGGGCGCAGCCCCACTAACCTGGCCAAGGT
GAAAGGAATCACCCAGGGCCCCAATGAGTCCCCTTCTGCATTCCTGGAGCGGC
TGAAGGAAGCCTACCGACGGTATACTCCCTACGATCCTGAGGACCCAGGCCAG
GAAACCAACGTGAGTATGAGCTTCATCTGGCAGTCCGCTCCTGACATTGGCCG

AAAACTGGAGCGGCTGGAAGATCTGAAGAACAAGACCCTGGGCGACCTGGTGC
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AAGAATTAGAAGGGAAACAGAGGAAAAGGAGGAACGCCGACGGACTGAGGAT
GAACAGAAGGAGAAAGAAAGAGACCGGCGGCGGCACCGGGAGATGTCTAAGC
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ACGGAGAAGCCAGCTCGATCGGGACCAGTGCGCCTATTGTAAGGAAAAAGGGC
ATTGGGCTAAGGACTGCCCCAAGAAACCCAGAGGCCCACGCGGGCCCCGACCT
CAGACTTCCCTGCTGACCCTGGACGATTGCGAGAGCCGGGGCCGGCGGTGCCCA
GAAATGATCTCTGTGCTGGGGCCCATTAGTGGACATGTGCTGAAGGCCGTCTTCTCC
AGGGGAGACACCCCCGTGCTGCCTCACGAGACTCGACTGCTGCAGACCGGCATCCA
TGTGCGGGTCTCCCAGCCCTCTCTGATTCTGGTGTCACAGTATACACCAGATAGCAC
TCCCTGCCACAGAGGAGACAATCAGCTCCAGGTGCAGCATACCTACTTTACAGGCTC
CGAGGTCGAAAACGTGTCTGTCAATGTGCACAACCCTACCGGCAGGAGCATCTGTC
CTAGCCAGGAGCCAATGAGCATCTACGTGTACGCCCTGCCTCTGAAGATGCTGAATA
TCCCATCAATTAACGTCCACCATTACCCTAGCGCAGCCGAACGGAAGCACAGACAT
CTGCCAGTGGCCGACGCTGTCATCCATGCCAGCGGCAAACAGATGTGGCAGGCAAG
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ACGTGTACTATACCAGCGCCTTCGTGTTCCCTACCAAAGACGTGGCCCTGAGACATG
TGGTGTGCGCACATGAGCTGGTGTGCAGCATGGAAAACACTAGGGCCACCAAGATG
CAGGTCATCGGCGATCAGTATGTCAAAGTGTACCTGGAGAGTTTTTGCGAAGACGTG
CCATCAGGGAAGCTGTTCATGCATGTGACCCTGGGCAGCGATGTCGAGGAAGACCT
GACCATGACAAGAAATCCACAGCCCTTTATGAGACCCCACGAGAGGAATGGGTTCA
CTGTGCTGTGCCCCAAGAACATGATCATTAAGCCTGGAAAAATCAGTCATATTATGC
TGGATGTGGCCTTTACATCACACGAGCATTTCGGACTGCTGTGCCCCAAATCCATCC
CTGGACTGAGCATTTCCGGCAATCTGCTGATGAACGGCCAGCAGATCTTCCTGGAAG
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TTCTTTTTCGACATCGACCTGCTGCTGCAGCGAGGACCACAGTACAGCGAGCACCCT
ACTTTTACCTCCCAGTATCGGATTCAGGGGAAGCTGGAGTACAGGCACACCTGGGAT
CGCCATGACGAAGGAGCCGCTCAGGGGGACGATGACGTGTGGACATCTGGCAGTGA
TTCAGACGAGGAACTGGTGACAACTGAGCGAAAAACCCCCCGGGTGACAGGAGGA
GGGGCAATGGCAGGGGCCAGCACCAGCGCAGGGCGGAAGCGAAAAAGCGCCAGCA
GCGCCACAGCATGTACCGCCGGCGTGATGACTAGAGGAAGGCTGAAGGCCGAGTCT
ACAGTCGCTCCCGAGGAAGATACTGACGAGGATAGTGACAATGAAATCCACAACCC
CGCCGTGTTCACCTGGCCACCTTGGCAGGCAGGGATTCTGGCTCGCAACCTGGTCCC
CATGGTGGCAACCGTCCAGGGACAGAATCTGAAGTATCAGGAGTTTTTCTGGGATGC
TAACGACATCTACCGGATTTTTGCAGAGCTGGAAGGCGTGTGGCAGCCAGCAGCCC
AGCCCAAACGACGGAGACATCGACAGGACGCTCTGCCAGGACCTTGTATCGCCAGC
ACACCAAAGAAGCACAGGGGCTAA (SEQ ID NO:6) (MMLV Gag nucleotide sequence bolded)

<u>Codon Optimized MMLV Gag – CMV pp65 Nucleotide Sequence (SEQ ID NO:22)</u>
ATGGGACAGACCGTCACAACACCCCTGAGCCTGACCCTGGGACATTGGAAAGA
CGTGGAGAGGATCGCACATAACCAGAGCGTGGACGTGAAGAAACGGAGATGG
GTCACATTCTGCAGTGCTGAGTGGCCAACTTTTAATGTGGGATGGCCCCGAGA
CGGCACTTTCAACAGGGATCTGATCACCCAGGTGAAGATCAAGGTCTTTAGCC

CAGGACCTCACGGACATCCAGACCAGGTGCCTTATATCGTCACCTGGGAGGCA
CTGGCCTTCGATCCCCCTCCATGGGTGAAGCCATTTGTCCACCCAAAACCACCT
CCACCACTGCCTCCAAGTGCCCCTTCACTGCCACTGGAACCACCCCGGAGCAC
ACCACCCCGCAGCTCCCTGTATCCTGCTCTGACTCCATCTCTGGGCGCAAAGCC
AAAACCACAGGTGCTGAGCGACTCCGGAGGACCACTGATTGACCTGCTGACAG
AGGACCCCCCACCATACCGAGATCCTCGGCCTCCACCAAGCGACCGCGATGGA
AATGGAGGAGAGGCTACTCCTGCCGGCGAAGCCCCTGACCCATCTCCAATGGC
TAGTAGGCTGCGCGGCAGGCGCGAGCCTCCAGTGGCAGATAGCACCACATCCC
AGGCCTTCCCTCTGAGGGCTGGGGGAAATGGGCAGCTCCAGTATTGGCCATTT
TCTAGTTCAGACCTGTACAACTGGAAGAACAATAACCCCTCTTTCAGTGAGGAC
CCCGGCAAACTGACCGCCCTGATCGAATCCGTGCTGATTACCCATCAGCCCAC
ATGGGACGATTGTCAGCAGCTCCTGGGCACCCTGCTGACCGGAGAGGAAAAGC
AGCGCGTGCTGCTGGAGGCTCGCAAAGCAGTCCGAGGGGACGATGGACGGCC
CACACAGCTCCCTAATGAGGTGGACGCCGCTTTTCCACTGGAAAGACCCGACT
GGGATTATACTACCCAGGCAGGGAGAAACCACCTGGTCCATTACAGGCAGCTC
CTGCTGGCAGGCCTGCAGAATGCCGGGAGATCCCCCACCAACCTGGCCAAGGT
GAAAGGCATCACACAGGGGCCTAATGAGTCACCAAGCGCCTTTCTGGAGAGGC
TGAAGGAAGCTTACCGACGGTATACCCCATACGACCCTGAGGACCCCGGACAG
GAAACAAACGTCTCCATGTCTTTCATCTGGCAGTCTGCCCCAGACATTGGGCG
GAAGCTGGAGAGACTGGAAGACCTGAAGAACAAGACCCTGGGCGACCTGGTG
CGGGAGGCTGAAAAGATCTTCAACAAACGGGAGACCCCCGAGGAAAGAGAGG
AAAGGATTAGAAGGGAAACTGAGGAAAAGGAGGAACGCCGACGGACCGAGGA
CGAACAGAAGGAGAAAGAACGAGATCGGCGGCGGCACCGGGAGATGTCAAAG
CTGCTGGCCACCGTGGTCAGCGGACAGAAACAGGACAGACAGGGAGGAGAGC
GACGGAGAAGCCAGCTCGACAGGGATCAGTGCGCATACTGTAAGGAAAAAGGC
CATTGGGCCAAGGATTGCCCCAAAAAGCCAAGAGGACCAAGAGGACCAAGACC
ACAGACATCACTGCTGACCCTGGACGACTGCGAGAGCCGGGGCCGGCGGTGCCC
AGAAATGATCTCTGTGCTGGGGCCCATTAGTGGACATGTGCTGAAGGCCGTCTTCTC
CAGGGGAGACACCCCCGTGCTGCCTCACGAGACTCGACTGCTGCAGACCGGCATCC
ATGTGCGGGTCTCCCAGCCCTCTCTGATTCTGGTGTCACAGTATACACCAGATAGCA
CTCCCTGCCACAGAGGAGACAATCAGCTCCAGGTGCAGCATACCTACTTTACAGGCT
CCGAGGTCGAAAACGTGTCTGTCAATGTGCACAACCCTACCGGCAGGAGCATCTGT
CCTAGCCAGGAGCCAATGAGCATCTACGTGTACGCCCTGCCTCTGAAGATGCTGAAT
ATCCCATCAATTAACGTCCACCATTACCCTAGCGCAGCCGAACGGAAGCACAGACA
TCTGCCAGTGGCCGACGCTGTCATCCATGCCAGCGGCAAACAGATGTGGCAGGCAA
GACTGACCGTGTCCGGGCTGGCCTGGACAAGGCAGCAGAATCAGTGGAAGGAGCCC
GACGTGTACTATACCAGCGCCTTCGTGTTCCCTACCAAAGACGTGGCCCTGAGACAT
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GCAGGTCATCGGCGATCAGTATGTCAAAGTGTACCTGGAGAGTTTTTGCGAAGACGT
GCCATCAGGGAAGCTGTTCATGCATGTGACCCTGGGCAGCGATGTCGAGGAAGACC
TGACCATGACAAGAAATCCACAGCCCTTTATGAGACCCCACGAGAGGAATGGGTTC
ACTGTGCTGTGCCCCAAGAACATGATCATTAAGCCTGGAAAAATCAGTCATATTATG
CTGGATGTGGCCTTTACATCACACGAGCATTTCGGACTGCTGTGCCCCAAATCCATC
CCTGGACTGAGCATTTCCGGCAATCTGCTGATGAACGGCCAGCAGATCTTCCTGGAA
GTGCAGGCCATCCGGGAGACCGTCGAACTGCGACAGTATGACCCAGTGGCTGCACT
GTTCTTTTTCGACATCGACCTGCTGCTGCAGCGAGGACCACAGTACAGCGAGCACCC

TACTTTTACCTCCCAGTATCGGATTCAGGGGAAGCTGGAGTACAGGCACACCTGGGA
TCGCCATGACGAAGGAGCCGCTCAGGGGGACGATGACGTGTGGACATCTGGCAGTG
ATTCAGACGAGGAACTGGTGACAACTGAGCGAAAAACCCCCCGGGTGACAGGAGG
AGGGGCAATGGCAGGGGCCAGCACCAGCGCAGGGCGGAAGCGAAAAAGCGCCAGC
AGCGCCACAGCATGTACCGCCGGCGTGATGACTAGAGGAAGGCTGAAGGCCGAGTC
TACAGTCGCTCCCGAGGAAGATACTGACGAGGATAGTGACAATGAAATCCACAACC
CCGCCGTGTTCACCTGGCCACCTTGGCAGGCAGGGATTCTGGCTCGCAACCTGGTCC
CCATGGTGGCAACCGTCCAGGGACAGAATCTGAAGTATCAGGAGTTTTTCTGGGATG
CTAACGACATCTACCGGATTTTTGCAGAGCTGGAAGGCGTGTGGCAGCCAGCAGCC
CAGCCCAAACGACGGAGACATCGACAGGACGCTCTGCCAGGACCTTGTATCGCCAG
CACACCAAAGAAGCACAGGGGCTAA (SEQ ID NO:22) (MMLV Gag nucleotide sequence bolded)

[0077] In some embodiments, the present invention provides nucleic acids which encode a Gag polypeptide or a characteristic portion of a Gag polypeptide. In certain embodiments, nucleic acids can be DNA or RNA, and can be single stranded or double-stranded. In some embodiments, inventive nucleic acids may include one or more non-natural nucleotides; in other embodiments, inventive nucleic acids include only natural nucleotides.

B. Envelope proteins

[0078] In some embodiments, the present invention utilizes VLPs comprised of one or more envelope polypeptides from HCMV (e.g., gB and/or gH). As used herein, the term "envelope polypeptide" refers to a polypeptide sequence whose amino acid sequence includes at least one characteristic sequence of an envelope glycoprotein. A wide variety of envelope glycoprotein sequences from various viruses, including, but not limited to HCMV, are known in the art and those of ordinary skill in the art, referring to such sequences, can readily identify sequences that are characteristic of envelope glycoproteins generally, and/or of particular envelope glycoproteins. In some embodiments, an envelope polypeptide comprises a cytoplasmic, transmembrane and/or extracellular portion or domain.

[0079] In some embodiments, an envelope polypeptide from HCMV includes a transmembrane and cytoplasmic domain that is not found in nature in the HCMV protein. For example, in some embodiments, an envelope polypeptide from HCMV includes a transmembrane and cytoplasmic domain from another HCMV protein (e.g., gB or gH). In some embodiments, an envelope polypeptide from HCMV includes a transmembrane domain and

cytoplasmic domain found in nature in vesicular stomatitis virus (VSV). As is known in the art, polypeptides sometimes have transmembrane, cytoplasmic, and/or extracellular domains. In general, a "transmembrane domain", as used herein, refers to a domain that has an attribute of being present in the membrane (e.g., spanning a portion or all of a cellular membrane). As will be appreciated, it is not required that every amino acid in a transmembrane domain be present in the membrane. For example, in some embodiments, a transmembrane domain is characterized in that a designated stretch or portion of a protein is substantially located in the membrane. As is well known in the art, amino acid or nucleic acid sequences may be analyzed using a variety of algorithms to predict protein subcellular localization (e.g., transmembrane localization). Exemplary such programs include psort (PSORT.org), Prosite (prosite.expasy.org), among others. In general, a "cytoplasmic domain", as used herein, refers to a domain that has an attribute of being present in the cytoplasm. As will be appreciated, it is not required that every amino acid in a cytoplasmic domain be present in the cytoplasm. For example, in some embodiments, a cytoplasmic domain is characterized in that a designated stretch or portion of a protein is substantially located in the cytoplasm. As is well known in the art, amino acid or nucleic acid sequences may be analyzed using a variety of algorithms to predict protein subcellular localization (e.g., cytoplasmic localization). Exemplary such programs include psort (PSORT.org), Prosite (prosite.expasy.org), among others.

[0080] The transmembrane domain of VSV-G functions to target the viral glycoprotein to the cell membrane (Compton T et al., 1989 Proc Natl Acad Sci USA 86:4112-4116). Swapping the transmembrane and cytoplasmic domains of VSV-G for the transmembrane and cytoplasmic domains of another protein has been used to direct a protein to the cell membrane when the native protein does not naturally do this or requires accessory co-expressed proteins to accomplish this (Garrone P et al., 2011 Sci Transl Med 94:).

[0081] Among other things, the present invention encompasses the recognition that VLPs containing a structural component of a virus (e.g., MLV) and one or more heterologous surface antigens (e.g., envelope protein) are especially effective for antigen delivery and induction of an immune response against the heterologous antigen.

C. Heterologous Antigens

[0082] Envelope proteins of HCMV, such as glycoproteins gB and gH, are important targets for production of neutralizing antibodies against HCMV, as neutralizing antibodies are generally able to prevent infection. Therapies for HCMV infection, such as a gB subunit vaccine, have been developed and tested in experimental animals and clinical studies. Results from such studies, however, have demonstrated that in humans, the antibody response was not long-lived and not sufficiently effective for the treatment of HCMV in all cases. The reasons which have been suggested for the limited efficacy of subunit vaccines based exclusively on gB of HCMV in turn are strain-specific variations in immune responses, inadequate induction of a cellular immune response, and structural restrictions on antigen used, whose epitopes are thought to be conformation-dependent. The present inventors recognized that development of an HCMV vaccine comprising one or more envelope polypeptide antigens presented in their native conformation on the surface of a VLP leads to induction of neutralizing antibodies (e.g., via a humoral immune response) and an HCMV vaccine comprising one or more structural protein antigens (e.g., tegument protein pp65) leads to induction of helper T cells (T_H lymphocytes) and cytotoxic T cells (CTL) (e.g., via a cell-mediated immune response). Neutralizing antibodies are generally formed against viral envelope proteins, and especially against HCMV glycoproteins gB and gH. T_H cells are stimulated by tegument structural proteins of a virus, such as, for example, HCMV pp65 (ppUL83). In addition, pp65 plays an important role in induction of a CTL response against HCMV.

[0083] It will be appreciated that provided VLPs may comprise any heterologous antigen, including heterologous antigens from HCMV. For example, in some embodiments, a VLP in accordance with the present invention comprises one or more HCMV envelope polypeptides. In some embodiments, a VLP in accordance with the present invention comprises one or more HCMV structural polypeptides. In some embodiments, a VLP in accordance with the present invention comprises one or more HCMV envelope polypeptides and one or more HCMV structural polypeptides. A list of exemplary, but non-limiting HCMV antigens is provided below.

gB – Glycoprotein Complex (gC) I

[0084] The most fully characterized glycoprotein complex of HCMV is the gB complex (gB;UL55). It has been demonstrated that sera from CMV-seropositive individuals contains antibodies to gB, and up to 70% of the neutralizing antibody response in convalescent sera is gB-specific (Marshall GS et al., 1994 J Med Virol 43:77-83).

[0085] An exemplary HCMV gB polypeptide amino acid and nucleic acid sequence is shown below as SEQ ID NO:7 and SEQ ID NO:8, respectively. In some embodiments, a suitable gB polypeptide is substantially homologous to a known HCMV gB polypeptide. For example, a gB polypeptide may be a modified HCMV gB polypeptide containing one or more amino acid substitutions, deletions, and/or insertions as compared to a wild-type or naturally-occurring gB polypeptide (e.g., SEQ ID NO:7). Thus, in some embodiments, a gB polypeptide suitable for the present invention is substantially homologous to an HCMV gB polypeptide (SEQ ID NO:7). In some embodiments, an HCMV polypeptide suitable for the present invention has an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous to SEQ ID NO:7. In some embodiments, a gB polypeptide suitable for the present invention is substantially identical to an HCMV gB polypeptide (SEQ ID NO:7). In some embodiments, a gB polypeptide suitable for the present invention has an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:7.

HCMV gB Amino Acid Sequence (SEQ ID NO:7)

MESRIWCLVVCVNLCIVCLGAAVSSSSTRGTSATHSHHSSHTTSAAHSRSGSVSQRVTSS
QTVSHGVNETIYNTTLKYGDVVGVNTTKYPYRVCSMAQGTDLIRFERNIVCTSMKPINE
DLDEGIMVVYKRNIVAHTFKVRVYQKVLTFRRSYAYIHTTYLLGSNTEYVAPPMWEIH
HINSHSQCYSSYSRVIAGTVFVAYHRDSYENKTMQLMPDDYSNTHSTRYVTVKDQWHS
RGSTWLYRETCNLNCMVTITTARSKYPYHFFATSTGDVVDISPFYNGTNRNASYFGENA
DKFFIFPNYTIVSDFGRPNSALETHRLVAFLERADSVISWDIQDEKNVTCQLTFWEASERT
IRSEAEDSYHFSSAKMTATFLSKKQEVNMSDSALDCVRDEAINKLQQIFNTSYNQTYEK
YGNVSVFETTGGLVVFWQGIKQKSLVELERLANRSSLNLTHNRTKRSTDGNNATHLSN
MESVHNLVYAQLQFTYDTLRGYINRALAQIAEAWCVDQRRTLEVFKELSKINPSAILSAI
YNKPIAARFMGDVLGLASCVTINQTSVKVLRDMNVKESPGRCYSRPVVIFNFANSSYVQ
YGQLGEDNEILLGNHRTEECQLPSLKIFIAGNSAYEYVDYLFKRMIDLSSISTVDSMIALD
IDPLENTDFRVLELYSQKELRSINVFDLEEIMREFNSYKQRVKYVEDKVVDPLPPYLKGL
DDLMSGLGAAGKAVGVAIGAVGGAVASVVEGVATFLKNPFGAFTIILVAIAVVIIIYLIY
TRQRRLCMQPLQNLFPYLVSADGTTVTSGNTKDTSLQAPPSYEESVYNSGRKGPGPPSS

DASTAAPPYTNEQAYQMLLALVRLDAEQRAQQNGTDSLDGQTGTQDKGQKPNLLDRL
RHRKNGYRHLKDSDEEENV* (SEQ ID NO:7) (TM and CD underlined)

HCMV gB Nucleotide Sequence (SEQ ID NO:8)

ATGGAATCCAGGATCTGGTGCCTGGTAGTCTGCGTTAACTTGTGTATCGTCTGTCTG
GGTGCTGCGGTTTCCTCATCTTCTACTCGTGGAACTTCTGCTACTCACAGTCACCATT
CCTCTCATACGACGTCTGCTGCTCATTCTCGATCCGGTTCAGTCTCTCAACGCGTAAC
TTCTTCCCAAACGGTCAGCCATGGTGTTAACGAGACCATCTACAACACTACCCTCAA
GTACGGAGATGTGGTGGGGGTCAACACCACCAAGTACCCCTATCGCGTGTGTTCTAT
GGCACAGGGTACGGATCTTATTCGCTTTGAACGTAATATCGTCTGCACCTCGATGAA
GCCCATCAATGAAGACCTGGACGAGGGCATCATGGTGGTCTACAAACGCAACATCG
TCGCGCACACCTTTAAGGTACGAGTCTACCAGAAGGTTTTGACGTTTCGTCGTAGCT
ACGCTTACATCCACACCACTTATCTGCTGGGCAGCAACACGGAATACGTGGCGCCTC
CTATGTGGGAGATTCATCATATCAACAGTCACAGTCAGTGCTACAGTTCCTACAGCC
GCGTTATAGCAGGCACGGTTTTCGTGGCTTATCATAGGGACAGCTATGAAAACAAA
ACCATGCAATTAATGCCCGACGATTATTCCAACACCCACAGTACCCGTTACGTGACG
GTCAAGGATCAATGGCACAGCCGCGGCAGCACCTGGCTCTATCGTGAGACCTGTAA
TCTGAATTGTATGGTGACCATCACTACTGCGCGCTCCAAGTATCCCTATCATTTTTTC
GCAACTTCCACGGGTGATGTGGTTGACATTTCTCCTTTCTACAACGGAACTAATCGC
AATGCCAGCTATTTTGGAGAAAACGCCGACAAGTTTTTCATTTTTCCGAACTACACT
ATCGTCTCCGACTTTGGAAGACCGAATTCTGCGTTAGAGACCCACAGGTTGGTGGCT
TTTCTTGAACGTGCGGACTCAGTGATCTCCTGGGATATACAGGACGAGAAGAATGTT
ACTTGTCAACTCACTTTCTGGGAAGCCTCGGAACGCACCATTCGTTCCGAAGCCGAG
GACTCGTATCACTTTTCTTCTGCCAAAATGACCGCCACTTTCTTATCTAAGAAGCAAG
AGGTGAACATGTCCGACTCTGCGCTGGACTGTGTACGTGATGAGGCCATAAATAAGT
TACAGCAGATTTTCAATACTTCATACAATCAAACATATGAAAAATATGGAAACGTGT
CCGTCTTTGAAACCACTGGTGGTTTGGTGGTGTTCTGGCAAGGTATCAAGCAAAAAT
CTCTGGTGGAACTCGAACGTTTGGCCAACCGCTCCAGTCTGAATCTTACTCATAATA
GAACCAAAAGAAGTACAGATGGCAACAATGCAACTCATTTATCCAACATGGAGTCG
GTGCACAATCTGGTCTACGCCCAGCTGCAGTTCACCTATGACACGTTGCGCGGTTAC
ATCAACCGGGCGCTGGCGCAAATCGCAGAAGCCTGGTGTGTGGATCAACGGCGCAC
CCTAGAGGTCTTCAAGGAACTTAGCAAGATCAACCCGTCAGCTATTCTCTCGGCCAT
CTACAACAAACCGATTGCCGCGCGTTTCATGGGTGATGTCCTGGGTCTGGCCAGCTG
CGTGACCATTAACCAAACCAGCGTCAAGGTGCTGCGTGATATGAATGTGAAGGAAT
CGCCAGGACGCTGCTACTCACGACCAGTGGTCATCTTTAATTTCGCCAACAGCTCGT
ACGTGCAGTACGGTCAACTGGGCGAGGATAACGAAATCCTGTTGGGCAACCACCGC
ACTGAGGAATGTCAGCTTCCCAGCCTCAAGATCTTCATCGCCGGCAACTCGGCCTAC
GAGTACGTGGACTACCTCTTCAAACGCATGATTGACCTCAGCAGCATCTCCACCGTC
GACAGCATGATCGCCCTAGACATCGACCCGCTGGAAAACACCGACTTCAGGGTACT
GGAACTTTACTCGCAGAAAGAATTGCGTTCCATCAACGTTTTTGATCTCGAGGAGAT
CATGCGCGAGTTCAATTCGTATAAGCAGCGGGTAAAGTACGTGGAGGACAAGGTAG
TCGACCCGCTGCCGCCCTACCTCAAGGGTCTGGACGACCTCATGAGCGGCCTGGGCG
CCGCGGGAAAGGCCGTTGGCGTAGCCATTGGGGCCGTGGGTGGCGCGGTGGCCTCC
GTGGTCGAAGGCGTTGCCACCTTCCTCAAAAACCCCTTCGGAGCCTTCACCATCATC
CTCGTGGCCATAGCCGTCGTCATTATCATTTATTTGATCTATACTCGACAGCGGCGTC

TCTGCATGCAGCCGCTGCAGAACCTCTTTCCCTATCTGGTGTCCGCCGACGGGACCA
CCGTGACGTCGGGCAACACCAAAGACACGTCGTTACAGGCTCCGCCTTCCTACGAG
GAAAGTGTTTATAATTCTGGTCGCAAAGGACCGGGACCACCGTCGTCTGATGCATCC
ACGGCGGCTCCGCCTTACACCAACGAGCAGGCTTACCAGATGCTTCTGGCCCTGGTC
CGTCTGGACGCAGAGCAGCGAGCGCAGCAGAACGGTACAGATTCTTTGGACGGACA
GACTGGCACGCAGGACAAGGGACAGAAGCCCAACCTGCTAGACCGACTGCGACACC
GCAAAAACGGCTACCGACACTTGAAAGACTCCGACGAAGAAGAGAACGTCTGA
(SEQ ID NO:8) (TM and CD underlined)

Codon Optimized HCMV gB Nucleotide Sequence (SEQ ID NO:9)

