(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

PCT

(43) International Publication Date
11 February 2010 (11.02.2010)

(10) International Publication Number
WO 2010/017542 A1

(51) International Patent Classification:
C12N 7/01 (2006.01) *C07H 21/00* (2006.01)
C07K 14/00 (2006.01)

(21) International Application Number:
PCT/US2009/053249

(22) International Filing Date:
10 August 2009 (10.08.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/087,504 8 August 2008 (08.08.2008) US
61/218,603 19 June 2009 (19.06.2009) US

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(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2010/017542 A1

(54) **Title**: VIRUS-LIKE PARTICLES COMPRISING COMPOSITE CAPSID AMINO ACID SEQUENCES FOR ENHANCED CROSS REACTIVITY

(57) **Abstract**: The present invention provides polypeptides having a composite amino acid sequence derived from a consensus sequence representing the capsid proteins of two or more circulating strains of a non-enveloped virus. In particular, the invention provides virus-like particles comprising at least one composite polypeptide. Such virus-like particles have antigenic epitopes of two or more circulating strains of a non-enveloped virus and produce an increase in antisera cross-reactivity to one or more circulating strains of the non-enveloped virus. Methods of making composite virus-like particles and vaccine formulations comprising composite virus-like particles are also disclosed.

VIRUS-LIKE PARTICLES COMPRISING COMPOSITE CAPSID AMINO ACID SEQUENCES FOR ENHANCED CROSS REACTIVITY

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] This application claims the benefit of U.S. Provisional Application No. 61/087,504, filed August 8, 2008, and U.S. Provisional Application No. 61/218,603, filed June 19, 2009, both of which are herein incorporated by reference in their entireties.

FIELD OF THE INVENTION

[002] The invention is in the field of vaccines, particularly vaccines comprising virus-like particles with a composite amino acid sequence derived from a consensus sequence representing two or more capsid proteins from non-enveloped viruses. In addition, the invention relates to methods of preparing vaccine compositions and methods of inducing a protective immune response using the vaccine compositions of the invention.

BACKGROUND OF THE INVENTION

[003] The prevalent approach to preparing vaccines for viruses with seasonal or year-to-year patterns is modeled by commercial Influenza vaccines which require the anticipation, publication, and subsequent synthesis of a new vaccine when the virus evolves to present a different antigenic profile. This approach causes significant timeline delays and cost as new antigens are synthesized in anticipation of the next years viral strain. Further, as evidenced by the failings of the 2008 influenza vaccine, errors in the predicted strain can result in significant disease related costs as patients are under-protected. Thus, improved methods for designing and preparing vaccines to protect against multiple circulating strains of disease-causing virus is desirable.

[004] Noroviruses are non-cultivatable human Caliciviruses that have emerged as the single most important cause of epidemic outbreaks of nonbacterial gastroenteritis (Glass *et al.* (2000) J Infect Dis, Vol. 181 (Sup 2): S254-S261; Hardy *et al.* (1999) Clin Lab Med, Vol. 19(3): 675-90). These viruses have been grouped into five different genogroups of which genogroups I and II are further subdivided into greater than 25 genotypes and are the agents for the vast majority of illness in humans attributed to this virus. There are significant challenges to the development of

vaccines against Norovirus, including the inability to propagate the virus in culture and suitable animal models of acute gastroenteritis. Standard virologic techniques including viral attenuation or *in vitro* neutralization assays are therefore not possible today.

[005] Noroviruses contain a 7.5 Kb single strand positive sense RNA genome that contains three open reading frames. The major viral capsid protein (VP1) is encoded by ORF2 and expression of this protein results in the spontaneous assembly of virus-like particles (VLPs), which mimic the structure of the virus but are incapable of replication. This structure is composed of 180 monomeric subunits of VP1 and are candidate vaccines to prevent acute gastroenteritis. The VP1 monomer has two domains: a shell (S) domain that forms the inner viral core and a prominent protruding (P) domain linked by a flexible hinge. The P domain is further subdivided into two subdomains P1 and P2, which is the most surface exposed region and is thought to contain important cell recognition and antigenic sites. Homology analysis indicates that the majority of the hypervariable amino acid regions of VP1 are located in the P2 domain (Allen *et al*. (2008) PLoS One, Vol. 1: 1-9).

[006] Recent epidemiology studies have lead to the hypothesis that Norovirus evolution is epochal with periods of stasis followed by emergence of novel epidemic strains, similar to that observed for Influenza virus. Most recent outbreaks appear to be related to emergence of variant virus in the GII.4 genotype at a persistence interval of around two years. There is a need in the art for a vaccine candidate that provides antigenic epitopes that would be cross protective for multiple Norovirus, or other non-enveloped virus strains, which would obviate the need for construction of vaccines for each contemporary outbreak strain.

SUMMARY OF THE INVENTION

[007] The present invention is based, in part, on the discovery that a polypeptide comprising a composite capsid sequence, which combines epitopes from a number of circulating viral strains, can be used to produce a more robust immunological response to multiple viral strains. Such a polypeptide can be used to prepare vaccine formulations that are protective against several circulating strains of the virus, and therefore improve strain-to-strain and year-to-year protection.

[008] The present invention provides at least one polypeptide having a composite amino acid sequence, wherein said composite amino acid sequence is derived from a consensus sequence representing the capsid proteins of two or more circulating strains of a non-enveloped virus, and

wherein the at least one polypeptide forms a virus-like particle when expressed in a host cell and contains at least 1 different amino acid as compared to each of the capsid sequences of said two or more circulating strains. In one embodiment, the virus-like particle comprising the at least one composite polypeptide has antigenic properties of the two or more circulating strains of the non-enveloped virus. In another embodiment, the composite polypeptide or composite virus-like particle provides an increase in antisera cross-reactivity to one or more circulating strains of the non-enveloped virus as compared to the antisera cross-reactivity obtained by immunizing with a virus-like particle containing only protein from said one or more circulating strains.

[009] The virus-like particle may comprise at least one polypeptide having a composite amino acid sequence derived from a consensus sequence representing capsid proteins of two or more circulating strains of a non-enveloped virus, wherein the non-enveloped virus is selected from the group consisting of Calicivirus, Picornavirus, Astrovirus, Adenovirus, Reovirus, Polyomavirus, Papillomavirus, Parvovirus, and Hepatitis E virus. In one embodiment, the non-enveloped virus is a Calicivirus. In another embodiment, the Calicivirus is a Norovirus or Sapovirus. The Norovirus may be a genogroup I or genogroup II Norovirus.

[010] The consensus sequence may be derived from two or more Norovirus strains classified in the same genogroup and genotype. In one embodiment, the consensus sequence is derived from genogroup II, genotype 4 Norovirus strains, such as Houston, Minerva, and Laurens strains. In another embodiment, the consensus sequence is derived from Norovirus strains from at least two different genotypes within a genogroup. In still another embodiment, the consensus sequence is derived from Norovirus strains from at least two different genogroups.

[011] The present invention also encompasses a virus-like particle comprising at least one composite polypeptide derived from two or more circulating Calicivirus strains and a capsid protein from a second non-enveloped virus, such as Norovirus. The capsid protein may be a VP1 and/or VP2 protein from a genogroup I or genogroup II Norovirus. In another embodiment, the virus-like particle comprises at least one composite polypeptide