ATGGAGTCAAGGATTTGGTGCCTGGTCGTGTGCGTCAATCTGTGCATCGTCTGTCTG
GGGGCTGCCGTGTCATCAAGTTCTACAAGAGGCACCAGCGCCACCCACTCACACCA
TAGCTCCCATACCACATCCGCCGCTCACTCCCGGTCTGGCAGCGTGAGCCAGAGAGT
CACATCTAGTCAGACCGTGAGCCACGGGGTCAACGAGACCATCTACAATACTACCC
TGAAGTATGGCGACGTGGTCGGGGTGAACACAACTAAATACCCATATAGGGTCTGC
AGTATGGCCCAGGGCACTGATCTGATTAGATTCGAAAGGAACATCGTGTGCACCAG
CATGAAGCCCATTAATGAGGACCTGGATGAAGGGATCATGGTGGTCTACAAACGCA
ATATTGTGGCCCATACCTTCAAGGTGCGAGTCTATCAGAAAGTGCTGACATTTCGGA
GATCTTACGCATATATCCACACCACATACCTGCTGGGGAGTAACACCGAGTATGTGG
CTCCCCCTATGTGGGAAATTCACCATATCAATAGCCATTCCCAGTGCTACTCAAGCT
ACAGCAGAGTGATCGCTGGAACAGTGTTCGTCGCATACCACAGAGACTCTTATGAG
AACAAGACTATGCAGCTCATGCCCGACGATTACAGCAATACACATTCCACTAGATAT
GTGACAGTCAAAGATCAGTGGCACTCAAGGGGCAGCACCTGGCTGTACCGCGAGAC
ATGCAACCTGAATTGTATGGTGACTATCACTACCGCTAGATCCAAGTACCCCTATCA
CTTCTTTGCAACTTCCACCGGGGACGTGGTCGATATTTCTCCTTTCTACAACGGCACA
AACCGGAATGCATCTTATTTTGGGGAGAACGCCGACAAGTTCTTTATTTTCCCAAAT
TACACCATCGTGTCTGATTTTGGCAGACCCAACAGTGCCCTGGAGACACATCGACTG
GTGGCATTCCTGGAACGGGCCGACTCCGTCATTTCTTGGGACATCCAGGATGAGAAG
AATGTGACCTGCCAGCTCACCTTCTGGGAGGCCAGCGAACGCACCATCCGATCCGA
GGCTGAAGATTCTTACCACTTCTCCTCTGCCAAAATGACAGCTACTTTTCTGAGCAA
GAAACAGGAGGTGAACATGTCTGACAGTGCTCTGGATTGCGTGCGGGACGAAGCAA
TTAATAAGCTGCAGCAGATCTTCAACACATCATACAACCAGACTTACGAGAAGTAC
GGAAACGTGAGCGTCTTCGAAACAACTGGCGGGCTGGTGGTCTTTTGGCAGGGCAT
CAAGCAGAAATCCCTGGTGGAGCTGGAAAGGCTGGCCAATCGCAGTTCACTGAACC
TGACTCATAATCGGACCAAGAGATCTACAGACGGAAACAATGCCACACATCTGTCT
AACATGGAGAGTGTGCACAATCTGGTCTACGCTCAGCTCCAGTTTACCTACGACACA
CTGAGAGGCTATATTAACAGGGCACTGGCCCAGATCGCTGAAGCATGGTGCGTGGA
TCAGAGGCGCACCCTGGAGGTCTTCAAGGAACTGTCCAAAATCAACCCTTCAGCAA
TTCTGAGCGCCATCTACAATAAGCCAATTGCAGCCAGGTTTATGGGAGACGTGCTGG
GCCTGGCCAGTTGCGTCACTATCAACCAGACCTCAGTGAAGGTCCTGCGCGATATGA
ATGTGAAAGAGAGTCCCGGCAGATGCTATTCACGGCCTGTGGTCATCTTCAACTTTG
CTAATAGCTCCTACGTGCAGTATGGACAGCTCGGCGAGGACAACGAAATTCTGCTG
GGGAATCACAGGACCGAGGAATGTCAGCTCCCTAGCCTGAAGATTTTCATCGCTGG
AAACTCCGCATACGAGTATGTGGATTACCTGTTCAAGCGGATGATTGACCTGTCTAG

TATCTCCACTGTGGATTCTATGATTGCCCTGGACATCGATCCACTGGAAAATACCGA
CTTCAGGGTGCTGGAGCTGTATAGCCAGAAGGAACTGCGCTCCATCAACGTGTTCGA
TCTGGAGGAAATTATGAGAGAGTTTAATAGCTACAAGCAGAGGGTGAAATATGTCG
AAGATAAGGTGGTCGACCCCCTGCCACCCTACCTGAAAGGCCTGGACGATCTGATG
AGCGGGCTGGGAGCTGCAGGGAAGGCAGTGGGAGTCGCTATCGGCGCAGTGGGAG
GAGCCGTGGCCAGCGTGGTCGAGGGAGTGGCAACATTCCTGAAAAACCCCTTCGGG
GCCTTCACCATCATTCTGGTGGCAATCGCCGTGGTCATCATTATCTACCTGATCTACA
CAAGGCAGCGGCGGCTGTGCATGCAGCCTCTGCAGAACCTGTTTCCATACCTGGTGA
GCGCCGACGGGACCACAGTCACCTCAGGAAATACTAAGGATACCTCTCTGCAGGCC
CCCCCAAGTTACGAGGAATCAGTGTATAACAGCGGCAGAAAAGGACCAGGACCACC
TTCAAGCGACGCCAGCACTGCCGCTCCACCCTACACCAATGAGCAGGCCTATCAGAT
GCTGCTGGCTCTGGTGCGCCTGGATGCCGAACAGCGAGCTCAGCAGAACGGGACCG
ACTCCCTGGATGGACAGACCGGAACACAGGACAAGGGACAGAAACCTAATCTGCTG
GATCGGCTGCGGCACAGAAAAAACGGGTATAGGCACCTGAAGGACTCCGACGAAG
AAGAAAATGTCTAA (SEQ ID NO:9) (TM and CD underlined)

[0086] In some embodiments, a gB polypeptide for use in accordance with the present invention lacks a transmembrane domain and/or cytoplasmic domain and/or contains a modified transmembrane domain and/or cytoplasmic domain. A gB polypeptide may optionally include one or more additional polypeptides (e.g., a heterologous transmembrane domain and/or cytoplasmic domain polypeptide). In some embodiments, a gB polypeptide is expressed as a fusion protein with a heterologous polypeptide. The gB polypeptide can be linked to a heterologous polypeptide to create a fusion protein without altering gB function and/or antigenicity. For example, a coding sequence for a heterologous polypeptide may be spliced into the gB polypeptide coding sequence, e.g., at the 3' end of the gB polypeptide coding sequence. In some embodiments, a coding sequence for a heterologous polypeptide may be spliced in frame into the gB polypeptide coding sequence. In some embodiments, a gB polypeptide-coding sequence and heterologous polypeptide may be expressed by a single promoter. In some embodiments, a heterologous polypeptide is inserted at (e.g., fused to) the C-terminus of a gB polypeptide.

[0087] In some embodiments, a heterologous polypeptide is or comprises a transmembrane domain and/or cytoplasmic domain found in Vesicular Stomatitis Virus (VSV). In some embodiments, a gB that is lacking a transmembrane domain and/or cytoplasmic domain is fused to a transmembrane domain and/or cytoplasmic domain from VSV. An exemplary gB-VSV fusion polypeptide for use in accordance with the present invention is shown below as SEQ

ID NO:10. In some embodiments, a suitable gB-VSV polypeptide fusion protein includes all or a portion of a gB polypeptide that is substantially homologous to a known gB polypeptide and all or a portion of a VSV polypeptide that is substantially homologous to a known VSV polypeptide. For example, a gB – VSV polypeptide fusion protein may contain one or more amino acid substitutions, deletions, and/or insertions as compared to a wild-type or naturally-occurring gB and/or VSV polypeptide. Thus, in some embodiments, a gB-VSV polypeptide fusion protein suitable for the present invention is substantially homologous to SEQ ID NO:10. In some embodiments, a gB-VSV polypeptide fusion protein suitable for the present invention has an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous to SEQ ID NO:10. In some embodiments, a gB-VSV polypeptide fusion protein suitable for the present invention is substantially identical to SEQ ID NO:10. In some embodiments, a gB-VSV polypeptide fusion protein suitable for the present invention has an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:10. As used herein, "gB-G" refers to a HMCV gB – VSV TM/CTD fusion protein.

HCMV gB-G Amino Acid Sequence (SEQ ID NO:10)

MESRIWCLVVCVNLCIVCLGAAVSSSSTRGTSATHSHHSSHTTSAAHSRSGSVSQRVTSS
QTVSHGVNETIYNTTLKYGDVVGVNTTKYPYRVCSMAQGTDLIRFERNIVCTSMKPINE
DLDEGIMVVYKRNIVAHTFKVRVYQKVLTFRRSYAYIHTTYLLGSNTEYVAPPMWEIH
HINSHSQCYSSYSRVIAGTVFVAYHRDSYENKTMQLMPDDYSNTHSTRYVTVKDQWHS
RGSTWLYRETCNLNCMVTITTARSKYPYHFFATSTGDVVDISPFYNGTNRNASYFGENA
DKFFIFPNYTIVSDFGRPNSALETHRLVAFLERADSVISWDIQDEKNVTCQLTFWEASERT
IRSEAEDSYHFSSAKMTATFLSKKQEVNMSDSALDCVRDEAINKLQQIFNTSYNQTYEK
YGNVSVFETTGGLVVFWQGIKQKSLVELERLANRSSLNLTHNRTKRSTDGNNATHLSN
MESVHNLVYAQLQFTYDTLRGYINRALAQIAEAWCVDQRRTLEVFKELSKINPSAILSAI
YNKPIAARFMGDVLGLASCVTINQTSVKVLRDMNVKESPGRCYSRPVVIFNFANSSYVQ
YGQLGEDNEILLGNHRTEECQLPSLKIFIAGNSAYEYVDYLFKRMIDLSSISTVDSMIALD
IDPLENTDFRVLELYSQKELRSINVFDLEEIMREFNSYKQRVKYVEDKVVDPLPPYLKGL
DDLMSGLGAAGKAVGVAIGAVGGAVASVVEGVATFLKNPFFFIIGLIIGLFLVLRVGIHL
CIKLKHTKKRQIYTDIEMNRLGK* (SEQ ID NO:10) (TM and CTD underlined)

HCMV gB – G Nucleotide Sequence (SEQ ID NO:11)

ATGGAATCCAGGATCTGGTGCCTGGTAGTCTGCGTTAACTTGTGTATCGTCTGTCTG
GGTGCTGCGGTTTCCTCATCTTCTACTCGTGGAACTTCTGCTACTCACAGTCACCATT
CCTCTCATACGACGTCTGCTGCTCATTCTCGATCCGGTTCAGTCTCTCAACGCGTAAC
TTCTTCCCAAACGGTCAGCCATGGTGTTAACGAGACCATCTACAACACTACCCTCAA
GTACGGAGATGTGGTGGGGGTCAACACCACCAAGTACCCCTATCGCGTGTGTTCTAT

GGCACAGGGTACGGATCTTATTCGCTTTGAACGTAATATCGTCTGCACCTCGATGAA
GCCCATCAATGAAGACCTGGACGAGGGCATCATGGTGGTCTACAAACGCAACATCG
TCGCGCACACCTTTAAGGTACGAGTCTACCAGAAGGTTTTGACGTTTCGTCGTAGCT
ACGCTTACATCCACACCACTTATCTGCTGGGCAGCAACACGGAATACGTGGCGCCTC
CTATGTGGGAGATTCATCATATCAACAGTCACAGTCAGTGCTACAGTTCCTACAGCC
GCGTTATAGCAGGCACGGTTTTCGTGGCTTATCATAGGGACAGCTATGAAAACAAA
ACCATGCAATTAATGCCCGACGATTATTCCAACACCCACAGTACCCGTTACGTGACG
GTCAAGGATCAATGGCACAGCCGCGGCAGCACCTGGCTCTATCGTGAGACCTGTAA
TCTGAATTGTATGGTGACCATCACTACTGCGCGCTCCAAGTATCCCTATCATTTTTTC
GCAACTTCCACGGGTGATGTGGTTGACATTTCTCCTTTCTACAACGGAACTAATCGC
AATGCCAGCTATTTTGGAGAAAACGCCGACAAGTTTTTCATTTTTCCGAACTACACT
ATCGTCTCCGACTTTGGAAGACCGAATTCTGCGTTAGAGACCCACAGGTTGGTGGCT
TTTCTTGAACGTGCGGACTCAGTGATCTCCTGGGATATACAGGACGAGAAGAATGTT
ACTTGTCAACTCACTTTCTGGGAAGCCTCGGAACGCACCATTCGTTCCGAAGCCGAG
GACTCGTATCACTTTTCTTCTGCCAAAATGACCGCCACTTTCTTATCTAAGAAGCAAG
AGGTGAACATGTCCGACTCTGCGCTGGACTGTGTACGTGATGAGGCCATAAATAAGT
TACAGCAGATTTTCAATACTTCATACAATCAAACATATGAAAAATATGGAAACGTGT
CCGTCTTTGAAACCACTGGTGGTTTGGTGGTGTTCTGGCAAGGTATCAAGCAAAAAT
CTCTGGTGGAACTCGAACGTTTGGCCAACCGCTCCAGTCTGAATCTTACTCATAATA
GAACCAAAAGAAGTACAGATGGCAACAATGCAACTCATTTATCCAACATGGAGTCG
GTGCACAATCTGGTCTACGCCCAGCTGCAGTTCACCTATGACACGTTGCGCGGTTAC
ATCAACCGGGCGCTGGCGCAAATCGCAGAAGCCTGGTGTGTGGATCAACGGCGCAC
CCTAGAGGTCTTCAAGGAACTTAGCAAGATCAACCCGTCAGCTATTCTCTCGGCCAT
CTACAACAAACCGATTGCCGCGCGTTTCATGGGTGATGTCCTGGGTCTGGCCAGCTG
CGTGACCATTAACCAAACCAGCGTCAAGGTGCTGCGTGATATGAATGTGAAGGAAT
CGCCAGGACGCTGCTACTCACGACCAGTGGTCATCTTTAATTTCGCCAACAGCTCGT
ACGTGCAGTACGGTCAACTGGGCGAGGATAACGAAATCCTGTTGGGCAACCACCGC
ACTGAGGAATGTCAGCTTCCCAGCCTCAAGATCTTCATCGCCGGCAACTCGGCCTAC
GAGTACGTGGACTACCTCTTCAAACGCATGATTGACCTCAGCAGCATCTCCACCGTC
GACAGCATGATCGCCCTAGACATCGACCCGCTGGAAAACACCGACTTCAGGGTACT
GGAACTTTACTCGCAGAAAGAATTGCGTTCCATCAACGTTTTTGATCTCGAGGAGAT
CATGCGCGAGTTCAATTCGTATAAGCAGCGGGTAAAGTACGTGGAGGACAAGGTAG
TCGACCCGCTGCCGCCCTACCTCAAGGGTCTGGACGACCTCATGAGCGGCCTGGGCG
CCGCGGGAAAGGCCGTTGGCGTAGCCATTGGGGCCGTGGGTGGCGCGGTGGCCTCC
GTGGTCGAAGGCGTTGCCACCTTCCTCAAAAACCCCTTTTTCTTTATCATAGGGTTAA
TCATTGGACTATTCTTGGTTCTCCGAGTTGGTATCCATCTTTGCATTAAATTAAAGCA
CACCAAGAAAAGACAGATTTATACAGACATAGAGATGAACCGACTTGGAAAGTAA
(SEQ ID NO:11) TM and CTD underlined)

Codon Optimized HCMV gB – G Nucleotide Sequence (SEQ ID NO:12)

ATGGAGTCAAGGATTTGGTGTCTGGTCGTCTGCGTCAACCTGTGCATTGTCTGCCTG
GGAGCCGCCGTCTCATCATCATCTACCCGAGGCACATCCGCCACTCACTCTCACCAT
AGCTCCCATACCACATCCGCCGCTCACTCAAGAAGCGGGTCCGTGTCTCAGAGGGTC
ACATCTAGTCAGACCGTGAGCCATGGAGTCAACGAGACAATCTACAATACTACCCT
GAAGTATGGAGACGTGGTCGGCGTGAACACAACTAAATACCCCTATAGGGTCTGCT
CTATGGCCCAGGGGACAGATCTGATCCGATTTGAACGGAACATCGTGTGCACTAGC

ATGAAGCCTATCAATGAGGACCTGGATGAAGGAATTATGGTGGTCTACAAACGAAA
TATCGTGGCCCATACTTTTAAGGTGAGAGTCTATCAGAAAGTGCTGACCTTCCGGAG
AAGCTACGCTTATATTCACACCACATACCTGCTGGGGTCCAACACCGAGTATGTGGC
ACCCCCTATGTGGGAAATCCACCATATTAATAGTCATTCACAGTGCTACTCAAGCTA
CAGCAGAGTGATCGCTGGAACCGTGTTCGTCGCATACCACAGAGACAGTTATGAGA
ACAAGACAATGCAGCTCATGCCCGACGATTACAGTAATACCCATTCAACAAGATAT
GTGACCGTCAAAGATCAGTGGCACTCTCGCGGCAGTACCTGGCTGTACCGAGAGAC
ATGCAACCTGAATTGTATGGTGACAATTACTACCGCCAGAAGCAAGTACCCTTATCA
CTTCTTTGCTACCTCAACAGGGGACGTGGTCGACATCAGCCCCTTCTACAACGGAAC
AAACCGGAATGCCTCCTATTTCGGCGAGAACGCTGACAAATTCTTTATCTTCCCCAA
CTACACTATCGTGAGCGATTTCGGCAGACCTAACAGTGCCCTGGAGACCCATCGGCT
GGTGGCATTTCTGGAAAGAGCCGACAGCGTGATCTCCTGGGACATTCAGGATGAGA
AGAATGTGACCTGCCAGCTCACCTTCTGGGAGGCCAGCGAAAGAACCATCAGGTCC
GAGGCAGAAGATTCTTACCACTTTTCCTCTGCAAAAATGACTGCCACCTTCCTGTCC
AAGAAACAGGAGGTGAACATGAGCGACTCCGCACTGGATTGCGTGCGGGACGAAGC
CATCAATAAGCTGCAGCAGATCTTCAACACATCTTACAACCAGACTTACGAGAAGTA
CGGCAACGTGAGTGTCTTTGAAACAACTGGCGGGCTGGTGGTCTTCTGGCAGGGGAT
CAAGCAGAAATCTCTGGTGGAGCTGGAACGGCTGGCCAATAGAAGTTCACTGAACC
TGACTCATAATCGCACCAAGCGATCCACAGACGGAAACAATGCAACTCATCTGAGC
AACATGGAGTCCGTGCACAATCTGGTCTACGCCCAGCTCCAGTTCACTTACGACACC
CTGCGAGGCTATATCAACCGGGCCCTGGCTCAGATTGCAGAAGCCTGGTGCGTGGAT
CAGAGGCGCACCCTGGAGGTCTTTAAGGAACTGAGCAAAATTAACCCATCTGCTATC
CTGAGTGCAATCTACAATAAGCCCATCGCAGCCAGGTTCATGGGGGACGTGCTGGG
ACTGGCCTCCTGCGTCACTATCAACCAGACCTCTGTGAAGGTCCTGCGCGATATGAA
TGTGAAAGAGAGTCCTGGCAGGTGTTATTCACGCCCAGTGGTCATCTTCAACTTCGC
TAATAGCTCCTACGTGCAGTATGGCCAGCTCGGGGAGGACAACGAAATCCTGCTGG
GAAATCACAGGACCGAGGAATGTCAGCTCCCAAGTCTGAAGATCTTTATTGCCGGC
AACTCAGCTTACGAGTATGTGGATTACCTGTTCAAACGCATGATCGACCTGTCTAGT
ATTTCAACAGTGGATAGCATGATCGCCCTGGACATTGATCCCCTGGAAAATACTGAC
TTCAGGGTGCTGGAGCTGTATAGCCAGAAGGAACTGCGCTCCATTAACGTGTTTGAT
CTGGAGGAAATCATGAGGGAGTTCAATTCCTACAAGCAGCGCGTGAAATATGTCGA
AGATAAGGTGGTCGACCCTCTGCCACCCTACCTGAAAGGCCTGGACGATCTGATGA
GCGGGCTGGGAGCTGCAGGCAAGGCAGTGGGAGTCGCCATCGGAGCTGTGGGAGGC
GCTGTCGCATCCGTGGTCGAGGGAGTGGCTACCTTTCTGAAGAACCCATTCTTTTTC
ATCATCGGCCTGATCATTGGGCTGTTCCTGGTGCTGAGAGTCGGCATCCACCTGTGC
ATTAAGCTGAAGCACACCAAGAAGAGGCAGATCTACACCGATATTGAAATGAACAG
ACTGGGCAAGTGA (SEQ ID NO:12) (TM and CTD underlined)

gH – Glycoprotein Complex (gC) III

[0088] The gc III complex contains glycoproteins gH (UL75), gL (UL115) and gO (UL74) (Urban M et al., 1996 J Gen Virol 77:1537 – 47). Like gB, gH is conserved among human pathogenic herpesviruses and plays an important role in a number of steps during HCMV replication. HCMV encodes two gH/gL complexes: gH/gL/gO and a

gH/gL/UL128/UL130/UL131 complex (Wang D and Shenk T 2005 Proc Natl Acad USA 102:18153-8). The gO-containing complex is generally sufficient for fibroblast infection with HCMV, whereas the UL128/UL130/UL131-containing complex is needed in order for HCMV to infect endothelial and epithelial cells (Wang D and Shenk T 2005 J Virol 79 10330-8). Natural infection with HCMV typically elicits high titer neutralizing antibodies specific for epithelial cell entry and it has been demonstrated that antibodies against gH/gL/UL128/UL130/UL131 epitopes may comprise a significant component of this activity (Macagno A et al., 2010 J Virol 84:1005-13). Immunological studies on gH have demonstrated that in mammalian cells the protein requires additional polypeptides (like gL) for correct processing and transport to the cellular membrane (Urban M et al., 1996 J Gen Virol 77:1537-1547). If expressed alone, gH is found exclusively in the cytoplasm and/or nuclear membrane (Cranage MP et al., 1988 J Virol 62: 1416-1422).

[0089] An exemplary HCMV gH polypeptide amino acid and nucleic acid sequence is shown below as SEQ ID NO:13 and SEQ ID NO:14, respectively. In some embodiments, a suitable gH polypeptide is substantially homologous to a known HCMV gH polypeptide. For example, a gH polypeptide may be a modified HCMV gH polypeptide containing one or more amino acid substitutions, deletions, and/or insertions as compared to a wild-type or naturally-occurring gH polypeptide (e.g., SEQ ID NO:13). Thus, in some embodiments, a gH polypeptide suitable for the present invention is substantially homologous to an HCMV gH polypeptide (SEQ ID NO:13). In some embodiments, an HCMV gH polypeptide suitable for the present invention has an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous to SEQ ID NO:13. In some embodiments, a gH polypeptide suitable for the present invention is substantially identical to an HCMV gH polypeptide (SEQ ID NO:13). In some embodiments, a gH polypeptide suitable for the present invention has an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:13.

HCMV gH Amino Acid Sequence (SEQ ID NO:13)

MRPGLPSYLIVLAVCLLSHLLSSRYGAEAISEPLDKAFHLLLNTYGRPIRFLRENTTQCT
YNSSLRNSTVVRENAISFNFFQSYNQYYVFHMPRCLFAGPLAEQFLNQVDLTETLERYQ
QRLNTYALVSKDLASYRSFSQQLKAQDSLGEQPTTVPPPIDLSIPHVWMPPQTTPHGWT
ESHTTSGLHRPHFNQTCILFDGHDLLFSTVTPCLHQGFYLIDELRYVKITLTEDFFVVTVSI

DDDTPMLLIFGHLPRVLFKAPYQRDNFILRQTEKHELLVLVKKDQLNRHSYLKDPDFLD
AALDFNYLDLSALLRNSFHRYAVDVLKSGRCQMLDRRTVEMAFAYALALFAAARQEE
AGAQVSVPRALDRQAALLQIQEFMITCLSQTPPRTTLLLYPTAVDLAKRALWTPNQITDI
TSLVRLVYILSKQNQQHLIPQWALRQIADFALKLHKTHLASFLSAFARQELYLMGSLVH
SMLVHTTERREIFIVETGLCSLAELSHFTQLLAHPHHEYLSDLYTPCSSSGRRDHSLERLT
RLFPDATVPTTVPAALSILSTMQPSTLETFPDLFCLPLGESFSALTVSEHVSYVVTNQYLI
KGISYPVSTTVVGQSLIITQTDSQTKCELTRNMHTTHSITAALNISLENCAFCQSALLEYD
DTQGVINIMYMHDSDDVLFALDPYNEVVVSSPRTHYLMLLKNGTVLEVTDVVVDATDS
RLLMMSVYALSAIIGIYLLYRMLKTC* (SEQ ID NO:13) (TM and CTD underlined)

HCMV gH Nucleotide Sequence (SEQ ID NO:14)

ATGCGGCCAGGCCTCCCCTCCTACCTCATCGTCCTCGCCGTCTGTCTCCTCAGCCACC
TACTTTCGTCACGATATGGCGCAGAAGCCATATCCGAACCGCTGGACAAAGCGTTTC
ACCTACTGCTCAACACCTACGGGAGACCCATCCGCTTCCTGCGTGAAAACACCACCC
AGTGTACCTACAATAGCAGCCTCCGTAACAGCACGGTCGTCAGGGAAAACGCCATC
AGTTTCAACTTTTTCCAAAGCTATAATCAATACTATGTATTCCATATGCCTCGATGTC
TTTTTGCGGGTCCTCTGGCGGAGCAGTTTCTGAACCAGGTAGATCTGACCGAAACCC
TGGAAAGATACCAACAGAGACTTAACACTTACGCGCTGGTATCCAAAGACCTGGCC
AGCTACCGATCTTTTTCGCAGCAGCTAAAGGCACAGGACAGCCTAGGTGAACAGCC
CACCACTGTGCCACCACCCATTGACCTGTCAATACCTCACGTTTGGATGCCACCGCA
AACCACTCCACACGGCTGGACAGAATCACATACCACCTCAGGACTACACCGACCAC
ACTTTAACCAGACCTGTATCCTCTTTGATGGACACGATCTACTATTCAGCACCGTCAC
ACCTTGTTTGCACCAAGGCTTTTACCTCATCGACGAACTACGTTACGTTAAAATAAC
ACTGACCGAGGACTTCTTCGTAGTTACGGTGTCCATAGACGACGACACACCCATGCT
GCTTATCTTCGGCCATCTTCCACGCGTACTCTTTAAAGCGCCCTATCAACGCGACAA
CTTTATACTACGACAAACTGAAAAACACGAGCTCCTGGTGCTAGTTAAGAAAGATC
AACTGAACCGTCACTCTTATCTCAAAGACCCGGACTTTCTTGACGCCGCACTTGACT
TCAACTACCTGGACCTCAGCGCACTACTACGTAACAGCTTTCACCGTTACGCCGTGG
ATGTACTCAAAAGCGGTCGATGTCAGATGCTGGACCGCCGCACGGTAGAAATGGCC
TTCGCCTACGCATTAGCACTGTTCGCAGCAGCCCGACAAGAAGAGGCCGGCGCCCA
AGTCTCCGTCCCACGGGCCCTAGACCGCCAGGCCGCACTCTTACAAATACAAGAATT
TATGATCACCTGCCTCTCACAAACACCACCACGCACCACGTTGCTGCTGTATCCCAC
GGCCGTGGACCTGGCCAAACGAGCCCTTTGGACACCGAATCAGATCACCGACATCA
CCAGCCTCGTACGCCTGGTCTACATACTCTCTAAACAGAATCAGCAACATCTCATCC
CCCAGTGGGCACTACGACAGATCGCCGACTTTGCCCTAAAACTACACAAAACGCAC
CTGGCCTCTTTTCTTTCAGCCTTCGCGCGTCAAGAACTCTACCTCATGGGCAGCCTCG
TCCACTCCATGCTAGTACATACGACGGAGAGACGCGAAATCTTCATCGTAGAAACG
GGCCTCTGTTCATTAGCCGAGCTATCACACTTTACGCAGTTGCTAGCTCATCCGCAC
CACGAATACCTCAGCGACCTGTACACACCCTGTTCCAGTAGCGGGCGACGCGATCA
CTCGCTCGAACGCCTCACACGTCTCTTCCCCGATGCCACCGTCCCCACTACCGTTCCC
GCCGCCCTCTCCATCCTATCTACCATGCAACCAAGCACGCTAGAAACCTTCCCCGAC
CTGTTTTGTCTGCCGCTCGGCGAATCCTTCTCCGCGCTGACCGTCTCCGAACACGTCA
GTTATGTCGTAACAAACCAGTACCTGATCAAAGGTATCTCCTACCCTGTCTCCACCA
CCGTCGTAGGCCAGAGCCTCATCATCACCCAGACGGACAGTCAAACTAAATGCGAA
CTGACGCGCAACATGCATACCACACACAGCATCACAGCGGCGCTCAACATTTCCCTA
GAAAACTGCGCCTTTTGCCAAAGCGCCCTACTAGAATACGACGACACGCAAGGCGT

CATCAACATCATGTACATGCACGACTCGGACGACGTCCTTTTCGCCCTGGATCCCTA
CAACGAAGTGGTGGTCTCATCTCCGCGAACTCACTACCTCATGCTTTTGAAAAACGG
TACGGTCCTAGAAGTAACTGACGTCGTCGTGGACGCTACCGACAGTCGTCTCCTCAT
GATGTCCGTCTACGCGCTATCGGCCATCATCGGCATCTATCTGCTCTACCGCATGCTC
AAGACATGCTGA (SEQ ID NO:14) (TM and CTD underlined)

Codon Optimized HCMV gH Nucleotide Sequence (SEQ ID NO:15)

ATGAGACCTGGACTGCCTTCTTATCTGATTGTGCTGGCCGTCTGCCTGCTGTCACATC
TGCTGAGTTCACGCTATGGGGCTGAGGCTATCTCCGAGCCACTGGACAAGGCTTTTC
ACCTGCTGCTGAACACCTACGGGAGACCCATTAGGTTCCTGCGCGAGAATACCACA
CAGTGCACATATAACAGCTCCCTGCGGAACAGCACTGTGGTCCGCGAAAACGCCAT
CTCTTTTAATTTCTTTCAGAGTTACAACCAGTACTACGTGTTCCATATGCCACGCTGT
CTGTTTGCAGGACCCCTGGCCGAGCAGTTCCTGAACCAGGTGGACCTGACCGAGAC
ACTGGAAAGATACCAGCAGAGGCTGAATACCTATGCCCTGGTGAGTAAGGATCTGG
CTTCATATCGGTCTTTCAGTCAGCAGCTCAAGGCCCAGGACTCACTGGGCGAGCAGC
CTACTACCGTGCCCCCTCCAATCGATCTGAGCATTCCACACGTCTGGATGCCCCCTC
AGACAACTCCCCACGGCTGGACCGAAAGCCATACCACATCCGGGCTGCACAGACCC
CATTTCAACCAGACATGCATCCTGTTTGATGGGCACGACCTGCTGTTCAGCACTGTG
ACCCCTTGTCTGCATCAGGGATTCTACCTGATCGATGAGCTGAGATATGTGAAAATT
ACACTGACTGAAGACTTCTTTGTGGTCACCGTGAGCATCGACGATGACACACCAATG
CTGCTGATTTTTGGACACCTGCCCCGGGTGCTGTTCAAGGCCCCCTACCAGCGAGAC
AACTTTATTCTGCGGCAGACCGAGAAACACGAACTGCTGGTGCTGGTCAAGAAAGA
TCAGCTCAACAGGCATAGCTATCTGAAGGACCCCGACTTTCTGGATGCCGCTCTGGA
CTTCAACTACCTGGACCTGTCAGCACTGCTGCGGAATAGCTTCCACAGATATGCCGT
GGATGTCCTGAAATCCGGAAGATGCCAGATGCTGGACCGGAGAACCGTGGAGATG
GCATTTGCCTACGCTCTGGCACTGTTCGCAGCCGCTAGGCAGGAGGAAGCAGGCGC
TCAGGTGTCCGTCCCTCGCGCACTGGATCGACAGGCAGCCCTGCTGCAGATCCAGGA
GTTCATGATTACCTGTCTGTCTCAGACACCACCCAGAACTACCCTGCTGCTGTACCC
CACTGCCGTGGACCTGGCTAAGAGGGCACTGTGGACCCCTAACCAGATCACTGATA
TTACCTCTCTGGTGCGCCTGGTCTATATCCTGAGTAAACAGAATCAGCAGCACCTGA
TCCCACAGTGGGCCCTGCGACAGATTGCCGACTTCGCTCTGAAGCTGCACAAAACCC
ATCTGGCTTCCTTCCTGTCTGCATTTGCCCGCCAGGAGCTGTACCTGATGGGCTCTCT
GGTGCACAGTATGCTGGTCCATACAACTGAGAGGCGCGAAATCTTTATTGTGGAGAC
AGGGCTGTGCAGCCTGGCTGAACTGTCCCACTTCACTCAGCTCCTGGCCCATCCTCA
CCATGAGTACCTGTCCGATCTGTATACCCCATGTTCTAGTTCAGGCCGACGGGACCA
CTCTCTGGAACGACTGACTCGGCTGTTTCCTGATGCAACCGTGCCTACCACCGTGCC
CGCCGCCCTGAGTATCCTGTCAACAATGCAGCCAAGCACACTGGAGACTTTCCCCGA
CCTGTTTTGCCTGCCTCTGGGGGAGTCATTCAGCGCCCTGACCGTGTCAGAACATGT
CAGCTACGTGGTCACAAACCAGTATCTGATCAAGGGAATTTCCTACCCCGTGTCTAC
TACCGTGGTCGGCCAGAGTCTGATCATTACCCAGACAGATTCACAGACTAAATGTGA
GCTGACCCGGAATATGCACACAACTCATAGCATCACCGCCGCTCTGAACATTTCCCT
GGAGAATTGCGCTTTTTGTCAGAGTGCACTGCTGGAATACGATGACACACAGGGCGT
GATCAACATTATGTATATGCACGATAGCGATGACGTGCTGTTCGCTCTGGACCCCTA
CAACGAGGTGGTCGTGAGCTCCCCTCGCACTCATTATCTGATGCTGCTGAAGAATGG
AACAGTGCTGGAAGTCACTGATGTCGTGGTCGATGCCACAGACTCCCGGCTGCTGAT

GATGTCTGTGTACGCACTGTCCGCCATCATCGGCATCTATCTGCTGTATCGAATGCTG AAAACCTGTTGA (SEQ ID NO:15) (TM and CTD underlined)

[0090] In some embodiments, a gH polypeptide for use in accordance with the present invention lacks a transmembrane domain and/or cytoplasmic domain and/or contains a modified transmembrane domain and/or cytoplasmic domain. A gH polypeptide may optionally include one or more additional polypeptides (e.g., a heterologous transmembrane domain and/or cytoplasmic domain polypeptide). In some embodiments, a gH polypeptide is expressed as a fusion protein with a heterologous polypeptide. The gH polypeptide can be linked to a heterologous polypeptide to create a fusion protein without altering gH function and/or antigenicity. For example, a coding sequence for a heterologous polypeptide may be spliced into the gH polypeptide coding sequence, e.g., at the 3' end of the gH polypeptide coding sequence. In some embodiments, a coding sequence for a heterologous polypeptide may be spliced in frame into the gH polypeptide coding sequence. In some embodiments, a gH polypeptide-coding sequence and heterologous polypeptide may be expressed by a single promoter. In some embodiments, a heterologous polypeptide is inserted at (e.g., fused to) the C-terminus of a gH polypeptide.

[0091] In some embodiments, a heterologous polypeptide is or comprises a transmembrane domain and/or cytoplasmic domain found in Vesicular Stomatitis Virus (VSV). In some embodiments, a gH that is lacking a transmembrane domain and/or cytoplasmic domain is fused to a transmembrane domain and/or cytoplasmic domain from VSV. An exemplary gH-VSV fusion polypeptide for use in accordance with the present invention is shown below as SEQ ID NO:16. In some embodiments, a suitable gH-VSV polypeptide fusion protein includes all or a portion of a gH polypeptide that is substantially homologous to a known HCMV gH polypeptide and all or a portion of a VSV polypeptide that is substantially homologous to a known VSV polypeptide. For example, a gH – VSV polypeptide fusion protein may contain one or more amino acid substitutions, deletions, and/or insertions as compared to a wild-type or naturally-occurring gH and/or VSV polypeptide. Thus, in some embodiments, a gH-VSV polypeptide fusion protein suitable for the present invention is substantially homologous to SEQ ID NO:16. In some embodiments, a gH-VSV polypeptide fusion protein suitable for the present invention has an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%,

90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous to SEQ ID NO:16. In some embodiments, a gH-VSV polypeptide fusion protein suitable for the present invention is substantially identical to SEQ ID NO:16. In some embodiments, a gH-VSV polypeptide fusion protein suitable for the present invention has an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:16. As used herein, "gH-G" refers to a HMCV gH – VSV TM/CTD fusion protein.

HCMV gH – G Amino Acid Sequence (SEQ ID NO:16)

MRPGLPSYLIVLAVCLLSHLLSSRYGAEAISEPLDKAFHLLLNTYGRPIRFLRENTTQCT
YNSSLRNSTVVRENAISFNFFQSYNQYYVFHMPRCLFAGPLAEQFLNQVDLTETLERYQ
QRLNTYALVSKDLASYRSFSQQLKAQDSLGEQPTTVPPPIDLSIPHVWMPPQTTPHGWT
ESHTTSGLHRPHFNQTCILFDGHDLLFSTVTPCLHQGFYLIDELRYVKITLTEDFFVVTVSI
DDDTPMLLIFGHLPRVLFKAPYQRDNFILRQTEKHELLVLVKKDQLNRHSYLKDPDFLD
AALDFNYLDLSALLRNSFHRYAVDVLKSGRCQMLDRRTVEMAFAYALALFAAARQEE
AGAQVSVPRALDRQAALLQIQEFMITCLSQTPPRTTLLLYPTAVDLAKRALWTPNQITDI
TSLVRLVYILSKQNQQHLIPQWALRQIADFALKLHKTHLASFLSAFARQELYLMGSLVH
SMLVHTTERREIFIVETGLCSLAELSHFTQLLAHPHHEYLSDLYTPCSSSGRRDHSLERLT
RLFPDATVPTTVPAALSILSTMQPSTLETFPDLFCLPLGESFSALTVSEHVSYVVTNQYLI
KGISYPVSTTVVGQSLIITQTDSQTKCELTRNMHTTHSITAALNISLENCAFCQSALLEYD
DTQGVINIMYMHDSDDVLFALDPYNEVVVSSPRTHYLMLLKNGTVLEVTDVVVDATDS
RFFFIIGLIIGLFLVLRVGIHLCIKLKHTKKRQIYTDIEMNRLGK* (SEQ ID NO:16) (TM and CTD underlined)

HCMV gH – G Nucleotide Sequence (SEQ ID NO:17)

ATGCGGCCAGGCCTCCCCTCCTACCTCATCGTCCTCGCCGTCTGTCTCCTCAGCCACC
TACTTTCGTCACGATATGGCGCAGAAGCCATATCCGAACCGCTGGACAAAGCGTTTC
ACCTACTGCTCAACACCTACGGGAGACCCATCCGCTTCCTGCGTGAAAACACCACCC
AGTGTACCTACAATAGCAGCCTCCGTAACAGCACGGTCGTCAGGGAAAACGCCATC
AGTTTCAACTTTTTCCAAAGCTATAATCAATACTATGTATTCCATATGCCTCGATGTC
TTTTTGCGGGTCCTCTGGCGGAGCAGTTTCTGAACCAGGTAGATCTGACCGAAACCC
TGGAAAGATACCAACAGAGACTTAACACTTACGCGCTGGTATCCAAAGACCTGGCC
AGCTACCGATCTTTTTCGCAGCAGCTAAAGGCACAGGACAGCCTAGGTGAACAGCC
CACCACTGTGCCACCACCCATTGACCTGTCAATACCTCACGTTTGGATGCCACCGCA
AACCACTCCACACGGCTGGACAGAATCACATACCACCTCAGGACTACACCGACCAC
ACTTTAACCAGACCTGTATCCTCTTTGATGGACACGATCTACTATTCAGCACCGTCAC
ACCTTGTTTGCACCAAGGCTTTTACCTCATCGACGAACTACGTTACGTTAAAATAAC
ACTGACCGAGGACTTCTTCGTAGTTACGGTGTCCATAGACGACGACACACCCATGCT
GCTTATCTTCGGCCATCTTCCACGCGTACTCTTTAAAGCGCCCTATCAACGCGACAA
CTTTATACTACGACAAACTGAAAAACACGAGCTCCTGGTGCTAGTTAAGAAAGATC
AACTGAACCGTCACTCTTATCTCAAAGACCCGGACTTTCTTGACGCCGCACTTGACT
TCAACTACCTGGACCTCAGCGCACTACTACGTAACAGCTTTCACCGTTACGCCGTGG
ATGTACTCAAAAGCGGTCGATGTCAGATGCTGGACCGCCGCACGGTAGAAATGGCC
TTCGCCTACGCATTAGCACTGTTCGCAGCAGCCCGACAAGAAGAGGCCGGCGCCCA

AGTCTCCGTCCCACGGGCCCTAGACCGCCAGGCCGCACTCTTACAAATACAAGAATT
TATGATCACCTGCCTCTCACAAACACCACCACGCACCACGTTGCTGCTGTATCCCAC
GGCCGTGGACCTGGCCAAACGAGCCCTTTGGACACCGAATCAGATCACCGACATCA
CCAGCCTCGTACGCCTGGTCTACATACTCTCTAAACAGAATCAGCAACATCTCATCC
CCCAGTGGGCACTACGACAGATCGCCGACTTTGCCCTAAAACTACACAAAACGCAC
CTGGCCTCTTTTCTTTCAGCCTTCGCGCGTCAAGAACTCTACCTCATGGGCAGCCTCG
TCCACTCCATGCTAGTACATACGACGGAGAGACGCGAAATCTTCATCGTAGAAACG
GGCCTCTGTTCATTAGCCGAGCTATCACACTTTACGCAGTTGCTAGCTCATCCGCAC
CACGAATACCTCAGCGACCTGTACACACCCTGTTCCAGTAGCGGGCGACGCGATCA
CTCGCTCGAACGCCTCACACGTCTCTTCCCCGATGCCACCGTCCCCACTACCGTTCCC
GCCGCCCTCTCCATCCTATCTACCATGCAACCAAGCACGCTAGAAACCTTCCCCGAC
CTGTTTTGTCTGCCGCTCGGCGAATCCTTCTCCGCGCTGACCGTCTCCGAACACGTCA
GTTATGTCGTAACAAACCAGTACCTGATCAAAGGTATCTCCTACCCTGTCTCCACCA
CCGTCGTAGGCCAGAGCCTCATCATCACCCAGACGGACAGTCAAACTAAATGCGAA
CTGACGCGCAACATGCATACCACACACAGCATCACAGCGGCGCTCAACATTTCCCTA
GAAAACTGCGCCTTTTGCCAAAGCGCCCTACTAGAATACGACGACACGCAAGGCGT
CATCAACATCATGTACATGCACGACTCGGACGACGTCCTTTTCGCCCTGGATCCCTA
CAACGAAGTGGTGGTCTCATCTCCGCGAACTCACTACCTCATGCTTTTGAAAAACGG
TACGGTCCTAGAAGTAACTGACGTCGTCGTGGACGCTACCGACAGTCGTTTTTTCTTT
ATCATAGGGTTAATCATTGGACTATTCTTGGTTCTCCGAGTTGGTATCCATCTTTGCA
TTAAATTAAAGCACACCAAGAAAAGACAGATTTATACAGACATAGAGATGAACCGA
CTTGGAAAGTAA (SEQ ID NO:17) (TM and CTD underlined)

Codon Optimized HCMV gH – G Nucleotide Sequence (SEQ ID NO:18)

ATGCGACCCGGACTGCCAAGCTACCTGATTGTCCTGGCTGTCTGTCTGCTGTCACAC
CTGCTGAGTTCAAGATATGGGGCCGAAGCCATCAGCGAGCCACTGGACAAGGCTTT
CCACCTGCTGCTGAACACCTACGGCAGACCCATTAGGTTTCTGCGCGAGAATACCAC
ACAGTGCACATATAACAGCTCCCTGAGGAATAGCACTGTGGTCCGCGAAAACGCCA
TCTCTTTCAATTTCTTTCAGAGTTACAACCAGTACTACGTGTTCCATATGCCACGCTG
TCTGTTCGCAGGACCCCTGGCCGAGCAGTTTCTGAACCAGGTGGACCTGACCGAGAC
ACTGGAAAGATACCAGCAGAGGCTGAATACCTATGCCCTGGTGAGTAAGGATCTGG
CTTCATATCGGTCTTTCAGTCAGCAGCTCAAGGCCCAGGACTCTCTGGGAGAGCAGC
CTACTACCGTGCCCCCTCCAATCGATCTGAGTATTCCACACGTCTGGATGCCCCCTC
AGACAACTCCCCACGGATGGACCGAAAGCCATACCACATCCGGCCTGCACAGACCC
CACTTCAACCAGACATGCATCCTGTTCGATGGCCACGACCTGCTGTTTTCCACTGTG
ACCCCTTGTCTGCATCAGGGGTTCTACCTGATCGATGAGCTGAGATATGTGAAGATT
ACACTGACTGAAGACTTCTTTGTGGTCACCGTGTCTATCGACGATGACACACCAATG
CTGCTGATTTTCGGACACCTGCCCCGGGTGCTGTTCAAGGCCCCCTACCAGCGAGAC
AACTTCATCCTGCGGCAGACCGAGAAACACGAACTGCTGGTGCTGGTCAAGAAAGA
TCAGCTCAACCGGCATTCCTATCTGAAGGACCCCGACTTCCTGGATGCCGCTCTGGA
CTTTAACTACCTGGACCTGTCAGCACTGCTGCGGAATAGCTTTCACAGATATGCCGT
GGATGTCCTGAAATCTGGGCGCTGCCAGATGCTGGACCGGAGAACCGTGGAGATGG
CATTCGCCTACGCTCTGGCACTGTTTGCAGCCGCTCGGCAGGAGGAAGCAGGAGCTC
AGGTGAGTGTCCCTCGCGCACTGGATCGACAGGCAGCCCTGCTGCAGATCCAGGAG
TTCATGATTACCTGTCTGAGCCAGACACCACCCAGAACTACCCTGCTGCTGTACCCC
ACTGCCGTGGACCTGGCTAAGAGGGCACTGTGGACCCCTAACCAGATCACTGATATT

ACCAGCCTGGTGAGACTGGTCTATATCCTGTCCAAACAGAATCAGCAGCACCTGATC
CCACAGTGGGCCCTGAGGCAGATTGCCGACTTTGCTCTGAAGCTGCACAAAACCCAT
CTGGCTTCCTTTCTGTCTGCATTCGCCAGACAGGAGCTGTACCTGATGGGATCTCTGG
TGCACAGTATGCTGGTCCATACAACTGAGAGGCGCGAAATCTTCATTGTGGAGACA
GGCCTGTGCAGCCTGGCTGAACTGTCCCACTTTACTCAGCTCCTGGCCCATCCTCAC
CATGAGTACCTGTCAGATCTGTATACCCCATGTTCTAGTTCAGGACGACGGGACCAC
AGCCTGGAACGACTGACTCGGCTGTTCCCTGATGCAACCGTGCCTACCACCGTGCCC
GCCGCCCTGAGTATCCTGTCAACAATGCAGCCAAGCACACTGGAGACTTTTCCCGAC
CTGTTCTGCCTGCCTCTGGGCGAGAGCTTCAGCGCCCTGACCGTGAGCGAACATGTC
AGCTACGTGGTCACAAACCAGTATCTGATCAAGGGGATTTCCTACCCCGTGTCTACT
ACCGTGGTCGGACAGTCCCTGATCATTACCCAGACAGATTCTCAGACTAAATGTGAG
CTGACCAGAAATATGCACACAACTCATAGTATCACCGCCGCTCTGAACATTTCACTG
GAGAATTGCGCTTTCTGTCAGTCCGCACTGCTGGAATACGATGACACACAGGGCGTG
ATCAACATTATGTATATGCACGATTCTGATGACGTGCTGTTTGCTCTGGACCCCTACA
ACGAGGTGGTCGTGAGCTCCCCCAGAACTCATTATCTGATGCTGCTGAAGAATGGCA
CAGTGCTGGAAGTCACTGATGTCGTGGTCGATGCCACAGACTCCCGCTTCTTTTTCAT
CATTGGCCTGATCATTGGGCTGTTCCTGGTGCTGCGAGTCGGCATCCACCTGTGCAT
CAAGCTGAAGCATACAAAGAAGAGACAGATCTACACCGATATTGAAATGAACAGGC
TGGGCAAATGA (SEQ ID NO:18) (TM and CTD underlined)

[0092] In some embodiments, a gH polypeptide includes a transmembrane domain and/or cytoplasmic domain found in gB. An exemplary nucleotide encoding an HCMV gH – HCMV gB TM/CTD fusion polypeptide for use in accordance with the present invention is shown below as SEQ ID NO:20. In some embodiments, an HCMV gH – HCMV gB TM/CTD polypeptide suitable for the present invention is substantially homologous to the polypeptide encoded by SEQ ID NO:20. In some embodiments, an HCMV gH – HCMV gB TM/CTD polypeptide suitable for the present invention has an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous to the polypeptide encoded by SEQ ID NO:20. In some embodiments, an HCMV gH – HCMV gB TM/CTD polypeptide suitable for the present invention is substantially identical to the polypeptide encoded by SEQ ID NO:20. In some embodiments, an HCMV gH – HCMV gB TM/CTD polypeptide suitable for the present invention has an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the polypeptide encoded by SEQ ID NO:20.

HCMV gH – HCMV gB TM/CTD Nucleotide Sequence (SEQ ID NO:20)

ATGCGGCCAGGCCTCCCCTCCTACCTCATCGTCCTCGCCGTCTGTCTCCTCAGCCACC
TACTTTCGTCACGATATGGCGCAGAAGCCATATCCGAACCGCTGGACAAAGCGTTTC
ACCTACTGCTCAACACCTACGGGAGACCCATCCGCTTCCTGCGTGAAAACACCACCC
AGTGTACCTACAATAGCAGCCTCCGTAACAGCACGGTCGTCAGGGAAAACGCCATC
AGTTTCAACTTTTTCCAAAGCTATAATCAATACTATGTATTCCATATGCCTCGATGTC
TTTTTGCGGGTCCTCTGGCGGAGCAGTTTCTGAACCAGGTAGATCTGACCGAAACCC
TGGAAAGATACCAACAGAGACTTAACACTTACGCGCTGGTATCCAAAGACCTGGCC
AGCTACCGATCTTTTTCGCAGCAGCTAAAGGCACAGGACAGCCTAGGTGAACAGCC
CACCACTGTGCCACCACCCATTGACCTGTCAATACCTCACGTTTGGATGCCACCGCA
AACCACTCCACACGGCTGGACAGAATCACATACCACCTCAGGACTACACCGACCAC
ACTTTAACCAGACCTGTATCCTCTTTGATGGACACGATCTACTATTCAGCACCGTCAC
ACCTTGTTTGCACCAAGGCTTTTACCTCATCGACGAACTACGTTACGTTAAAATAAC
ACTGACCGAGGACTTCTTCGTAGTTACGGTGTCCATAGACGACGACACACCCATGCT
GCTTATCTTCGGCCATCTTCCACGCGTACTCTTTAAAGCGCCCTATCAACGCGACAA
CTTTATACTACGACAAACTGAAAAACACGAGCTCCTGGTGCTAGTTAAGAAAGATC
AACTGAACCGTCACTCTTATCTCAAAGACCCGGACTTTCTTGACGCCGCACTTGACT
TCAACTACCTGGACCTCAGCGCACTACTACGTAACAGCTTTCACCGTTACGCCGTGG
ATGTACTCAAAAGCGGTCGATGTCAGATGCTGGACCGCCGCACGGTAGAAATGGCC
TTCGCCTACGCATTAGCACTGTTCGCAGCAGCCCGACAAGAAGAGGCCGGCGCCCA
AGTCTCCGTCCCACGGGCCCTAGACCGCCAGGCCGCACTCTTACAAATACAAGAATT
TATGATCACCTGCCTCTCACAAACACCACCACGCACCACGTTGCTGCTGTATCCCAC
GGCCGTGGACCTGGCCAAACGAGCCCTTTGGACACCGAATCAGATCACCGACATCA
CCAGCCTCGTACGCCTGGTCTACATACTCTCTAAACAGAATCAGCAACATCTCATCC
CCCAGTGGGCACTACGACAGATCGCCGACTTTGCCCTAAAACTACACAAAACGCAC
CTGGCCTCTTTTCTTTCAGCCTTCGCGCGTCAAGAACTCTACCTCATGGGCAGCCTCG
TCCACTCCATGCTAGTACATACGACGGAGAGACGCGAAATCTTCATCGTAGAAACG
GGCCTCTGTTCATTAGCCGAGCTATCACACTTTACGCAGTTGCTAGCTCATCCGCAC
CACGAATACCTCAGCGACCTGTACACACCCTGTTCCAGTAGCGGGCGACGCGATCA
CTCGCTCGAACGCCTCACACGTCTCTTCCCCGATGCCACCGTCCCCACTACCGTTCCC
GCCGCCCTCTCCATCCTATCTACCATGCAACCAAGCACGCTAGAAACCTTCCCCGAC
CTGTTTTGTCTGCCGCTCGGCGAATCCTTCTCCGCGCTGACCGTCTCCGAACACGTCA
GTTATGTCGTAACAAACCAGTACCTGATCAAAGGTATCTCCTACCCTGTCTCCACCA
CCGTCGTAGGCCAGAGCCTCATCATCACCCAGACGGACAGTCAAACTAAATGCGAA
CTGACGCGCAACATGCATACCACACACAGCATCACAGCGGCGCTCAACATTTCCCTA
GAAAACTGCGCCTTTTGCCAAAGCGCCCTACTAGAATACGACGACACGCAAGGCGT
CATCAACATCATGTACATGCACGACTCGGACGACGTCCTTTTCGCCCTGGATCCCTA
CAACGAAGTGGTGGTCTCATCTCCGCGAACTCACTACCTCATGCTTTTGAAAAACGG
TACGGTCCTAGAAGTAACTGACGTCGTCGTGGACGCTACCGACAGTCGT**TTCGGAG**
CCTTCACCATCATCCTCGTGGCCATAGCCGTCGTCATTATCATTTATTTGATCT
ATACTCGACAGCGGCGTCTCTGCATGCAGCCGCTGCAGAACCTCTTTCCCTATC
TGGTGTCCGCCGACGGGACCACCGTGACGTCGGGCAACACCAAAGACACGTCG
TTACAGGCTCCGCCTTCCTACGAGGAAAGTGTTTATAATTCTGGTCGCAAAGGA
CCGGGACCACCGTCGTCTGATGCATCCACGGCGGCTCCGCCTTACACCAACGA
GCAGGCTTACCAGATGCTTCTGGCCCTGGTCCGTCTGGACGCAGAGCAGCGAG
CGCAGCAGAACGGTACAGATTCTTTGGACGGACAGACTGGCACGCAGGACAAG

GGACAGAAGCCCAACCTGCTAGACCGACTGCGACACCGCAAAAACGGCTACCG ACACTTGAAAGACTCCGACGAAGAAGAGAACGTCTGA (SEQ ID NO:20) (gH peptide signal underlined; gB TM-CTD bolded)

Others (including gN and gM – Glycoprotein Complex (gC) II)

[0093] In addition to gB and gH, other envelope glycoproteins may be useful in vaccine development. For example, the gcII complex, containing gN (UL73) and gM (UL100), is of particular interest. Proteomic analyses of HCMV virions has demonstrated that gcII is the most abundantly expressed glycoprotein in virus particles, emphasizing its potential importance in protective immunity (Varnum SM et al., 2005 Human Gene Ther 16:1143-50). HCMV infection elicits a gcII-specific antibody response in a majority of seropositive individuals (Shimamura M et al., 2006 J Virol 80:4591-600), and DNA vaccines containing gcII antigens gM and gN have been shown to elicit neutralizing antibody responses in rabbits and mice (Shen S et al., 2007 Vaccine 25:3319-27).

pp65

[0094] The cellular immune response to HCMV includes MHC class II restricted $CD4^+$ and MHC class I restricted, cytotoxic $CD8^+$ T-lymphocyte responses to a number of viral antigens, many of which are found in the viral tegument, the region of the viral particle that lies between the envelope and nucleocapsid. For example, HCMV pp65 protein (UL83) has been shown to elicit a significant $CD8^+$ T-lymphocyte response following HCMV infection (McLaughlin-Taylor E 1994 J Med Virol 43:103-10). This 65 kDa viral protein is one of the most abundantly expressed structural proteins of HCMV. Exemplary pp65 sequences are described herein.

Others (including IE1, pp150)

[0095] Other proteins that elicit T-lymphocyte responses include the immediate early-1 (IE1) protein (UL123) and pp150 (UL32) (Gibson L et al., 2004 J Immunol 172:2256-64; La Rosa C et al., 2005 Human Immunol 66:116-26).

[0096] As described above, a Gag polypeptide may optionally include one or more additional polypeptides (e.g., a heterologous antigen). In some embodiments, a Gag polypeptide is co-expressed with a heterologous antigen (e.g., under separate promoters and/or as separate

proteins). The Gag polypeptide can be co-expressed with a heterologous antigen without altering Gag function. Without wishing to be bound by any theory, it is thought that co-expression of a self-assembling Gag polypeptide with a heterologous envelope antigen will allow the antigen to be incorporated into the envelope or lipid bilayer of a resulting VLP. In some embodiments, VLP envelope components serve as effective immunogens (e.g., for induction of humoral immune response). In some embodiments, a Gag polypeptide is expressed as a fusion protein with a heterologous antigen. For example, a coding sequence for a heterologous antigen may be spliced into the Gag polypeptide coding sequence, e.g., at the 3' end of the Gag polypeptide coding sequence. In some embodiments, a coding sequence for a heterologous antigen may be spliced in frame into the Gag polypeptide coding sequence. In some embodiments, a Gag polypeptide-coding sequence and heterologous antigen may be expressed by a single promoter. In some embodiments, a heterologous antigen is inserted at (e.g., fused to) the C-terminus of a Gag polypeptide. Without wishing to be bound by any theory, it is thought that fusion of a self-assembling Gag polypeptide to a heterologous antigen will allow the antigen to be incorporated into the structural components of a resulting VLP. In some embodiments, VLP structural components serve as effective immunogens (e.g., for induction of cellular immune response). For example, provided VLPs may comprise a retroviral gag polypeptide (e.g., MLV gag) and a structural component of HCMV (e.g., pp65). In some such embodiments, pp65 is incorporated into the VLP and serves as an antigen for eliciting an immune response against HCMV.

[0097] Provided VLPs may contain a structural retroviral protein (e.g., Gag polypeptide) that is arranged and constructed such that it self-assembles to form the VLP and is positioned in the VLP interior. In some embodiments, provided VLPs contain an envelope protein (e.g., gB and/or gH) that is arranged and constructed such that one or more epitopes of the envelope protein (e.g., gB and/or gH) is positioned on the VLP surface. In some embodiments, provided VLPs contain a fusion structural protein (e.g., Gag/pp65) that is arranged and constructed such that one or more epitopes of the structural protein (e.g., pp65) is positioned in the VLP interior.

II. Production of VLPs

[0098] It will be appreciated that a composition comprising VLPs will typically include a mixture of VLPs with a range of sizes. It is to be understood that the diameter values listed

below correspond to the most frequent diameter within the mixture. In some embodiments > 90% of the vesicles in a composition will have a diameter which lies within 50% of the most frequent value (e.g., 1000 ± 500 nm). In some embodiments the distribution may be narrower, e.g., > 90% of the vesicles in a composition may have a diameter which lies within 40, 30, 20, 10 or 5% of the most frequent value. In some embodiments, sonication or ultra-sonication may be used to facilitate VLP formation and/or to alter VLP size. In some embodiments, filtration, dialysis and/or centrifugation may be used to adjust the VLP size distribution.

[0099] In general, VLPs produced in accordance with the methods of the present disclosure may be of any size. In certain embodiments, the composition may include VLPs with diameter in range of about 20 nm to about 300 nm. In some embodiments, a VLP is characterized in that it has a diameter within a range bounded by a lower limit of 20, 30, 40, 50, 60, 70, 80, 90, or 100 nm and bounded by an upper limit of 300, 290, 280, 270, 260, 250, 240, 230, 220, 210, 200, 190, 180, or 170 nm. In some embodiments, VLPs within a population show an average diameter within a range bounded by a lower limit of 20, 30, 40, 50, 60, 70, 80, 90, or 100 nm and bounded by an upper limit of 300, 290, 280, 270, 260, 250, 240, 230, 220, 210, 200, 190, 180, or 170 nm. In some embodiments, VLPs in a population have a polydispersity index that is less than 0.5 (e.g., less than 0.45, less than 0.4, or less than 0.3). In some embodiments, VLP diameter is determined by nanosizing. In some embodiments, VLP diameter is determined by electron microscopy.

A. In vitro / Ex vivo VLP production

[0100] Provided VLPs in accordance with the present invention may be prepared according to general methodologies known to the skilled person. For example, various nucleic acid molecules, genomes or reconstituted vectors or plasmids may be prepared using sequences of known viruses. Such sequences are available from banks, and material may be obtained from various collections, published plasmids, etc. These elements can be isolated and manipulated using techniques well known to the skilled artisan, or isolated from plasmids available in the art. Various synthetic or artificial sequences may also be produced from computer analysis or through (high throughput) screening of libraries. Recombinant expression of the polypeptides for VLPs requires construction of an expression vector containing a polynucleotide that encodes

one or more polypeptide(s). Once a polynucleotide encoding one or more polypeptides has been obtained, the vector for production of the polypeptide may be produced by recombinant DNA technology using techniques known in the art. Expression vectors that may be utilized in accordance with the present invention include, but are not limited to mammalian expression vectors, baculovirus expression vectors, plant expression vectors (e.g., Cauliflower Mosaic Virus (CaMV), Tobacco Mosaic Virus (TMV)), plasmid expression vectors (e.g., Ti plasmid), among others.

[0101] An exemplary VLP expression plasmid that may be used in accordance with the present invention is shown below as SEQ ID NO:19:

Propol II Expression Plasmid (SEQ ID NO:19)

CTAGAGAGCTTGGCCCATTGCATACGTTGTATCCATATCATAATATGTACATTTATAT
TGGCTCATGTCCAACATTACCGCCATGTTGACATTGATTATTGACTAGTTATTAATAG
TAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAA
CTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCA
ATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGG
GTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCA
AGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAG
TACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTA
TTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACT
CACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACC
AAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATG
GGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCG
TCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGA
CCGATCCAGCCTCCGGTCGACCGATCCTGAGAACTTCAGGGTGAGTTTGGGGACCCT
TGATTGTTCTTTCTTTTTCGCTATTGTAAAATTCATGTTATATGGAGGGGGCAAAGTT
TTCAGGGTGTTGTTTAGAATGGGAAGATGTCCCTTGTATCACCATGGACCCTCATGA
TAATTTTGTTTCTTTCACTTTCTACTCTGTTGACAACCATTGTCTCCTCTTATTTTCTTT
TCATTTTCTTGTAACTTTTTCGTTAAACTTTAGCTTGCATTTGTAACGAATTTTTAAAT
TCACTTTTGTTTATTTGTCAGATTGTAAGTACTTTCTCTAATCACTTTTTTTTCAAGGC
AATCAGGGTATATTATATTGTACTTCAGCACAGTTTTAGAGAACAATTGTTATAATT
AAATGATAAGGTAGAATATTTCTGCATATAAATTCTGGCTGGCGTGGAAATATTCTT
ATTGGTAGAAACAACTACATCCTGGTCATCATCCTGCCTTTCTCTTTATGGTTACAAT
GATATACACTGTTTGAGATGAGGATAAAATACTCTGAGTCCAAACCGGGCCCCTCTG
CTAACCATGTTCATGCCTTCTTCTTTTTCCTACAGCTCCTGGGCAACGTGCTGGTTAT
TGTGCTGTCTCATCATTTTGGCAAAGAATTCCTCGAGCGTACGCCTAGGGGATCCAG
CGCTATTTAAATGCTAGCATGCATGTTAACCCTGCAGGGGTACCGCGGCCGCAAGCT
TAGATCCGTCGAGGAATTCACTCCTCAGGTGCAGGCTGCCTATCAGAAGGTGGTGGC
TGGTGTGGCCAATGCCCTGGCTCACAAATACCACTGAGATCTTTTTCCCTCTGCCAA
AAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAA

ATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAGGACA
TATGGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCA
ACATATGCCCATATGCTGGCTGCCATGAACAAAGGTTGGCTATAAAGAGGTCATCA
GTATATGAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGACTTG
AGGTTAGATTTTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAAAT
TTTCCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTACTCCCAGTCATA
GCTGTCCCTCTTCTCTTATGGAGATCCCTCGACGGATCGGCCGCAATTCGTAATCATG
TCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGA
GCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATT
AATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCA
TTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGC
TTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGC
TCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGA
ACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCT
GGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAG
TCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAA
GCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTT
TCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCG
GTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGAC
CGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTA
TCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGG
TGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATT
TGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTG
ATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGAT
TACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGA
CGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAA
GGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTA
TATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCT
CAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAAC
TACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACC
CACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAG
CGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGG
GAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCT
ACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCC
AACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGGTTAGCTC
CTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTT
ATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGA
CTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCT
CTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTG
CTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTG
AGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTT
TCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGG
AATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTG
AAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAA
AAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTAAATTGT
AAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTT

AACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGAT
AGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTC
CAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCAT
CACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTA
AAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAA
GGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTC
ACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTC
CCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTC
GCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAA
CGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAGCGCGCGTAA
TACGACTCACTATAGGGCGAATTGGAGCTCCACCGCGGTGGCGGCCGCT (SEQ ID NO:19)

[0102] Provided VLPs may be prepared according to techniques known in the art. For example, in some embodiments, provided VLPs may be produced in any available protein expression system. Typically, the expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce VLPs. In some embodiments, VLPs are produced using transient transfection of cells. In some embodiments, VLPs are produced using stably transfected cells. Typical cell lines that may be utilized for VLP production include, but are not limited to, mammalian cell lines such as human embryonic kidney (HEK) 293, WI 38, Chinese hamster ovary (CHO), monkey kidney (COS), HT1080, C10, HeLa, baby hamster kidney (BHK), 3T3, C127, CV-1, HaK, NS/O, and L-929 cells. Specific non-limiting examples include, but are not limited to, BALB/c mouse myeloma line (NSO/l, ECACC No: 85110503); human retinoblasts (PER.C6 (CruCell, Leiden, The Netherlands)); monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., *J. Gen Virol.*, 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells +/-DHFR (CHO, Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.*, 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1 587); human cervical carcinoma cells (HeLa, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., *Annals N.Y. Acad. Sci.*, 383:44-68 (1982)); MRC 5

cells; FS4 cells; and a human hepatoma line (Hep G2). In some embodiments, cell lines that may be utilized for VLP production include insect (e.g., Sf-9, Sf-21, Tn-368, Hi5) or plant (e.g., *Leguminosa*, cereal, or tobacco) cells. It will be appreciated in some embodiments, particularly when glycosylation is important for protein function, mammalian cells are preferable for protein expression and/or VLP production (see, e.g., Roldao A et al., 2010 Expt Rev Vaccines 9:1149-76).

[0103] It will be appreciated that a cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in a specific way. Such modifications (e.g., glycosylation) and processing (e.g., cleavage or transport to the membrane) of protein products may be important for generation of a VLP or function of a VLP polypeptide or additional polypeptide (e.g., an adjuvant or additional antigen). Different cells have characteristic and specific mechanisms for post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. Generally, eukaryotic host cells (also referred to as packaging cells (e.g., 293T human embryo kidney cells) which possess appropriate cellular machinery for proper processing of the primary transcript, glycosylation and phosphorylation of the gene product may be used in accordance with the present invention.

[0104] VLPs may be purified according to known techniques, such as centrifugation, gradients, sucrose-gradient ultracentrifugation, tangential flow filtration and chromatography (e.g., ion exchange (anion and cation), affinity and sizing column chromatography), or differential solubility, among others. Alternatively or additionally, cell supernatant may be used directly, with no purification step. Additional entities, such as additional antigens or adjuvants may be added to purified VLPs.

[0105] In some embodiments, provided polynucleotide sequences are codon optimized. Codon optimization is well known in the art and involves modification of codon usage so that higher levels of protein are produced.

B. In vivo VLP production

[0106] Provided VLPs in accordance with the present invention may be prepared as DNA vaccines according to methods well known in the art. For example, in some embodiments, one or more vectors or plasmids, e.g., such as those described above, is administered to a subject such that recipient cells express polypeptides encoded by the vector or plasmid. In some embodiments, recipient cells expressing such polypeptides produce VLPs comprising the polypeptides.

C. Mono-, di-, trivalent eVLPs

[0107] In accordance with the present invention, cells may be transfected with a single expression vector as described herein. In some embodiments, a single expression vector encodes more than one element of a VLP (e.g., more than one of structural polyprotein, CMV tegument polypeptide, CMV glycoprotein, etc.). For example, in some embodiments, a single expression vector encodes two or more elements of a VLP. In some embodiments, a single expression vector encodes three of more elements of a VLP.

[0108] In some embodiments, cells are transfected with two or more expression vectors. For example, in some embodiments, cells are transfected with a first vector encoding a Gag polypeptide and a second vector encoding an HCMV envelope glycoprotein (e.g., gB or gB-G or gH-G). In some such embodiments, "monovalent" VLPs comprising an HCMV envelope glycoprotein (e.g., gB or gB-G or gH-G) are produced. In some embodiments, cells are transfected with a first vector encoding a Gag polypeptide, a second vector encoding an HCMV envelope glycoprotein (e.g., gB or gB-G or gH-G) and a third vector encoding another HCMV envelope glycoprotein (e.g., gB or gB-G or gH-G). In some such embodiments, "bivalent" VLPs comprising 2 HCMV envelope glycoproteins (e.g., gB and gH-G or gB-G and gH-G) are produced. In some embodiments, cells are transfected with a first vector encoding a Gag-pp65 fusion polypeptide and a second vector encoding an HCMV envelope glycoprotein (e.g., gB or gB-G or gH-G). In some such embodiments, "bivalent" VLPs comprising an HCMV structural protein and an HCMV envelope glycoprotein (e.g., gB or gB-G or gH-G) are produced. In some embodiments, cells are transfected with a first vector encoding a Gag-pp65 fusion polypeptide, a

second vector encoding an HCMV envelope glycoprotein (e.g., gB or gB-G or gH-G), and a third vector encoding another HCMV envelope glycoprotein (e.g., gB or gB-G or gH-G). In some such embodiments, "trivalent" VLPs comprising an HCMV structural protein and 2 HCMV envelope glycoproteins (e.g., gB and gH-G or gB-G and gH-G) are produced.

[0109] In some embodiments, monovalent, bivalent, or trivalent VLPs are admixed. For example, in some embodiments, monovalent and bivalent VLPs are admixed to form a trivalent VLP mixture. In some embodiments two bivalent VLPs are admixed to form a trivalent VLP mixture.

III. HCMV Infection and Treatment

[0110] Human cytomegalovirus (HCMV), a β-herpesvirus, is a ubiquitously occurring pathogen. In general, entry of herpesviruses into cells is a complex process initiated by adsorption and receptor binding and followed by fusion of the virus envelope with a cell membrane. Fusion generally occurs at either the plasma membrane or an endosomal membrane. HCMV infects multiple cell types *in vivo*, including epithelial cells, endothelial cells and fibroblasts (Plachter B et al., 1996 Adv Virus Res 46:195-261). It fuses with the plasma membranes of fibroblasts (Compton T et al., 1992 Virology 191:387-395), but enters retinal pigmented epithelial cells and umbilical vein endothelial cells via endocytosis (Bodaghi B et al., 1999 J Immunol 162:957-964; Ryckman BJ et al., 2006 J Virol 80:710-722). The mechanism by which herpesviruses' choose their route of entry remains unclear. It is generally assumed that entry pathways are mainly determined by the host cell, but there is evidence for tropic roles of virion glycoproteins (Wang X et al., 1998 J Virol 72:5552-5558). As mentioned previously, HCMV encodes two gH/gL complexes: gH/gL/gO and gH/gL/UL128/UL130/UL131. The gO-containing complex is sufficient for fibroblast infection, whereas the pUL128/UL130/UL131-containing complex is important for HCMV infection of endothelial and epithelial cells.

[0111] HCMV infects 50-85% of adults by 40 years of age (Gershon AA et al., 1997 in *Viral Infections of Humans*, 4th edition, New York; Plenum Press:229-251). Most healthy individuals who acquire HCMV after birth develop few, if any, symptoms. However, HCMV disease is the cause of significant morbidity and mortality in immunocompromised individuals,

such as recipients of hematopoietic cell transplants (HCT) and solid-organ transplants (SOT) (Pass RF 2001 Cytomegalovirus. In Fields Virology. 4th edition, Philadelphia; Lippincott Williams & Wilkens:2675-2705). In SOT or HCT populations, HCMV disease can occur either from new infection transmitted from the donor organ or HCT, or can recur as a result of reactivation of latent virus in the recipient. In HIV-infected individuals, HCMV infection accelerates progression to AIDS and death, despite availability of antiretroviral therapy (Deayton JR et al., 2004 Lancet 363:2116-2121). In addition in the US, HCMV is the most common intrauterine infection and causes congenital abnormalities resulting in death or severe birth defects, including deafness and mental retardation, in approximately 8000 infants each year (Stagon S et al., 1986 JAMA 256:1904-1908).

[0112] Immune responses which control HCMV are incompletely understood. By analogy to other human herpesviruses it can be assumed that both cellular and humoral immune responses play an important role (Kohl S 1992 Current topics in Microbiology and Immunology 179:75-88). For murine CMV it was shown that either a cytotoxic T cell response or the passive transfer of neutralizing antibodies is sufficient to protect against a lethal challenge (Rapp M et al., 1993 Multidisciplinary Approach to Understanding Cytomegalovirus Disease:327-332; Reddehase MJ et al., 198 J Virology 61:3102-3108).

[0113] Control of CMV in immunocompromised persons is primarily associated with cellular immune responses; both $CD8^+$ and $CD4^+$ T lymphocytes appear to be important for protection against CMV disease (Gamadia LE et al., 2003 Blood 101:2686-2692; Cobbold M et al., 2005 J Exp Med 202:379-386). The cellular immune response to CMV includes $CD4^+$ helper T-lymphocyte and $CD8^+$ Cytotoxic T-lymphocyte responses to a number of antigens, found in the viral tegument, the region of the viral particle between the envelope and capsid. A recent study of CMV-specific $CD4^+$ and $CD8^+$ T cells from healthy donors used overlapping peptides from a series of CMV open reading frames to identify antigens recognized after CMV infection (Sylwester AW et al., 2005 J Exp Med 202:673-685). The CMV tegument phosphoprotein 65 (pp65) and surface glycoprotein gB were the antigens most frequently recognized by $CD4^+$ T cells, and pp65 was also one of the antigens most frequently recognized by $CD8^+$ T cells.

[0114] In contrast to the transplant setting, the maternal humoral immune response against the virus seems to be important in preventing HCMV disease in the newborn. Antibodies to surface glycoproteins, especially gB, appear to be critical for protection against the maternal-fetal transfer of CMV (Fowler KB et al., 2003 JAMA 289:1008-1011). Moreover, in an earlier vaccination study it was shown that protection from re-infection is correlated with neutralizing antibodies (Adler SP et al., 1995 J Infectious Diseases 171:26-32). The humoral immune response to CMV is dominated by responses to viral envelope glycoproteins present in the outer envelope of the virus particle (e.g., gB and gH).

[0115] In the case of HCMV, direct evaluation of immunological effector functions is difficult since the virus is strictly species specific and no animal model system is available. However, murine CMV and guinea pig CMV have been used to evaluate vaccine strategies in these host species.

[0116] A CMV vaccine that induces both protective T cell and neutralizing antibody responses has the potential to prevent infection or ameliorate CMV disease due to congenital infection or transplantation.

[0117] The first live, attenuated HCMV vaccine candidate tested in humans was based on the laboratory-adapted AD169 strain. Subsequent trials with another laboratory-adapted clinical isolate, the Towne strain, confirmed that live attenuated vaccines could elicit neutralizing antibodies, as well as CD4+ and CD8+ T lymphocyte responses. The efficacy of the Towne vaccine was assessed in a series of studies in renal transplant recipients. Although the Towne vaccine did provide a protective impact on HCMV disease it failed to prevent HCMV infection after transplantation (Plotkin SA et al., 1984 Lancet 1:528-530). Towne vaccine was also evaluated in a placebo-controlled study of seronegative mothers who had children attending group daycare where it failed to prevent these women from acquiring infection from their HCMV-infected children (Adler SP et al., 1995 J Infectious Diseases 171:26-32). An interpretation of these studies was that the Towne vaccine was overattenuated. To explore this possibility a series of genetic recombinants in which regions of the unattenuated "Toledo" strain of CMV were substituted for the corresponding regions of the Towne genome, resulting in the construction of Towne/Toledo "chimeras" that contain some, but not all, of the mutations that

contribute to the Towne vaccine attenuation (Heineman TC et al. 2006 J Infect Disease 193:1350-1360). The safety and tolerability of four Towne/Toledo "chimeras" is being tested in a Phase I trial. Long-term safety concerns about the potential risk of establishing a latent HCMV infection have hindered the further development of live, attenuated vaccines.

[0118] The leading subunit CMV vaccine candidate is based on the envelope glycoprotein, gB, (purified recombinant gB vaccine is manufactured by Sanofi-Pasteur Vaccines) due to this protein's ability to elicit high-titer, virus-neutralizing antibody responses during natural infection. The recombinant gB vaccine elicits neutralizing antibody responses and has an excellent safety profile, however, it excludes other glycoprotein targets of neutralizing antibody response and more importantly T-lymphocyte targets. The vaccine requires MF59 adjuvant to optimize immunogenicity. In the most recent trial, this vaccine provided an overall 50% efficacy for prevention of CMV infection in a Phase 2 clinical trial in young woman (Pass RF et al., 2009 N Engl J Med 360:1191-1199). Other viral proteins being evaluated as subunit vaccine candidates include pp65 and IE1, both of which elicit T-cell responses.

[0119] DNA vaccines elicit robust cellular and humoral immune responses in animals and are well suited to specificity and precision in vaccine design. DNA vaccines have been developed for CMV and have focused on gB, IE1 and pp65 proteins as the candidate target immunogens. A bivalent CMV DNA vaccine candidate (Wloch MK 2008 J Infectious Diseases 297:1634-1642), using plasmid DNA encoding pp65 and gB and a trivalent vaccine candidate (Jacobson MA 2009 Vaccine 27:1540-1548) that also includes a third plasmid encoding the IE1 gene product have been developed by Vical Vaccines (patent US 7,410,795). The trivalent DNA vaccine alone had minimal immunogenicity irrespective of route of administration. However the CMV DNA vaccine did appear to safely prime for a memory response to CMV antigens observed after administration of a live, attenuated CMV (Towne).

[0120] In a vectored vaccine approach, the gene product of interest is expressed in the context of a non-replicating (usually viral) carrier. One example of this is a canarypox vector called ALVAC developed by Virogenetics and Sanofi-Pasteur Vaccines, which is an attenuated poxvirus that replicates abortively in mammalian cells. ALVAC expressing CMV gB and ALVAC expressing pp65 (US 6,267,965) have been tested in clinical trials. ALVAC-CMV(gB)

did not induce neutralizing antibodies but did prime for higher neutralizing antibody titers after subsequent infection with the Towne strain CMV (Adler SP et al. 1999 J Infectious Diseases 180:843-846), although it did not appear to boost neutralizing antibody titers after subsequent immunization with gB subunit/MF59 vaccine (Bernstein DI et al. 2002 J Infectious Diseases 185:686-690). A canarypox vector expressing pp65, ALVAC-CMV(pp64), induced long-lasting CTL responses in all originally seronegative volunteers, at frequencies comparable to naturally seropositive individuals (Berencsi K et al. 2001 J Infectious Diseases 183:1171-1179). Another approach used to express gB as a vectored vaccine is the use of an alphavirus replicon system by AlphaVax Inc (US 7,419,674). This approach involves a propagation-defective single-cycle RNA replicon vector system derived from an attenuated strain of an alphavirus, Venezuelan Equine Encephalitis (VEE) virus, to produce virus-like replicon particles (VRPs) expressing pp65, IE1 or gB protein (Berstein et al., 2010 Vaccine 28:484-493). A two component alphavirus replicon vaccine was used to express the three CMV proteins as a soluble form of CMV gB (Towne strain) and a pp65/IE1 fusion protein (Reap EA et al. 2007 Vaccine 25:7441-7449) was found to be safe and induced high levels of neutralizing antibody and polyfunctional CD4+ and CD8+ antigen-specific T cell responses. The Geometric Mean Titre (GMT) for the high dose group was about half the GMT in 12 naturally infected, CMV seropositive individuals tested in the assay.

[0121] A novel candidate for vaccination against HCMV currently in preclinical development is the "dense body" vaccine. Dense bodies (DBs) are enveloped, replication-defective particles formed during the replication of CMVs in cell culture. They contain both envelope glycoproteins and large quantities of pp65 protein. DBs are non-infectious and immunogenic but incapable of establishing latent HCMV infection in the vaccine recipient. DBs have been shown to be capable of inducing virus neutralizing antibodies and T-cell responses in mice in the absence of viral gene expression (Pepperl S et al., 2000 J Virol 74:6132-6146 – WO 00/53729 and US 6,713,070).

IV. Pharmaceutical Compositions

[0122] The present invention provides pharmaceutical compositions comprising provided VLPs and/or provided glycoprotein variants. In some embodiments, the present invention provides a VLP and at least one pharmaceutically acceptable excipient. Such pharmaceutical compositions may optionally comprise and/or be administered in combination with one or more additional therapeutically active substances. In some embodiments, provided pharmaceutical compositions are useful in medicine. In some embodiments, provided pharmaceutical compositions are useful as prophylactic agents (*i.e.*, vaccines) in the treatment or prevention of HCMV or of negative ramifications associated or correlated with HCMV infection. In some embodiments, provided pharmaceutical compositions are useful in therapeutic applications, for example in individuals suffering from or susceptible to HCMV infection. In some embodiments, pharmaceutical compositions are formulated for administration to humans.

[0123] For example, pharmaceutical compositions provided here may be provided in a sterile injectible form (e.g., a form that is suitable for subcutaneous injection or intravenous infusion). For example, in some embodiments, pharmaceutical compositions are provided in a liquid dosage form that is suitable for injection. In some embodiments, pharmaceutical compositions are provided as powders (*e.g.* lyophilized and/or sterilized), optionally under vacuum, which are reconstituted with an aqueous diluent (e.g., water, buffer, salt solution, etc.) prior to injection. In some embodiments, pharmaceutical compositions are diluted and/or reconstituted in water, sodium chloride solution, sodium acetate solution, benzyl alcohol solution, phosphate buffered saline, etc. In some embodiments, powder should be mixed gently with the aqueous diluent (e.g., not shaken).

[0124] In some embodiments, provided pharmaceutical compositions comprise one or more pharmaceutically acceptable excipients (e.g., preservative, inert diluent, dispersing agent, surface active agent and/or emulsifier, buffering agent, etc.). Suitable excipients include, for example, water, saline, dextrose, sucrose, trehalose, glycerol, ethanol, or similar, and combinations thereof. In addition, if desired, the vaccine may contain auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants which enhance the effectiveness of the vaccines. In some embodiments, pharmaceutical compositions comprise one

or more preservatives. In some embodiments, pharmaceutical compositions comprise no preservative.

[0125] In some embodiments, pharmaceutical compositions are provided in a form that can be refrigerated and/or frozen. In some embodiments, pharmaceutical compositions are provided in a form that cannot be refrigerated and/or frozen. In some embodiments, reconstituted solutions and/or liquid dosage forms may be stored for a certain period of time after reconstitution (e.g., 2 hours, 12 hours, 24 hours, 2 days, 5 days, 7 days, 10 days, 2 weeks, a month, two months, or longer). In some embodiments, storage of VLP formulations for longer than the specified time results in VLP degradation.

[0126] Liquid dosage forms and/or reconstituted solutions may comprise particulate matter and/or discoloration prior to administration. In some embodiments, a solution should not be used if discolored or cloudy and/or if particulate matter remains after filtration.

[0127] Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In some embodiments, such preparatory methods include the step of bringing active ingredient into association with one or more excipients and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping and/or packaging the product into a desired single- or multi-dose unit.

[0128] A pharmaceutical composition in accordance with the invention may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to a dose which would be administered to a subject and/or a convenient fraction of such a dose such as, for example, one-half or one-third of such a dose.

[0129] Relative amounts of active ingredient, pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the invention may vary, depending upon the identity, size, and/or condition of the subject treated

and/or depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

[0130] Pharmaceutical compositions of the present invention may additionally comprise a pharmaceutically acceptable excipient, which, as used herein, may be or comprise solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro, (Lippincott, Williams & Wilkins, Baltimore, MD, 2006) discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional excipient medium is incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention.

[0131] In some embodiments, a pharmaceutical composition is sufficiently immunogenic as a vaccine (e.g., in the absence of an adjuvant). In some embodiments, immunogenicity of a pharmaceutical composition is enhanced by including an adjuvant. Any adjuvant may be used in accordance with the present invention. A large number of adjuvants are known; a useful compendium of many such compounds is prepared by the National Institutes of Health and can be found (NIAID Strategic Plan for Research on Vaccine Adjuvants; NIAID, 2011). See also Allison (1998, Dev. Biol. Stand., 92:3-11), Unkeless et al. (1998, Annu. Rev. Immunol., 6:251-281), and Phillips et al. (1992, Vaccine, 10:151-158). Hundreds of different adjuvants are known in the art and may be employed in the practice of the present invention. Exemplary adjuvants that can be utilized in accordance with the invention include, but are not limited to, cytokines, gel-type adjuvants (e.g., aluminum hydroxide, aluminum phosphate, calcium phosphate, etc.); microbial adjuvants (e.g., immunomodulatory DNA sequences that include CpG motifs; endotoxins such as monophosphoryl lipid A; exotoxins such as cholera toxin, E. coli heat labile toxin, and pertussis toxin; muramyl dipeptide, etc.); oil-emulsion and emulsifier-based adjuvants (e.g., Freund's Adjuvant, MF59 [Novartis], SAF, etc.); particulate adjuvants (e.g., liposomes, biodegradable microspheres, saponins, etc.); synthetic adjuvants (e.g., nonionic block copolymers, muramyl

peptide analogues, polyphosphazene, synthetic polynucleotides, etc.); and/or combinations thereof. Other exemplary adjuvants include some polymers (e.g., polyphosphazenes; described in U.S. Patent 5,500,161), Q57, QS21, squalene, tetrachlorodecaoxide, etc. Pharmaceutically acceptable excipients have been previously described in further detail in the above section entitled "Pharmaceutical Compositions."

V. Administration

[0132] Provided compositions and methods of the present disclosure are useful for prophylaxis and/or treatment of HCMV infection in a subject, including human adults and children. In general however they may be used with any animal. In certain embodiments, the methods herein may be used for veterinary applications, e.g., canine and feline applications. If desired, the methods herein may also be used with farm animals, such as ovine, avian, bovine, porcine and equine breeds.

[0133] As used herein, the terms "subject," "individual" or "patient" refer to a human or a non-human mammalian subject. The individual (also referred to as "patient" or "subject") being treated is an individual (fetus, infant, child, adolescent, or adult) suffering from a disease, for example, HCMV infection. In some embodiments, the subject is at risk for HCMV infection. In some embodiments, the subject is an immunosuppressed subject. For example, in some embodiments, the immunosuppressed subject is selected from the group consisting of an HIV-infected subject, an AIDS patient, a transplant recipient, a pediatric subject, and a pregnant subject. In some embodiments, the subject has been exposed to HCMV infection. In some embodiments, the subject is a human.

[0134] Compositions described herein will generally be administered in such amounts and for such a time as is necessary or sufficient to induce an immune response. Dosing regimens may consist of a single dose or a plurality of doses over a period of time. The exact amount of an immunogenic composition (e.g., VLP) to be administered may vary from subject to subject and may depend on several factors. Thus, it will be appreciated that, in general, the precise dose used will be as determined by the prescribing physician and will depend not only on the weight of the subject and the route of administration, but also on the age of the subject and the severity

of the symptoms and/or the risk of infection. In certain embodiments a particular amount of a VLP composition is administered as a single dose. In certain embodiments, a particular amount of a VLP composition is administered as more than one dose (e.g., 1-3 doses that are separated by 1-12 months). In certain embodiments a particular amount of a VLP composition is administered as a single dose on several occasions (e.g., 1-3 doses that are separated by 1-12 months).

[0135] In some embodiments, a provided composition is administered in an initial dose and in at least one booster dose. In some embodiments, a provided composition is administered in an initial dose and two booster doses. In some embodiments, a provided composition is administered in an initial dose and three booster doses. In some embodiments, a provided composition is administered in an initial dose and four booster doses. In some embodiments, a provided composition is administered in an initial dose and in at least one booster dose about one month, about two months, about three months, about four months, about five months, or about six months following the initial dose. In some embodiments, a provided composition is administered in a second booster dose about six months, about seven months, about eight months, about nine months, about ten months, about eleven months, or about one year following the initial dose. In some embodiments, a provided composition is administered in a booster dose every 1 year, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, or 10 years.

[0136] In certain embodiments, provided compositions may be formulated for delivery parenterally, e.g., by injection. In such embodiments, administration may be, for example, intravenous, intramuscular, intradermal, or subcutaneous, or via by infusion or needleless injection techniques. In certain embodiments, the compositions may be formulated for peroral delivery, oral delivery, intranasal delivery, buccal delivery, sublingual delivery, transdermal delivery, transcutaneous delivery, intraperitoneal delivery, intravaginal delivery, rectal delivery or intracranial delivery.

Examples

[0137] The following examples describe some exemplary modes of making and practicing certain compositions that are described herein. It should be understood that these examples are for illustrative purposes only and are not meant to limit the scope of the compositions and methods described herein.

Example 1: Construction of DNA Expression Plasmids

[0138] This Example describes development of expression plasmids and constructs for expression of recombinant HCMV gene sequences (e.g., gB, gB-G, gH-G, and Gag/pp65 fusion gene sequences). A standard expression plasmid generally consists of a promoter sequence of mammalian origin, an intron sequence, a PolyAdenylation signal sequence (PolyA), a pUC origin of replication sequence (pUC – pBR322 is a colE1 origin/site of replication initiation and is used to replicate plasmid in bacteria such as E Coli (DH5α)), and an antibiotic resistance gene as a selectable marker for plasmid plaque selection. Within the plasmid following the Intron are a variety of restriction enzyme sites that can be used to splice in a gene or partial gene sequence of interest.

[0139] The Propol II expression plasmid contains the pHCMV (early promoter for HCMV), a Beta-Globin Intron (BGL Intron), a rabbit Globin polyAdenylation signal sequence (PolyA), a pUC origin of replication sequence (pUC – pBR322 is a colE1 origin/site of replication initiation and is used to replicate plasmid in bacteria such as E. coli (DH5α)), and an ampicillin resistance gene β-lactamase (Amp R – selectable marker for plasmid confers resistance to ampicillin) (100 μg/ml) (Figure 1A).

[0140] Figure 1B depicts exemplary recombinant expression plasmids. For example, to develop a pHCMV-Gag MMLV expression construct ("MLV-Gag"), a complementary DNA (cDNA) sequence encoding a Gag polyprotein of MMLV (Gag without its C terminus Pol sequence) was cloned in a Propol II (pHCMV) expression vector. To develop a gB expression construct ("gB"), the full-length sequence of gB was codon-optimized for human expression (GenScript) and was cloned in a Propol II expression vector including the extracellular portion, transmembrane domain (TM) and cytoplasmic portion (Cyto) of gB. To develop a gH-G

expression construct ("gH-G"), the truncated sequence of gH encoding only the extracellular portion was fused together with TM and Cyto portions of VSV-G and codon-optimized for human expression (GenScript) and cloned in a Propol II expression vector. Similarly, to develop a gB-G expression construct ("gB-G"), the truncated sequence of gB encoding only the extracellular portion was fused together with TM and Cyto portions of VSV-G and codon-optimized for human expression (GenScript) and cloned in a Propol II expression vector . To develop a Gag/pp65 expression construct ("Gag/pp65"), a sequence encoding the Gag polyprotein of MMLV (Gag without its C terminus Pol sequence) was fused with the full-length sequence of pp65 and codon-optimized for human expression (GenScript) and cloned in a Propol II expression vector.

[0141] DNA plasmids were amplified in competent E. coli (DH5α) and purified with endotoxin-free preparation kits according to standard protocols.

Example 2: Production of Virus-Like Particles (VLPs)

[0142] This Example describes methods for production of virus-like particles (VLPs) containing various recombinant HCMV antigens described in Example 1.

[0143] HEK 293T cells (ATCC, CRL-11268) were transiently transfected using calcium phosphate methods with an MMLV-Gag DNA expression plasmid and co-transfected with either a gB or a gB-G (data not shown) or a gH-G DNA expression plasmid. Alternatively cells were transfected with a Gag/pp65 DNA expression plasmid and co-transfected with either a gB or a gB-G (data not shown) or a gH-G DNA expression plasmid. It will be appreciated that cells can be transfected with an MMLV-Gag DNA expression plasmid and cotransfected with both a gB and a gH-G or gB-G and a gH-G DNA expression plasmid. Expression of various HCMV antigens by the HEK 293 cells was confirmed by flow cytometry (Figure 2A). After 48 to 72 hours of transfection, supernatants containing the VLPs were harvested and filtered through 0.45 μm pore size membranes and further concentrated and purified by ultracentrifugation through a 20% sucrose cushion in a SW32 Beckman rotor (25,000 rpm, 2 hours, 4°C). Pellets were resuspended in sterile endotoxin-free PBS (GIBCO) to obtain 500 times concentrated VLP stocks. Total protein was determined on an aliquot by a Bradford assay quantification kit

(BioRad). Purified VLPs were stored at -80°C until used. Each lot of purified VLPs was analyzed for the expression of MMLV-Gag, gB, gH-G and/or MMLV-Gag/pp65 fusion protein by SDS-Page and Western Blot with specific antibodies to gB (CH 28 mouse monoclonal antibody to gB; Virusys Corporation; Pereira, L et al. 1984 Virology 139:73-86), gH-G (mouse monoclonal antibody to Anti-VSV-G tag antibody P5D4; Abcam plc) and pp65 (CH12 mouse monoclonal antibody to UL83/pp65; Virusys Corporation; Pereira, L. et al. 1982 Infect Immun 36: 924-932) (Figure 2B). Antibodies were detected using enhanced chemilluminescence (ECL).

Example 3: Physico-chemical Characterization of Virus-Like Particles (VLPs)

[0144] Physico-chemical analysis of VLPs included particle size determination and Polydispersity Index assessment using a Malvern Instrument Zeta-Sizer Nano series (ZEN3600). Exemplary results obtained from nanosizing analysis are shown in Figures 3A and 3B. An exemplary VLP composition (gH-G/pp65 bivalent VLP composition) was produced in two different labs using the same recombinant expression vectors and both VLP preparations gave an average particle size of 150-180 nm in diameter. This is consistent with the size of a CMV virion which is reported to be 150-200 nm in size (1997 J Pathol 182: 273-281). The low Polydispersity Index (PdI) of 0.214-0.240 indicates product homogeneity or a narrow size distribution.

Example 4: Immunogenicity and Neutralization Activity of VLPs in Mice

[0145] VLP compositions prepared as described in Example 2 were tested in female BALB/C mice 6-8 weeks old (minimum 6 animals per test group). Mice were immunized intraperitoneally with 200 µl of VLP compositions twice, once on day 0 (Prime) and once on day 56 (week 8 Boost). Mice were treated with 10 µg, 25 µg or 50 µg (total protein) of a bivalent gB/Gag/pp65, a bivalent gH-G/Gag/pp65 or a trivalent gB/gH-G/Gag/pp65 VLP composition. To assess humoral immune responses in mice, blood was collected from all mice in the study groups pre-immunization and then post-1st and -2nd immunizations at 0, 2, 3, 4, 6, 8, 9, 10, 12 and 14 weeks. The study design is summarized in Table 1.

Table 1

Test Article #	Dose	Test Article Description	Immunization Schedule (weeks)
1	50 µg	pp65/gB bivalent VLPs	0, 8
2	25 µg	pp65/gB bivalent VLPs	0, 8
3	10 µg	pp65/gB bivalent VLPs	0, 8
4	50 µg	pp65/gH-G bivalent VLPs	0, 8
5	25 µg	pp65/gH-G bivalent VLPs	0, 8
6	10 µg	pp65/gH-G bivalent VLPs	0, 8
7	50 µg	Trivalent pp65/gB/gH-G VLPs	0, 8
8	25 µg	Trivalent pp65/gB/gH-G VLPs	0, 8
9	10 µg	Trivalent pp65/gB/gH-G VLPs	0, 8

[0146] Enzyme-linked Immunosorbent Assay (ELISA) was performed using commercially available ELISA plates (IBL International) coated with lysates derived from MRC-5 cells infected with HCMV strain AD169. Crude commercial HCMV lysate, which contains all CMV related antigens and is useful to detect IgG immune responses, was used as a positive control to determine mouse serum HCMV IgG content by ELISA. Serial dilutions of mouse sera (dilution in TBS-T/BSA/DMEM 10% FCS) were incubated with the coated plates for 2 hours at room temperature. After the plates were washed, anti-mouse Horse Radish Peroxidase (HRP) conjugated secondary antibody was added at a dilution of 1/10,000 and incubated for 1 hour, followed by the addition of Tetramethylbenzidine (TMB) substrate solution. The reaction was stopped by addition of HCL 1N and absorbance was read at 450 nm in an ELISA microwell plate reader. Figure 4 shows evidence of persistent antibodies and strong boosting of mice after 2nd immunization at 8 weeks for each of the bivalent and the trivalent VLP compositions.

[0147] Neutralizing antibody responses to HCMV were determined using a microneutralization assay. A standard amount of HCMV strain VR1814 (an endothelial cell-tropic CMV strain - Revello MG et al. J Gen Virol 82:1429-1438) was diluted with infection medium (DME containing 1% heat-inactivated FBS, 1% amino acid mixture, 1 % Penicillin-Streptomycin and Ca^{+2} and Mg^{+2} free PBS), and added to an equal volume of serial dilutions of heat-inactivated test serum and incubated for 1 hour at 37°C on a rotator. The serum/HCMV mixtures were added to human foreskin fibroblasts (HFF) or retinal pigmented epithelial cells (ARPE-19 cells) grown on coverslips in 24 well tissue culture plates and incubated for 2 hours at 37°C, 5% CO_2. The plates were washed with PBS twice and then cultured for 48 hours at 37°C, 5% CO_2. Cells were fixed with 4% paraformaldehyde, reacted with an anti-IE1 monoclonal antibody (CH160 Mouse Monoclonal antibody to IE1/2 or CH443 Monoclonal antibody to IE1; Virusys Corporation) for 1 hour at room temperature followed by FITC-labelled goat anti-mouse antibody for 45 minutes at room temperature. The number of cells expressing IE1 was determined by fluorescent microscopy. Pooled mouse sera (1:6 dilution) was tested for neutralizing activity at each bleed time point (0, 3, 6, 8 and 9 weeks) in duplicate. Figure 5 shows induction of neutralizing antibodies in mice (assayed in fibroblast cells) after 2nd immunization at 8 weeks for each of the bivalent and the trivalent VLP compositions. Figure 6 shows induction of neutralizing antibodies in mice (assayed in epithelial cells) after 2nd immunization at 8 weeks for each of the bivalent VLP compositions.

[0148] In another study, monovalent gB-G VLP compositions prepared as described in Example 2 were tested in female BALB/C mice 6-8 weeks old (minimum 8 animals per test group). Mice were immunized intraperitoneally with 200 µl of VLP compositions twice, once on day 0 (Prime) and once on day 56 (week 8 Boost). Mice were treated with equivalent amounts of 20 µg of gB content (determined by ELISA) per injection. To assess humoral immune responses in mice, blood was collected from all mice in the study groups pre-1st immunization and then post-1st immunization at 3 and 6 weeks and pre-2nd immunization at 8 weeks and then post 2nd immunization at 9 weeks from study start. The study design is summarized in Table 2.

Table 2

Test Article #	Dose	Test Article Description	Immunization Schedule (weeks)

1	20 μg	gB-G monovalent VLPs	0, 8
2	20 μg	Recombinant gB	0, 8

[0149] Neutralizing antibody responses to HCMV were determined using a microneutralization assay in fibroblast cells based on a green fluorescent protein (GFP) - expressing HCMV virus (TB40) and flow cytometric analysis of infected (GFP+) HFF cells. To assess the presence of neutralizing antibodies in serum samples, the serum was pre-incubated with GFP-expressing HCMV for a period of time sufficient for neutralizing antibodies to reduce the infectivity of HCMV. Serial dilutions of the pre-incubated mixture of serum and HCMV were used to contact a host cell (fibroblast or epithelial) susceptible to infection by HCMV. The number of cells that express the reporter gene construct (GFP) were determined by flow cytometry to calculate the infectious titer of the virus preparation. Figure 7 depicts Neutralizing Antibody titers for mice immunized twice at 0 and 8 weeks with monovalent gB-G VLPs versus recombinant gB. Sera collected pre- and post-immunizations as described were pooled and tested relative to a positive control CMV hyperglobulin, Cytogam™, in the presence of 10% guinea pig complement. As shown in Figure 7, the monovalent gB-G VLP composition elicited a more rapid and potent neutralization of fibroblast cell infection than that elicited by a recombinant gB protein.

[0150] As can be seen (or as will be appreciated by those skilled in the art having read the present specification), the data demonstrate, among other things, surprisingly good activity of VLPs, such as by VLPs that include gB-G as compared with recombinant gB.

[0151] In another study, bivalent gB/Gag/pp65 VLP compositions prepared as described in Example 2 were tested in female BALB/C mice 6-8 weeks old (minimum 4 animals per test group). Mice were immunized intraperitoneally with 200 μl of VLP compositions twice, once on day 0 (Prime) and once on day 56 (week 8 Boost). Mice were treated with bivalent gB/Gag/pp65 VLPs and splenocytes were collected 14 days later. Enriched $CD8^+$ T cells were stimulated (1:5 ratio) with splenocytes transfected for 24 hours with plasmid encoding Gag/pp65 or Gag to determine frequencies of CTLs directed against pp65 or Gag. CTL frequencies were determined

based on CFSE decay, gating on $CD3^+$ $CD8^+$ T cells (Figure 8A). The scatter plot shows the frequency of proliferating, pp65-specific CTLs after subtracting responses directed against Gag (Figure 8B). As depicted in Figure 8, bivalent gB/Gag/pp65 VLPs elicited pp65-specific CTLs in immunized mice.

Example 5: Immunogenicity and Neutralization Activity of VLPs in Rabbits

[0152] Bivalent gB/Gag/pp65 and gH-G/Gag/pp65 VLP compositions prepared as described in Example 2 were tested in New Zealand White rabbits 6-8 weeks old (minimum 6 animals per test group). Rabbits were immunized intramuscularly with 0.5 ml of VLP compositions three times, once on day 0 (Prime) and once on day 56 (week 8 Boost) and once on day 168 (week 24 Boost). Rabbits were treated with 25 μg or 50 μg or 100 μg (total protein) of either a bivalent gB/Gag/pp65, a bivalent gH-G/Gag/pp65 or a trivalent gB/gH-G/Gag/pp65 (bivalent gB/Gag/pp65 and gH-G/Gag/pp65 mixed together at a 1:1 ratio) VLP composition. To assess humoral immune responses in rabbits, blood was collected from all rabbits in the study groups pre-1^{st} immunization and then post-1^{st} immunization at 2, 4, 6 and 8 weeks and post-2^{nd} immunization at 10, 13, 16, 20 and 24 weeks from study start and then post-3^{rd} immunization at 26 and 28 weeks from study start. The study design is summarized in Table 3.

Table 3

Test Article #	Dose	Test Article Description	Immunization Schedule (weeks)
1	100 μg	gB/pp65 bivalent VLPs	0, 8, 24
2	50 μg	gB/pp65 bivalent VLPs	0, 8, 24
3	25 μg	gB/pp65 bivalent VLPs	0, 8, 24
4	100 μg	gH-G/pp65 bivalent VLPs	0, 8, 24
5	25 μg	gH-G/pp65 bivalent VLPs	0, 8, 24

6	100 μg each	gB/pp65 bivalent VLPs + gH-G/pp65 bivalent VLPs (1:1 ratio)	0, 8, 24
7	25 μg each	gB/pp65 bivalent VLPs + gH-G/pp65 bivalent VLPs (1:1 ratio)	0, 8, 24

[0153] Individual rabbit sera was tested for reactivity against recombinant gB antigen by ELISA (plotted on left axis Figure 9A). Neutralizing antibody responses to HCMV was determined using a microneutralization assay in fibroblast cells based on a GFP-expressing CMV virus (TB40) and flow cytometric analysis of infected (GFP+) HFF cells as described in Example 4 (plotted on right axis Figure 9B). Rabbit sera collected pre- and post-immunizations as described were pooled and tested for neutralizing activity in the presence of complement against HCMV expressing GFP in HFF fibroblasts relative to a positive control CMV hyperglobulin, Cytogam™. Endpoint titers are plotted and represent a 50% reduction in CMV-infected cells relative to matched pre-immunization sera (plotted on left axis Figure 9A). 50,000 cells were collected during flow cytometric analysis of infected (GFP^+) cells (plotted on right axis Figure 9B). As shown in Figure 9, the bivalent gB/Gag/pp65 VLP composition elicited high titer binding (A) and high titer neutralizing antibody (B) responses in rabbits against fibroblast cell infection.

[0154] Pooled rabbit sera was also tested for neutralizing antibody responses to HCMV using a microneutralization assay in epithelial cells based on a GFP-expressing HCMV virus (Towne TS15-rR) and flow cytometric analysis of infected (GFP+) ARPE-19 cells. Rabbit sera collected pre- and post-immunizations as described were pooled and tested for neutralizing activity in the presence of complement against HCMV expressing GFP in ARPE-19 epithelial cells relative to a positive control CMV hyperglobulin, Cytogam™. As shown in Figure 10, surprisingly, the combination of bivalent gB/Gag/pp65 and bivalent gH-G/Gag/pp65 VLP composition elicited a synergistic and more rapid neutralizing antibody response in rabbits against epithelial cell infection, relative to gB/Gag/pp65 or gH/Gag/pp65.

[0155] As can be seen (or as will be appreciated by those skilled in the art having read the present specification), the data demonstrate, among other things, surprisingly good activity of VLPs, such as by a combination of VLPs that include gB/Gag/pp65 and VLPs that include gH-G/Gag/pp65, even as compared with VLPs that include gB/Gag/pp65 or VLPs that include gH-G/Gag/pp65.

Example 6: Scaled-Up Production and Purification of Virus-Like Particles (VLPs)

[0156] VLPs were produced and purified as follows. CHO cells were transfected at a cell density between 1.5E06 to 2.0E06 cells/mL with plasmids of choice (prepared as described in Example 1) at predetermined concentrations and ratios. Stuffer DNA was added to make total DNA concentration up to 1 μg/mL cell culture. The plasmids used for transfection were first purified by MaxiPrep or GigaPrep plasmid purification kits (Qiagen). The PEIMAX used for transfection to deliver DNA to the cells was provided at a ratio of 6:1 (PEI: DNA wt/wt). The cells were cultured for 72 hours, and then the cultures were centrifuged at 4000 rpm for 20 minutes, using rotor JS-4.2A by Beckman Coulter, in 1 L bottles. The supernatant was filtered through 0.8/0.45 μm filter (AcroPak™ 500 Capsule, Pall). The filtered supernatant was then concentrated by Tangential Flow Filtration (TFF) and diafiltered against histidine-containing buffer. The diafiltered material was loaded onto an anion exchange chromatography column (AEX) where the flowthrough was collected. The flowthrough was then sterile filtered through 0.45 μm to be aliquoted in different volumes.

[0157] The TFF procedure involved overnight sanitization of two TFF membranes (Pellicon™ 2 Mini 500 kDa cutoff, 0.1 m^2 surface area) in 0.5 M NaOH by fixing them in parallel in a stainless-steel housing. After running water to neutral pH on the retentate as well as on the permeate side, the phosphate buffer (PBS) was used to equilibrate the membrane. The filtered supernatant was then loaded into the TFF retentate recirculation loop. The starting inlet pressure was 10.5 psi at a permeate flow rate of 400 mL/min that reduced to about 200 mL/min at the end of concentration. After concentration to about 10 to 20 times, diafiltration with 5 volumes of 20 mM Histidine +150 mM NaCl, pH 5.5 was done. The diafiltered material was collected and rinsed with an equal volume of his-buffer after recirculating for another 5 minutes with permeate flow closed. The retentate was then collected and pooled with the previously collected retentate.

To maintain the functionality of the membranes, the membranes were rinsed with PBS for 10 minutes; with 0.5 M NaOH for 40 minutes (after flushing 200 mL to waste through permeate and retentate); and finally with water to neutral pH in retentate and permeate. The membranes were then stored in 0.1 M NaOH solution in a refrigerator.

[0158] AEX column chromatography was used to reduce DNA content, using a 20 mL HiLoad™ 16/10 Sepharose™ HP column at a flow rate of 1.6 mL/min for equilibration with equilibration buffer (20 mM Histidine +150 mM NaCl , pH 5.5), and a flow rate of 3.2 mL/min for the loading, washing and stripping steps. Cleaning procedures were performed at 0.8 mL/min. A chart recorder was used to monitor the UV absorbance at 280 nm. A Gradifrac™ chromatography system (GE Healthcare) was used, which was sanitized before use. First ethanol (20% v/v present in the column as a storage buffer) was removed from the column using 5 column volumes of Super Q™ Water (low endotoxins). The equilibrium buffer was passed for 5 column volumes, followed by 5 column volumes of stripping buffer (20 mM Histidine +1000 mM NaCl , pH 5.5) to condition the column. The equilibration buffer was passed for 5 column volumes again to prepare the column for loading. Loading was performed at 3.2 mL/min, after which the column was washed with 5 column volumes of equilibration buffer, or until the base line was observed. The flowthrough was collected from the onset of the UV peak until the drop of UV peak to about 10% of the maximum peak height of the UV absorbance during the loading of the material. The column was then stripped by 5 column volumes of stripping buffer (20 mM Histidine +1000 mM NaCl , pH 5.5) and the peak was collected. This was followed by 5 column volumes of another stripping buffer (20 mM Histidine +2000 mM NaCl , pH 5.5) to remove any strongly bound proteins and nucleic acids and the peak was collected. The column was then cleaned with 1M NaOH to remove any precipitated proteins at 0.8 mL/min for 4 column volumes. The column was then rinsed with water at 0.8 ml/min to neutral pH (normally 4-5 column volumes). The column was then passed by 20% ethanol (4 column volumes at 0.8 mL/min) to remove any lipoproteins or lipids (2 column volumes). At this stage the column was either stored or rinsed with water (4 column volumes) to restart the cycle for a second batch.

[0159] Figure 11 depicts images of a purified gB-G monovalent VLP produced from CHO cells and then subjected to TFF (Figure 11A) and AEX (Figure 11B) purification methods followed by negative staining Electron Microscopy analytical methods. As shown in Figure 11,

intact gB-G monovalent VLPs are present after TFF (Figure 11A) and AEX purification (Figure 11B).

Example 7: Immunogenicity and Neutralization Activity of Purified VLPs in Rabbits

[0160] Monovalent gB-G VLP compositions prepared as described in Example 6 were tested in New Zealand White rabbits 6-8 weeks old (minimum 6 animals per test group). Rabbits were immunized intramuscularly with 0.5 ml (50 μg gB content) of VLP compositions three times, once on day 0 (Prime) and once on day 56 (week 8 Boost) and once on day 168 (week 24 Boost). To assess humoral immune responses in rabbits, blood was collected from all rabbits in the study groups pre-1st immunization and then post-1st immunization at 2, 4, 6 and 8 weeks and post-2nd immunization at 10, 13, 16, 20 and 24 weeks from study start. The study design is summarized in Table 4.

Table 4

Test Article #	Dose	Test Article Description	Immunization Schedule (weeks)
1	50 μg gB content	gB-G monovalent VLPs (TFF & AEX purified)	0, 8, 24

[0161] Figure 12 depicts the potent neutralization of fibroblast cell infection that was elicited by serum from rabbits immunized with TFF/AEX purified CHO cell-produced gB-G monovalent VLPs ("gB eVLPs" in Figure 12). This neutralization was superior to that achieved with a positive control CMV hyperglobulin, Cytogam™. Figure 12 also includes published neutralization titer data for Towne Vaccine and adjuvanted gB subunit vaccine (gB+MF59™) (Cui X et al. 2008 Vaccine 26:5760-5766).

[0162] Figure 13 illustrates potent neutralization of epithelial cell infection that was elicited by serum from rabbits immunized with TFF/AEX purified CHO cell-produced gB-G monovalent VLPs ("gB eVLPs" in Figure 13). This neutralization was comparable to that achieved with a positive control CMV hyperglobulin, Cytogam™. Figure 13 also includes

published neutralization titer data for Towne Vaccine and adjuvanted gB subunit vaccine (gB+MF59™) (Cui X et al. 2008 Vaccine 26:5760-5766).

[0163] Figure 14 depicts the avidity index of antibodies elicited in rabbits immunized with TFF/AEX purified CHO cell-produced gB-G monovalent VLP compositions. Pooled rabbit sera and a positive control CMV hyperglobulin, Cytogam™ were diluted 1:600,000 and tested against full length recombinant gB antigen by ELISA in the presence or absence of 5M urea. Antibody avidity was determined as previously described (Marshall BC and Adler S 2003 Viral Immunol 16:491-500). As shown in Figure 14, a rapid induction of high avidity neutralizing antibodies was elicited in rabbits by immunization with TFF/AEX purified CHO cell-produced gB-G monovalent VLPs. Maximal antibody avidity was achieved after two gB-G VLP immunizations.

Other Embodiments

[0164] Other embodiments of the disclosure will be apparent to those skilled in the art from a consideration of the specification or practice of the disclosure disclosed herein. It is intended that the specification and examples be considered as exemplary only, with the true scope of the disclosure being indicated by the following claims.

BRIEF DESCRIPTION OF SEQUENCE LISTING

SEQ ID NO:1 depicts an MMLV Gag Amino Acid Sequence.

SEQ ID NO:2 depicts an MMLV Gag Nucleotide Sequence.

SEQ ID NO:3 depicts a Codon Optimized MMLV Gag Nucleotide Sequence.

SEQ ID NO:4 depicts an MMLV Gag – CMV pp65 Amino Acid Sequence.

SEQ ID NO:5 depicts an MMLV Gag – CMV pp65 Nucleotide Sequence.

SEQ ID NO:6 depicts a Codon Optimized MMLV Gag – CMV pp65 Nucleotide Sequence.

SEQ ID NO:7 depicts an HCMV gB Amino Acid Sequence.

SEQ ID NO:8 depicts an HCMV gB Nucleotide Sequence.

SEQ ID NO:9 depicts a Codon Optimized HCMV gB Nucleotide Sequence.

SEQ ID NO:10 depicts an HCMV gB-G Amino Acid Sequence.

SEQ ID NO:11 depicts an HCMV gB – G Nucleotide Sequence.

SEQ ID NO:12 depicts a Codon Optimized HCMV gB – G Nucleotide Sequence.

SEQ ID NO:13 depicts an HCMV gH Amino Acid Sequence.

SEQ ID NO:14 depicts an HCMV gH Nucleotide Sequence.

SEQ ID NO:15 depicts a Codon Optimized HCMV gH Nucleotide Sequence.

SEQ ID NO:16 depicts an HCMV gH – G Amino Acid Sequence.

SEQ ID NO:17 depicts an HCMV gH – G Nucleotide Sequence.

SEQ ID NO:18 depicts a Codon Optimized HCMV gH – G Nucleotide Sequence.

SEQ ID NO:19 depicts a Propol II Expression Plasmid Nucleotide Sequence.

SEQ ID NO:20 depicts an HCMV gH – HCMV gB TM/CTD Nucleotide Sequence.

SEQ ID NO:21 depicts a Codon Optimized MMLV Gag Nucleotide Sequence.

SEQ ID NO:22 depicts a Codon Optimized MMLV Gag – CMV pp65 Nucleotide Sequence.

CLAIMS:

1. A virus like particle (VLP) comprising:

a fusion protein comprising an N-terminal portion of a gag protein found in a murine leukemia virus (MLV) fused upstream of a pp65 protein found in human cytomegalovirus (HCMV) wherein the N-terminal portion of the gag protein comprises an amino acid sequence that is at least 85% identical to SEQ ID NO:1 over its full length; and

a polypeptide comprising a glycoprotein (gB) protein found in HCMV.

2. The VLP of claim 1, wherein the N-terminal portion of the gag protein comprises the amino acid sequence of SEQ ID NO:1.

3. The VLP of claim 1 or 2, wherein the polypeptide comprising the gB protein comprises the amino acid sequence of SEQ ID NO:7.

4. The VLP of claim 1 or 2, wherein the polypeptide comprising the gB protein comprises an amino acid sequence that is at least 85% identical to SEQ ID NO:7 over its full length.

5. A VLP comprising:

a fusion protein comprising an amino acid sequence at least 85% identical to SEQ ID NO:4 over its full length; and

a polypeptide comprising an amino acid sequence at least 85% identical to SEQ ID NO:7.

6. The VLP of claim 5, wherein the fusion protein comprises the amino acid sequence of SEQ ID NO:4, and the polypeptide comprises the amino acid sequence of SEQ ID NO:7.

7. A pharmaceutical composition comprising the VLP of any one of claims 1 to 6, and a pharmaceutically acceptable carrier.

Date reçue/date received 2021-10-22

8. The pharmaceutical composition of claim 7, further comprising an adjuvant selected from the group consisting of a cytokine, a gel-type adjuvant, a microbial adjuvant, an oil-emulsion adjuvant, an emulsifier-based adjuvant, a particulate adjuvant, a synthetic adjuvant, a polymer adjuvant, and a combination thereof.

9. The pharmaceutical composition of claim 7 or 8, for use in reducing frequency or severity or delaying onset of symptoms of HCMV infection in a subject.

10. The pharmaceutical composition of claim 9, wherein the subject is an immunosuppressed subject.

11. The pharmaceutical composition of claim 10, wherein the immunosuppressed subject is selected from the group consisting of an HIV-infected subject, an AIDS patient, a transplant recipient, a pediatric subject, and a pregnant subject.

12. The pharmaceutical composition of any one of claims 9 to 11, wherein the subject has been exposed to HCMV.

13. The pharmaceutical composition of any one of claims 9 to 12, wherein the subject is a human.

14. Use of the pharmaceutical composition of claim 7 or 8, to reduce the frequency or severity or to delay onset of symptoms of HCMV infection in a subject.

15. The use of claim 14, wherein the subject is an immunosuppressed subject.

16. The use of claim 15, wherein the immunosuppressed subject is selected from the group consisting of an HIV-infected subject, an AIDS patient, a transplant recipient, a pediatric subject, and a pregnant subject.

Date reçue/date received 2021-10-22

17. The use of any one of claims 14 to 16, wherein the subject has been exposed to HCMV.

18. The use of any one of claims 14 to 17, wherein the subject is a human.

19. Use of the VLP of any one of claims 1 to 6, in the manufacture of a medicament for use in reducing frequency or severity or delaying onset of symptoms of HCMV infection.

20. A method for the production of a VLP, the method comprising:

co-transfecting a host cell with a vector comprising a nucleotide sequence encoding an MLV gag-HCMV pp65 fusion protein, wherein the gag protein is fused upstream of the pp65 protein and wherein the nucleotide sequence is at least 85% identical to SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:22 over its full length, and a vector comprising a nucleotide sequence encoding a gB protein found in HCMV wherein the nucleotide sequence is at least 85% identical to SEQ ID NO:8 or SEQ ID NO:9 over its full length; and

cultivating the host cell in a suitable medium under conditions allowing the expression of the proteins encoded by the vectors.

21. The method of claim 20, wherein the vector comprising a nucleotide sequence encoding an MLV gag-HCMV pp65 fusion protein comprises a polynucleotide sequence of SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:22.

22. The method of claim 20 or 21, wherein the vector comprising a nucleotide sequence encoding a gB polypeptide found in HCMV comprises a polynucleotide sequence of SEQ ID NO:8 or SEQ ID NO:9.

Date reçue/date received 2021-10-22

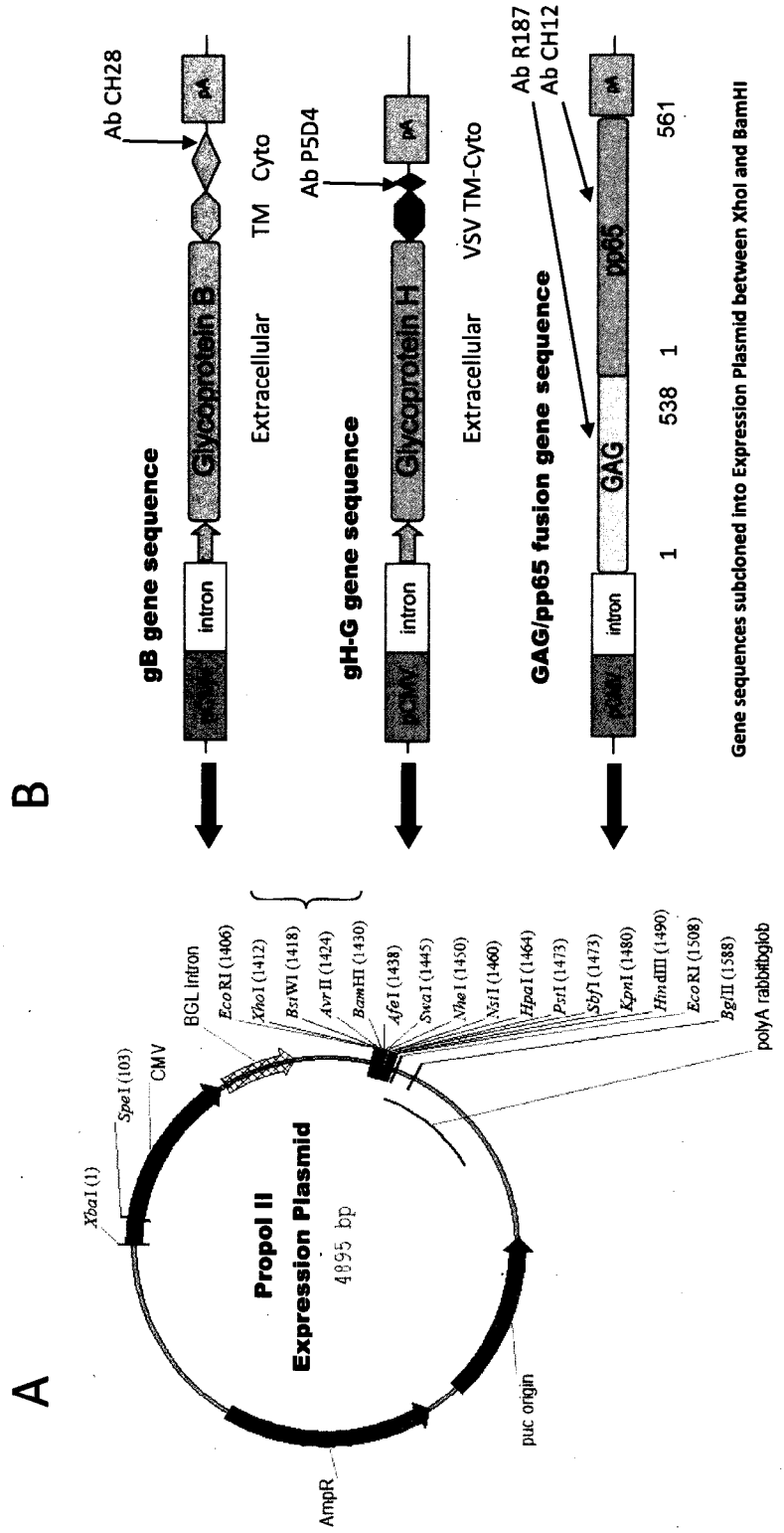

A
Propol II
Expression Plasmid
4895 bp
XbaI (1)
SpeI (103)
CMV
BGL Intron
EcoRI (1406)
XhoI (1412)
BstW1 (1418)
AvrII (1424)
BamHI (1430)
AfeI (1438)
SwaI (1445)
NheI (1450)
NsiI (1460)
HpaI (1464)
PstI (1473)
SbfI (1473)
KpnI (1480)
HindIII (1490)
EcoRI (1508)
BglII (1588)
polyA rabbitbglob
puc origin
AmpR
B
gB gene sequence
Glycoprotein B
intron
Extracellular
TM
Cyto
Ab CH28
gH-G gene sequence
Glycoprotein H
Extracellular
VSV TM-Cyto
Ab P5D4
GAG/pp65 fusion gene sequence
GAG
pp65
Ab R187
Ab CH12
1
538
1
561
Gene sequences subcloned into Expression Plasmid between XhoI and BamHI

Figure 1

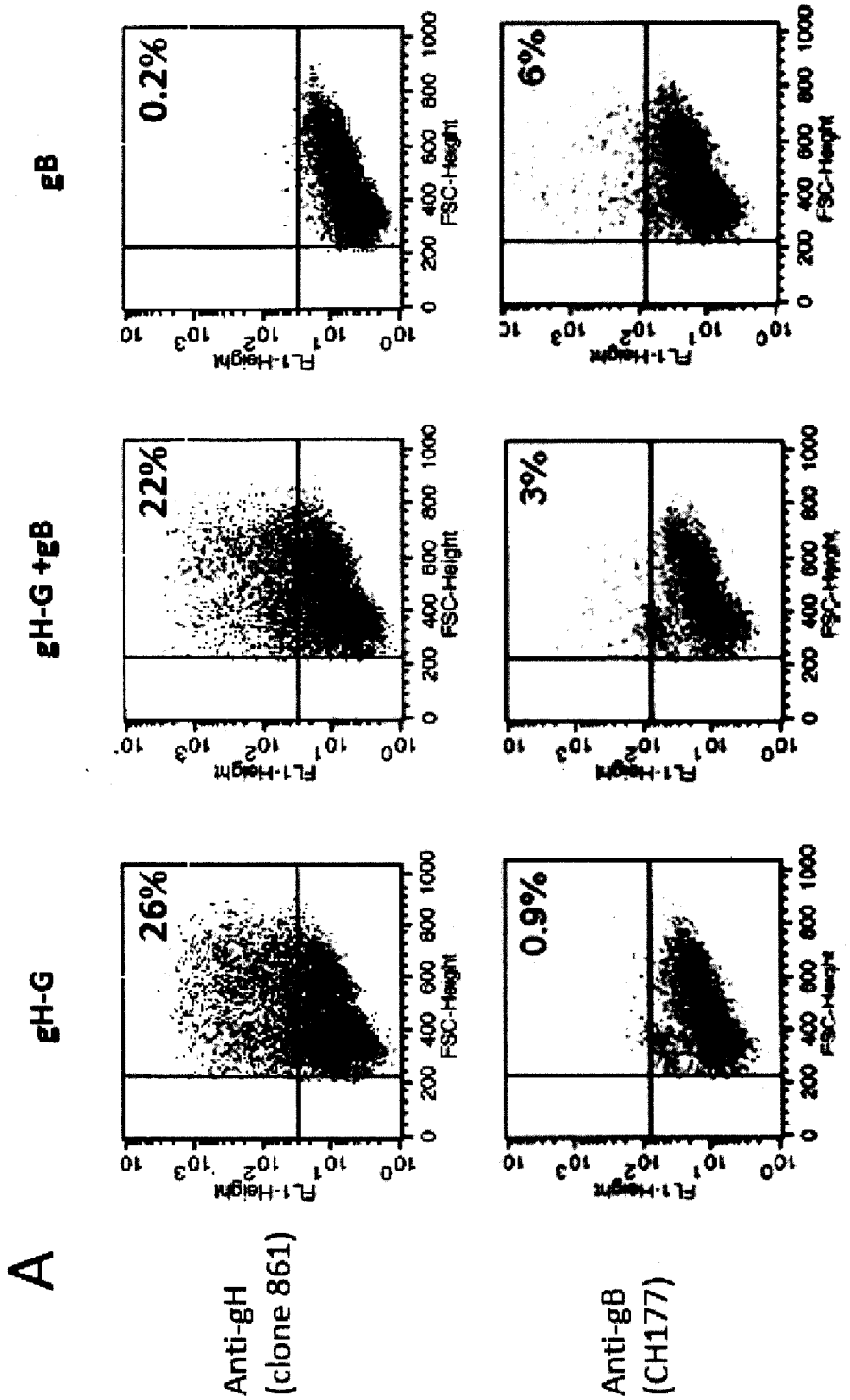

A
gH-G
gH-G +gB
gB
Anti-gH
(clone 861)
Anti-gB
(CH177)
26%
22%
0.2%
0.9%
3%
6%
FSC-Height
FL1-Height

Figure 2

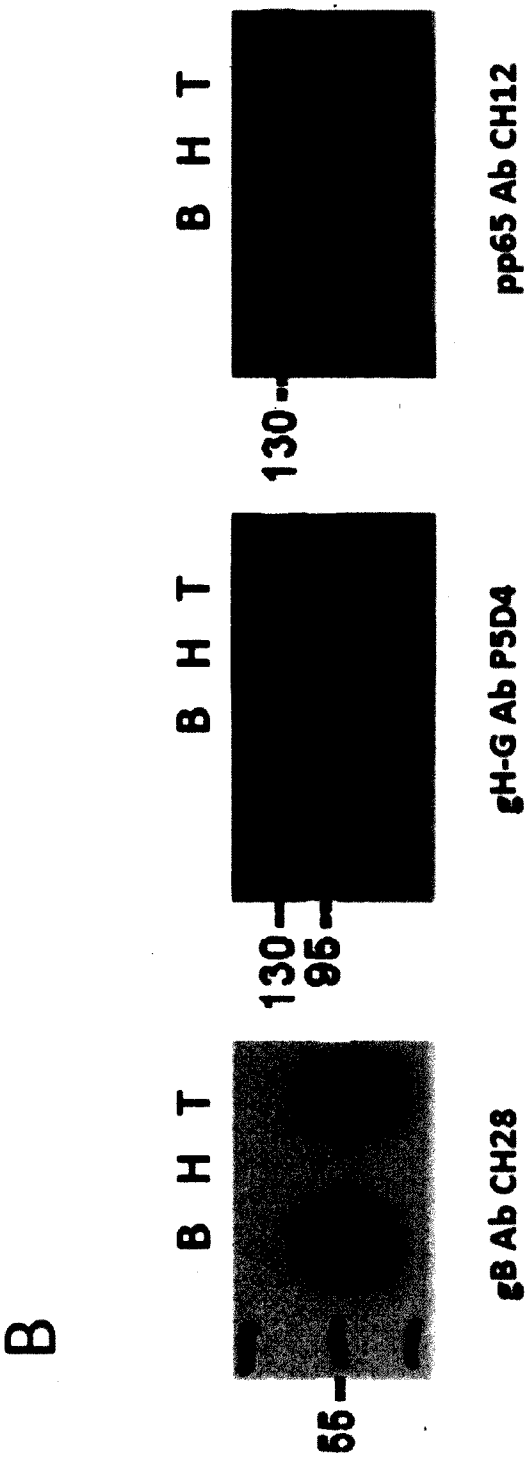

B
B H T
55
gB Ab CH28
B H T
130
96
gH-G Ab P5D4
B H T
130
pp65 Ab CH12
B: gB/pp65 bivalent VLP
H: gH-G/pp65 bivalent VLP
T: gB/gH-G/pp65 trivalent VLP

Figure 2

A

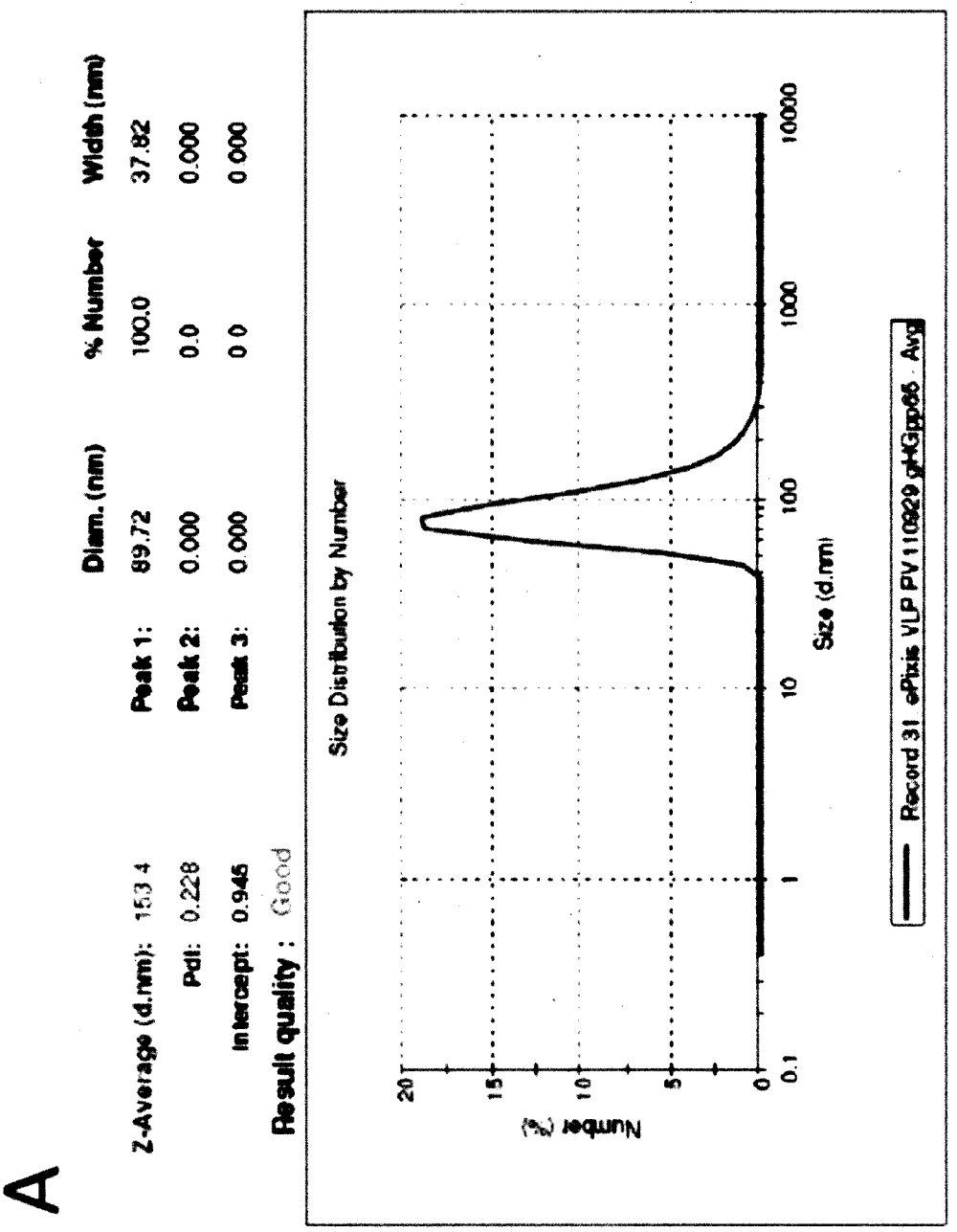

Diam. (nm)
% Number
Width (nm)
Z-Average (d.nm): 153.4
Peak 1: 89.72
100.0
37.82
PdI: 0.228
Peak 2: 0.000
0.0
0.000
Intercept: 0.945
Peak 3: 0.000
0.0
0.000
Result quality : Good
Size Distribution by Number
Number (%)
Size (d.nm)
Record 31: ePlus VLP PV110829 gHGpp86 Avg

Figure 3

B

	Diam. (nm)	% Number	Width (nm)
Z-Average (d.nm): 162.4	Peak 1: 101.8	100.0	45.31
PdI: 0.214	Peak 2: 0.000	0.0	0.000
Intercept: 0.948	Peak 3: 0.000	0.0	0.000

Result quality : Good

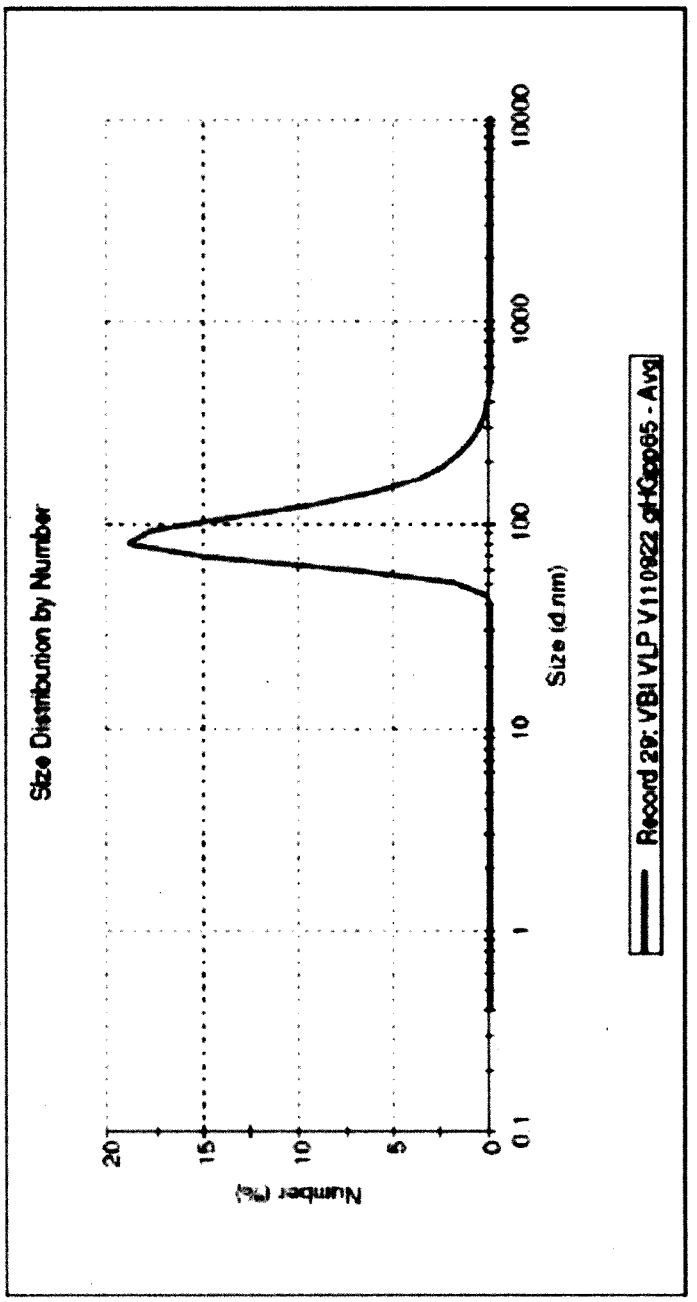
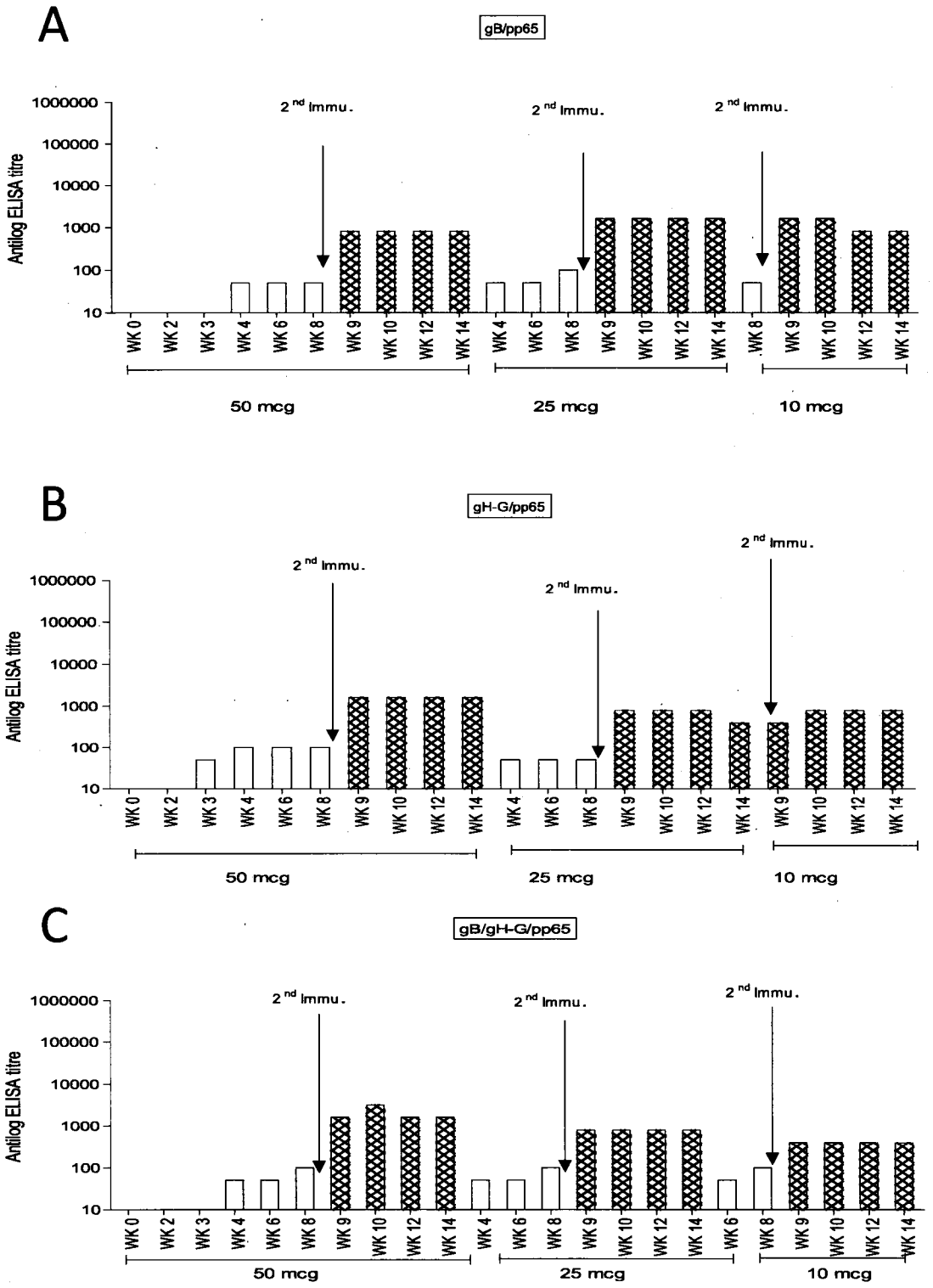


Figure 3


A
gB/pp65
Antilog ELISA titre
1000000
100000
10000
1000
100
10
2nd Immu.
2nd Immu.
2nd Immu.
WK 0
WK 2
WK 3
WK 4
WK 6
WK 8
WK 9
WK 10
WK 12
WK 14
WK 4
WK 6
WK 8
WK 9
WK 10
WK 12
WK 14
WK 8
WK 9
WK 10
WK 12
WK 14
50 mcg
25 mcg
10 mcg
B
gH-G/pp65
Antilog ELISA titre
1000000
100000
10000
1000
100
10
2nd Immu.
2nd Immu.
2nd Immu.
WK 0
WK 2
WK 3
WK 4
WK 6
WK 8
WK 9
WK 10
WK 12
WK 14
WK 4
WK 6
WK 8
WK 9
WK 10
WK 12
WK 14
WK 9
WK 10
WK 12
WK 14
50 mcg
25 mcg
10 mcg
C
gB/gH-G/pp65
Antilog ELISA titre
1000000
100000
10000
1000
100
10
2nd Immu.
2nd Immu.
2nd Immu.
WK 0
WK 2
WK 3
WK 4
WK 6
WK 8
WK 9
WK 10
WK 12
WK 14
WK 4
WK 6
WK 8
WK 9
WK 10
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WK 14
WK 6
WK 8
WK 9
WK 10
WK 12
WK 14
50 mcg
25 mcg
10 mcg

Figure 4

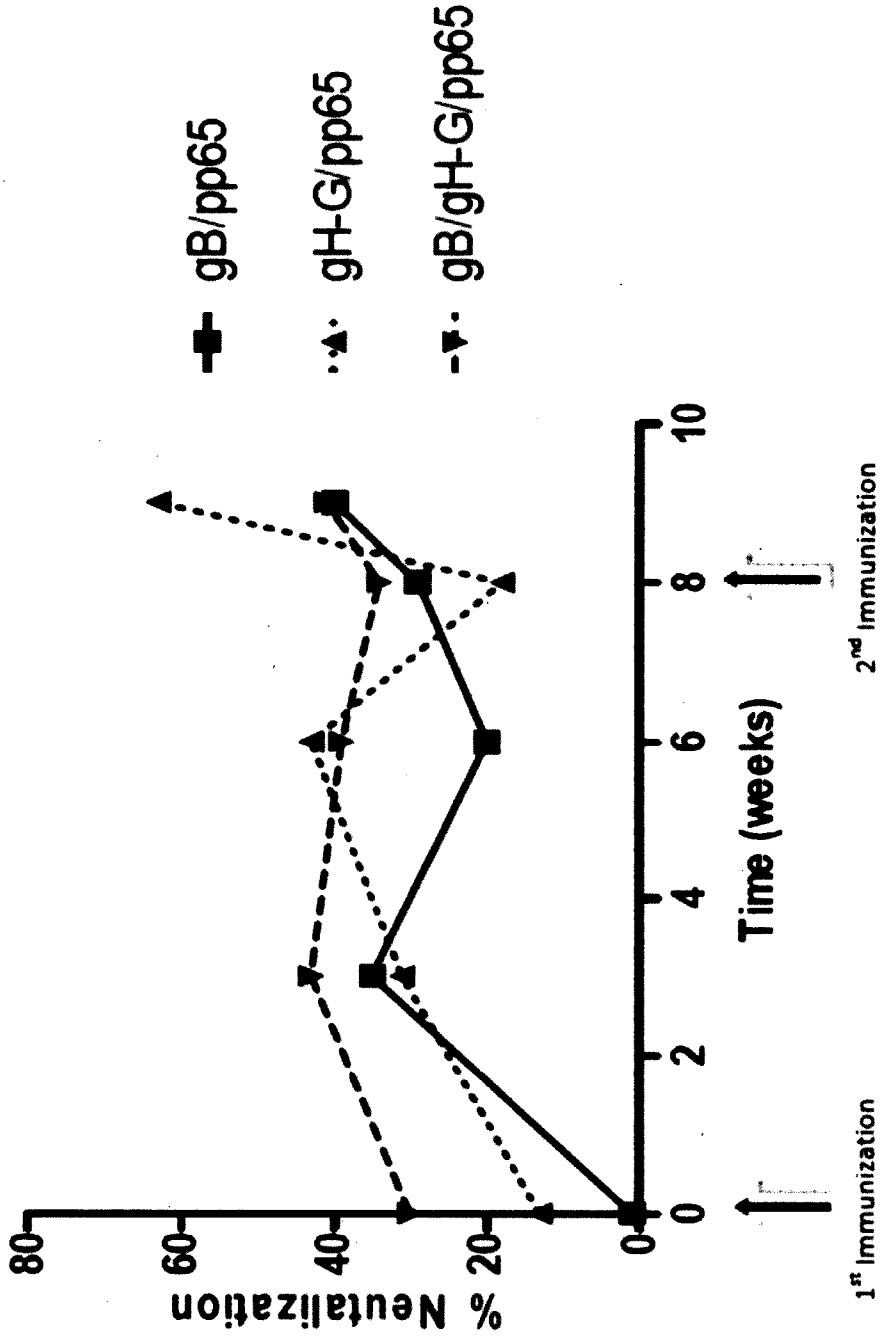

gB/pp65
gH-G/pp65
gB/gH-G/pp65
% Neutralization
0
20
40
60
80
0
2
4
6
8
10
Time (weeks)
1st Immunization
2nd Immunization

Figure 5

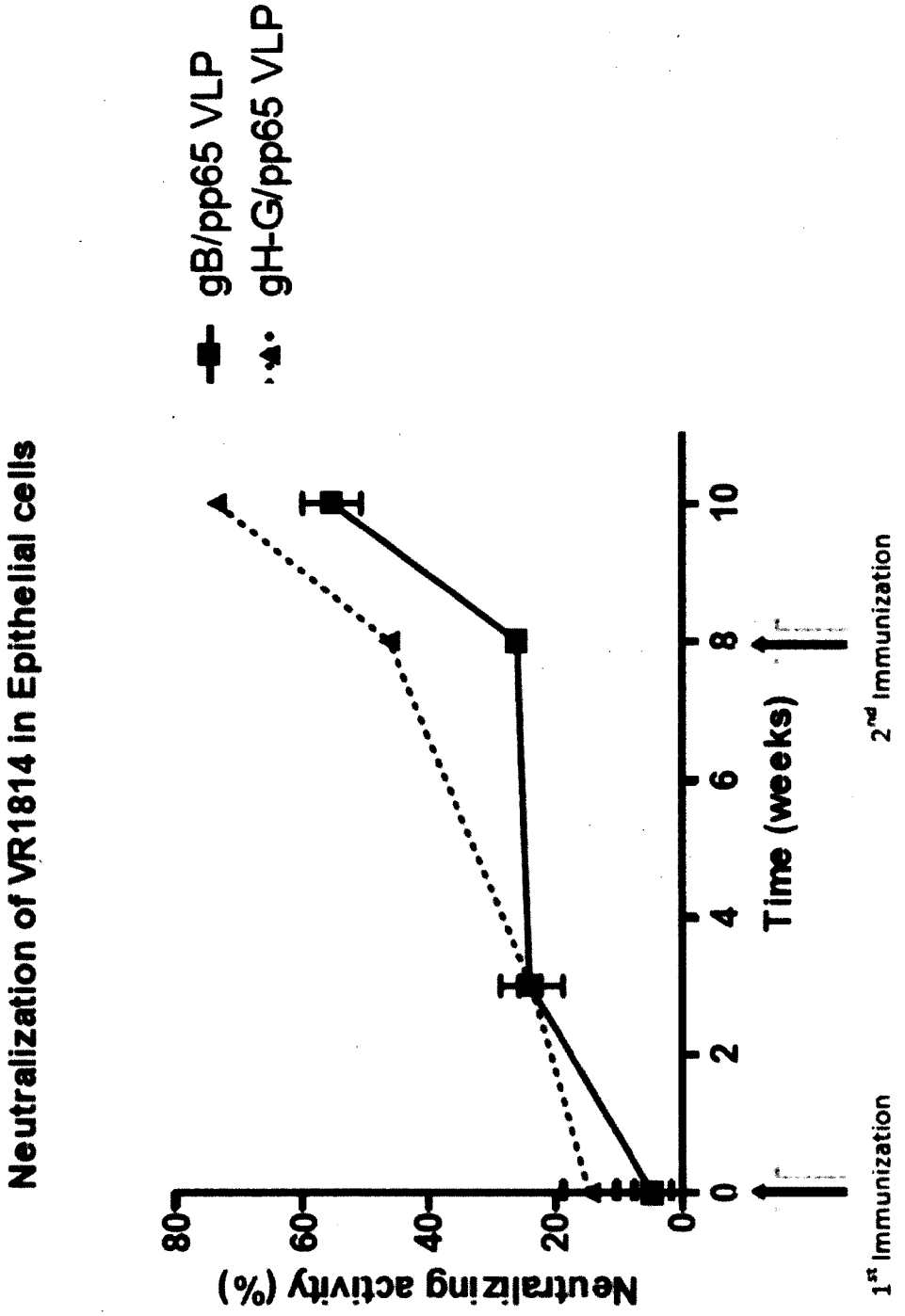

Neutralization of VR1814 in Epithelial cells
gB/pp65 VLP
gH-G/pp65 VLP
Neutralizing activity (%)
80
60
40
20
0
0
2
4
6
8
10
Time (weeks)
1st Immunization
2nd Immunization

Figure 6

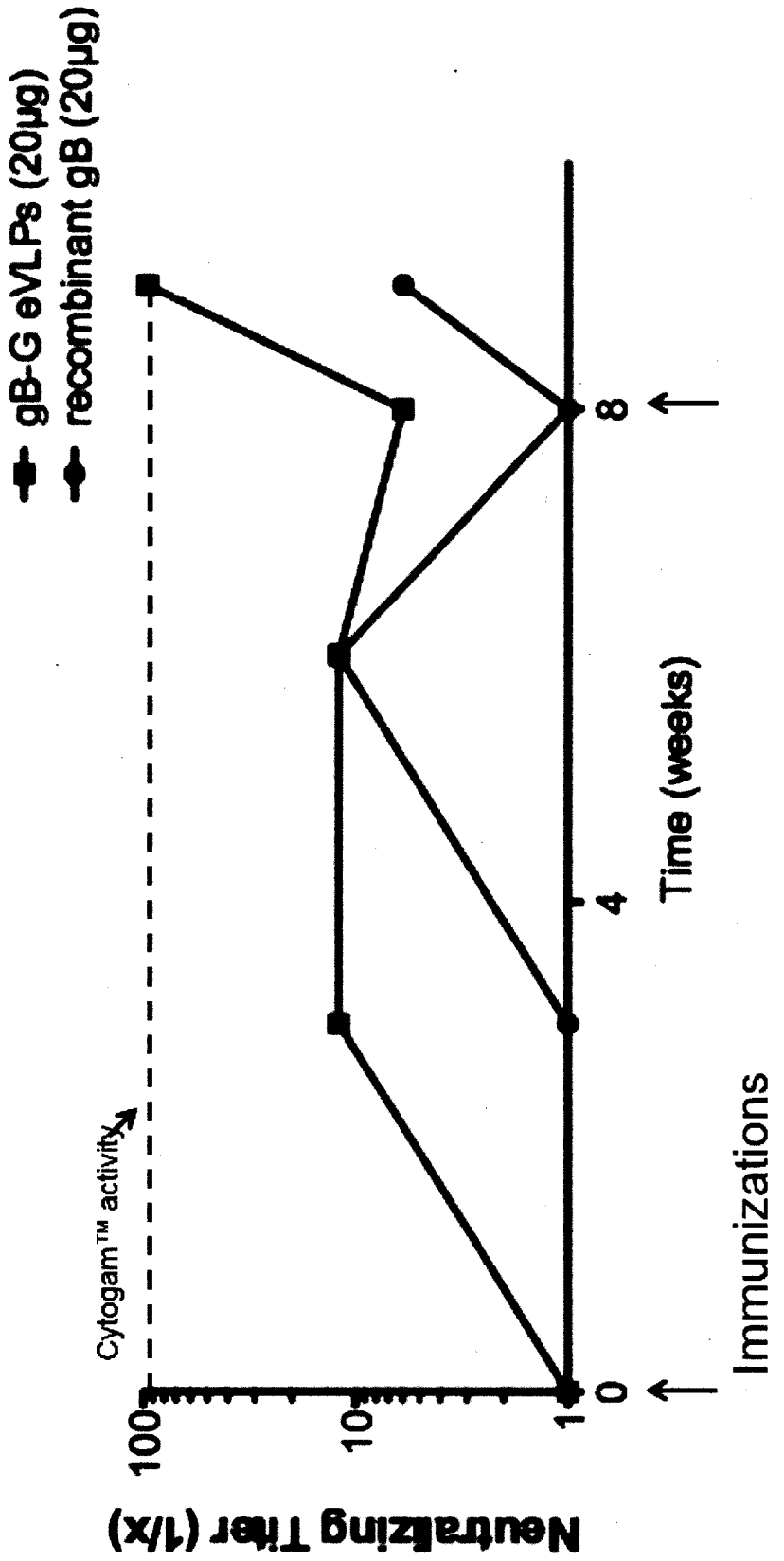

gB-G eVLPs (20µg)
recombinant gB (20µg)
Neutralizing Titer (1/x)
100
10
1
Cytogam™ activity
0
4
8
Time (weeks)
Immunizations

Figure 7

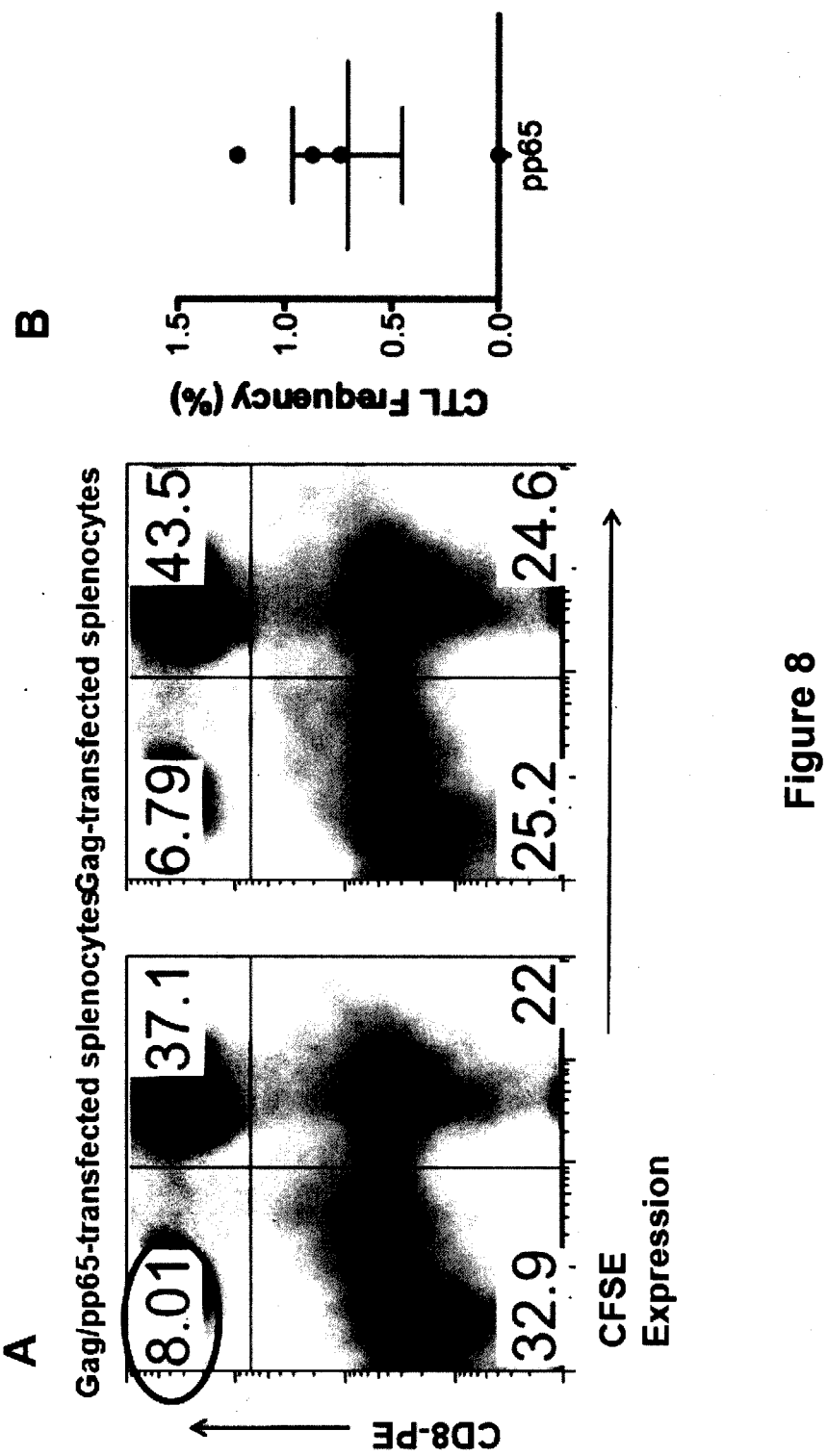

A
Gag/pp65-transfected splenocytes
Gag-transfected splenocytes
8.01
37.1
32.9
22
6.79
43.5
25.2
24.6
CD8-PE
CFSE
Expression
B
CTL Frequency (%)
1.5
1.0
0.5
0.0
pp65

Figure 8

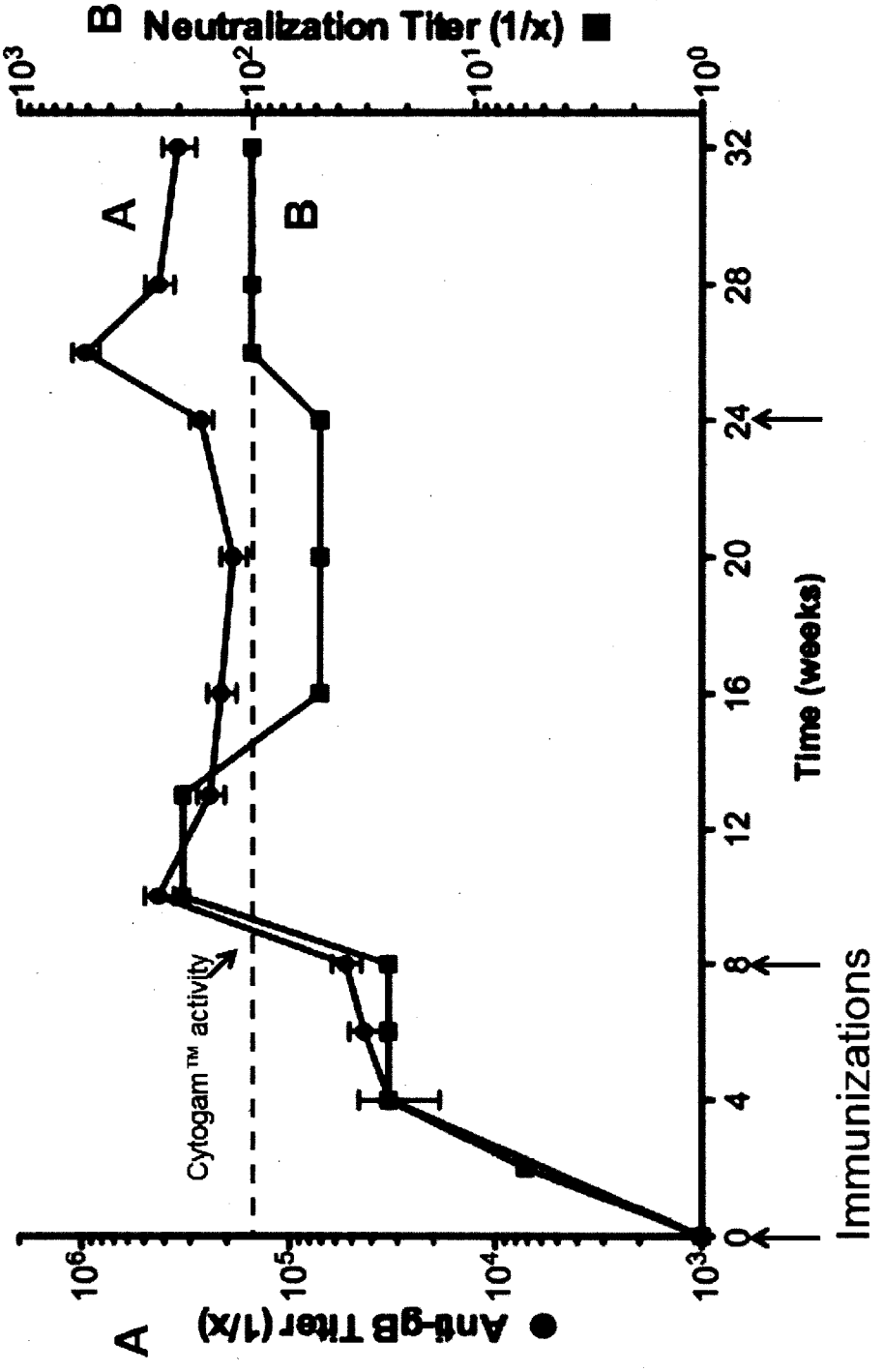

B Neutralization Titer (1/x) ■
A
B
Cytogam™ activity
● Anti-gB Titer (1/x)
Immunizations
Time (weeks)
0
4
8
12
16
20
24
28
32

Figure 9

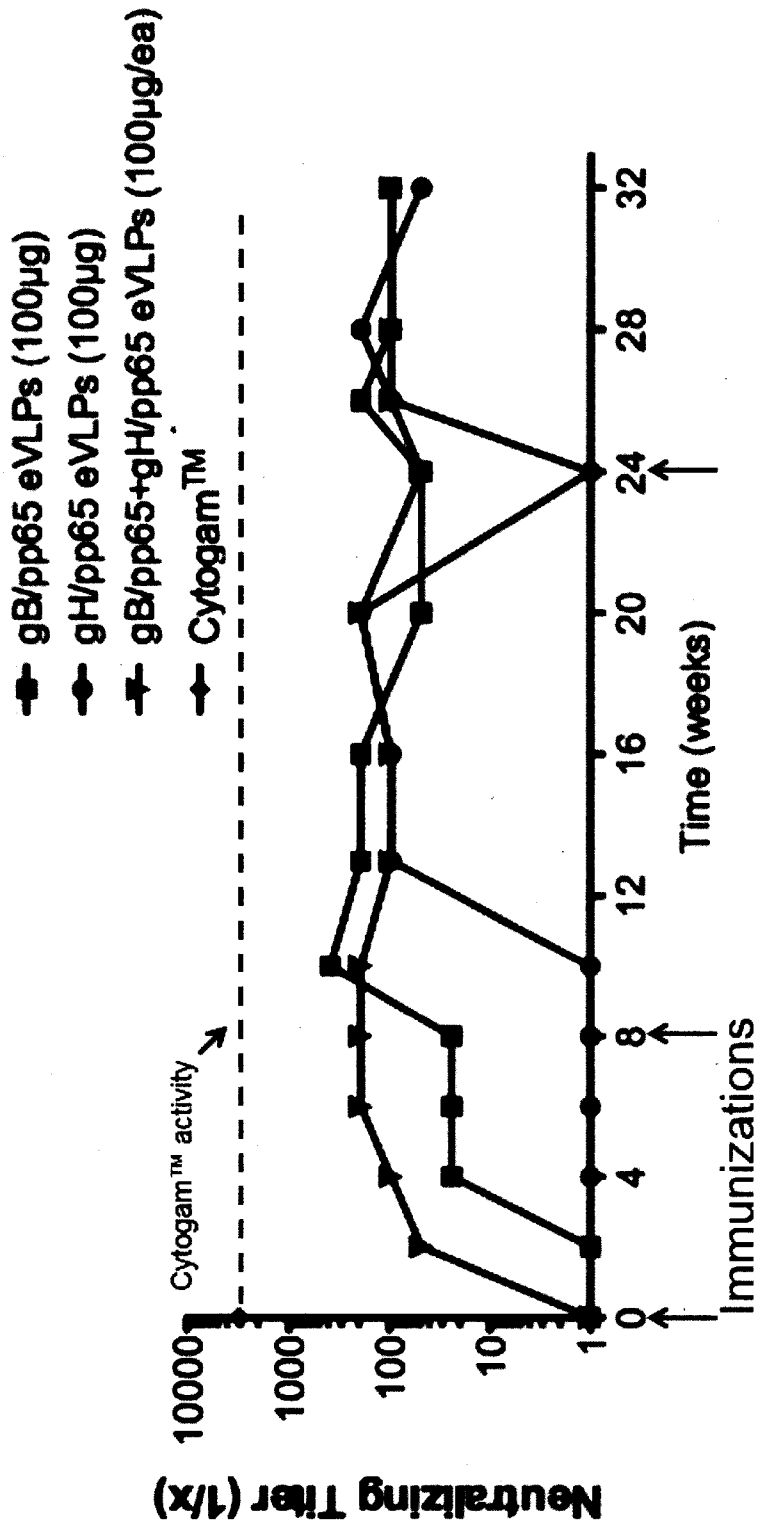
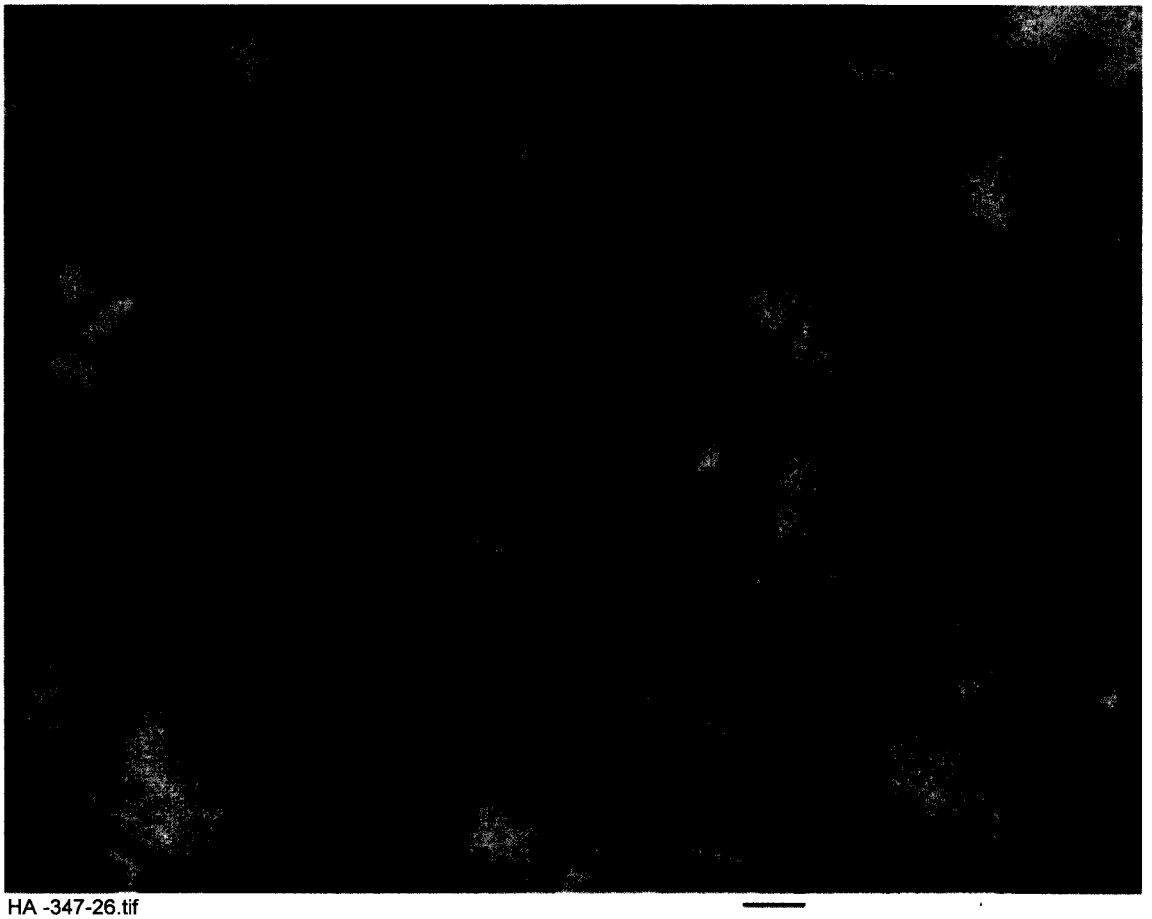

gB/pp65 eVLPs (100µg)
gH/pp65 eVLPs (100µg)
gB/pp65+gH/pp65 eVLPs (100µg/ea)
Cytogam™
Neutralizing Titer (1/x)
10000
1000
100
10
1
Cytogam™ activity
0
4
8
12
16
20
24
28
32
Immunizations
Time (weeks)

Figure 10

10L gB-G Diafiltration Retentate
Flowthrough (6.5x conc)

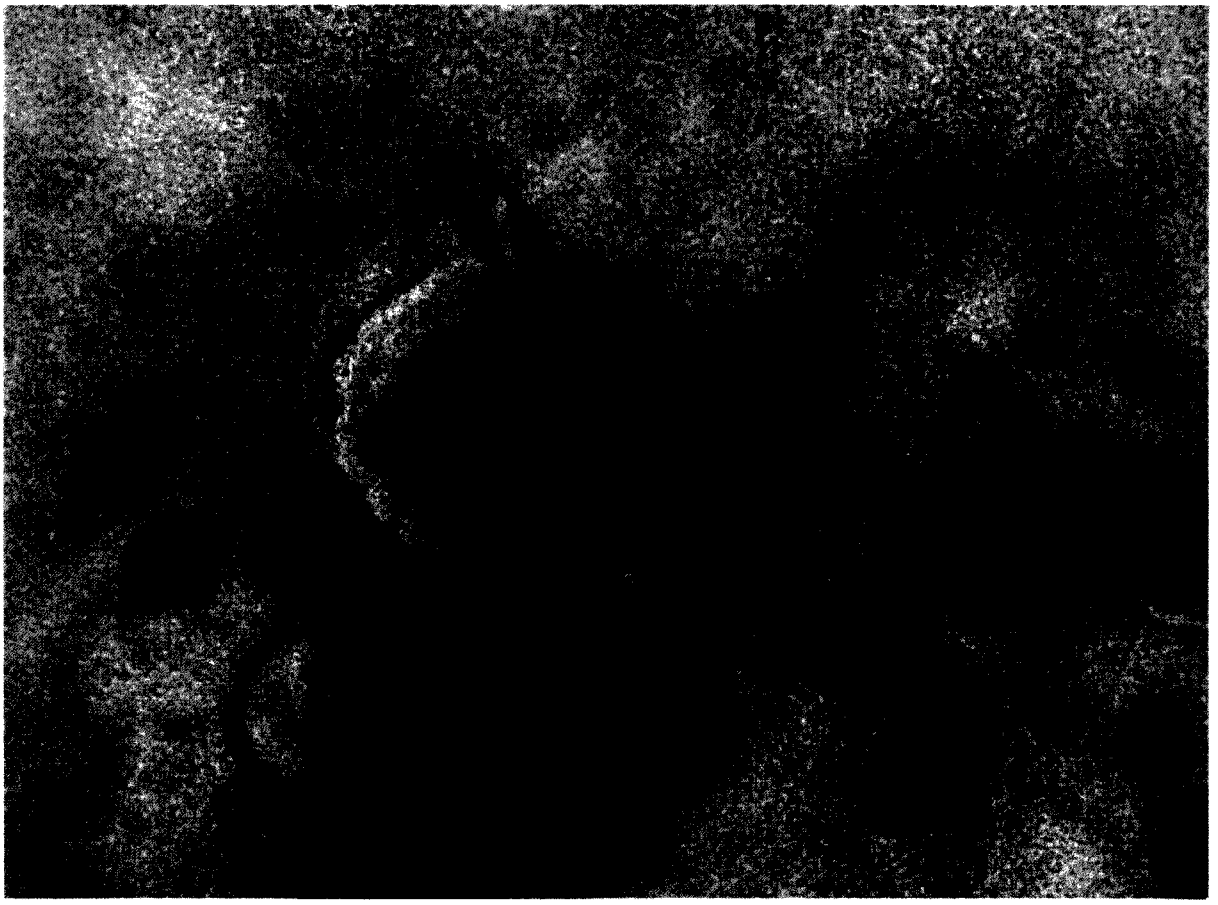


HA -347-26.tif
PSC20120420 DiafRentate 1/10
VBI Vaccines
Print Mag: 152000x @ 11.0 in
10:20 09-28-12
Microscopist : M Letarte
Retrovirus-like particles

100 nm
HV=75.0kV
Direct Mag : 40000x
SME - INRS - Institut Armand Frappier

Figure 11A

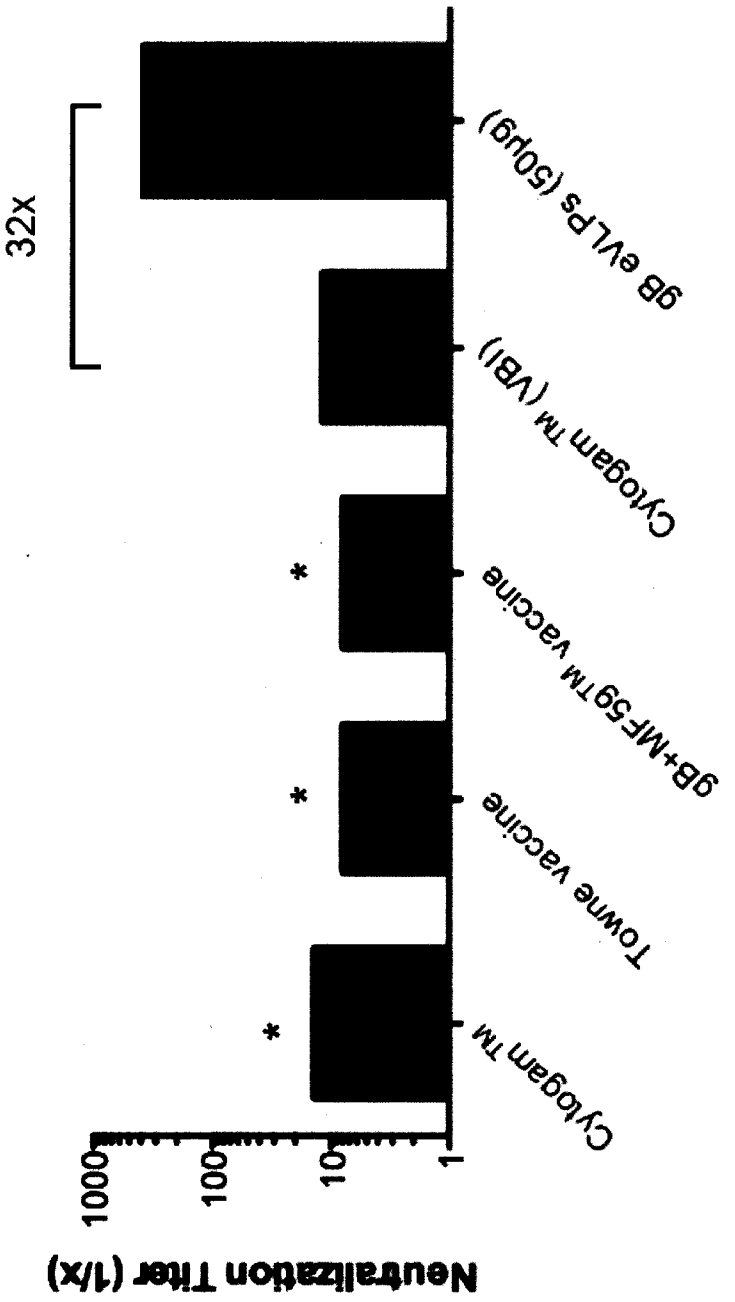
10L gB-G AEX



HA-348-06.tif
PSC20120529 conc 6.5x 1/5
VBI Vaccines
Print Mag: 152000x @11.0 in
12:48 09-28-12
Microscopist: M Letarte
Retrovirus-like particles

100 nm
HV=75.0kV
Direct Mag: 40000x
SME-INRS-Institut Frappier

Figure 11B



* Data source: Potency data of Cytogam™, live-attenuated Towne vaccine, and adjuvanted gB subunit vaccine adapted from *Cui X et al.*, 2008 Vaccine 26:5760-5766

Figure 12

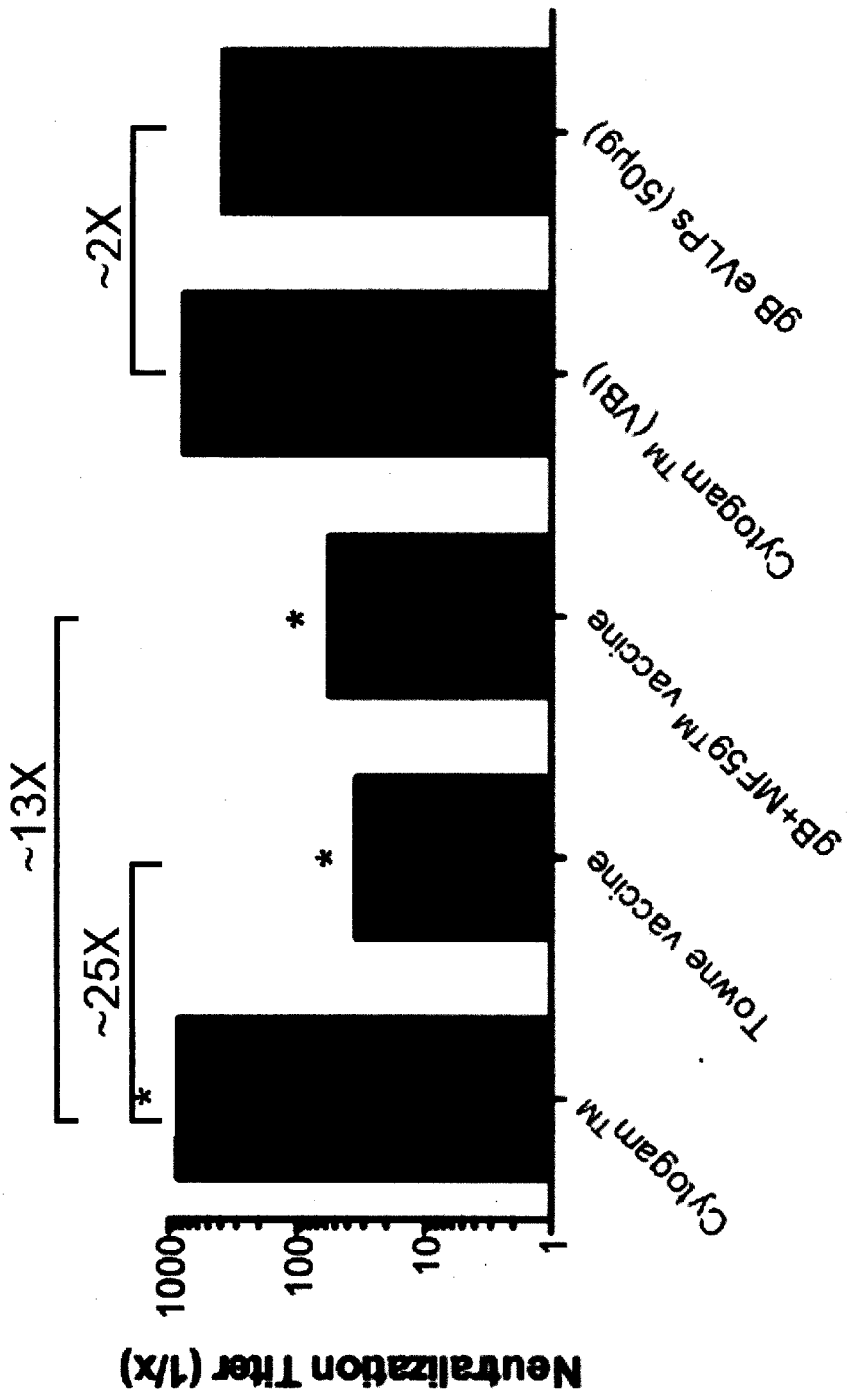


Figure 13

* Data source: Potency data of Cytogam™, live-attenuated Towne vaccine, and adjuvanted gB subunit vaccine adapted from *Cui X et al.*, 2008 Vaccine 26:5760-5766

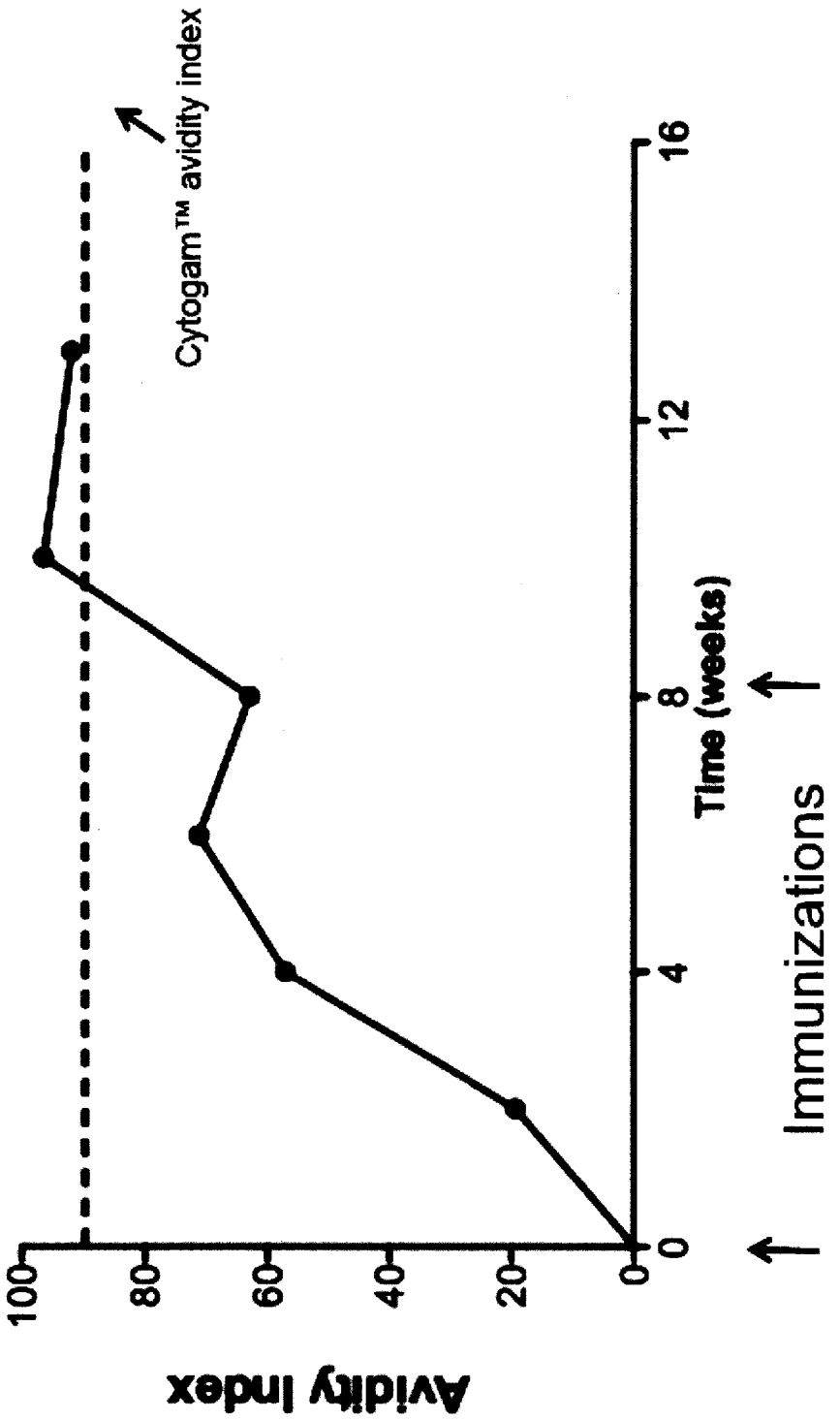
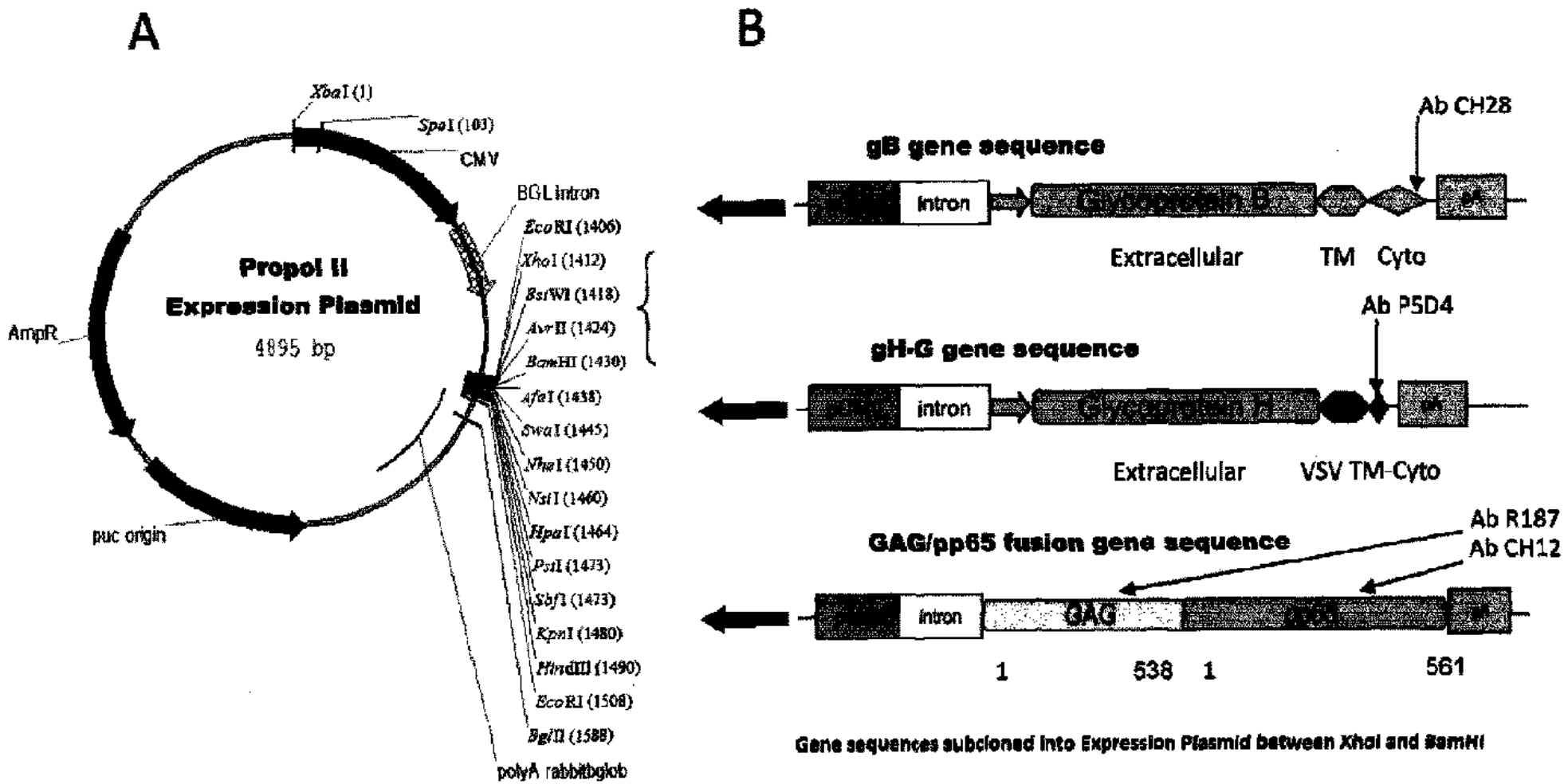

Avidity Index
100
80
60
40
20
0
0
4
8
12
16
Time (weeks)
Immunizations
Cytogam™ avidity index

Figure 14


A
XbaI (1)
SpeI (103)
CMV
BGL intron
EcoRI (1406)
XhoI (1412)
BsiWI (1418)
AvrII (1424)
BamHI (1430)
AflII (1438)
SwaI (1445)
NheI (1450)
NsiI (1460)
HpaI (1464)
PstI (1473)
SbfI (1473)
KpnI (1480)
HindIII (1490)
EcoRI (1508)
BglII (1588)
polyA rabbitbglob
Propol II
Expression Plasmid
4895 bp
AmpR
puc origin
B
gB gene sequence
Ab CH28
intron
Extracellular
TM
Cyto
gH-G gene sequence
Ab P5D4
intron
Extracellular
VSV TM-Cyto
GAG/pp65 fusion gene sequence
Ab R187
Ab CH12
intron
GAG
1
538
1
561
Gene sequences subcloned into Expression Plasmid between XhoI and BamHI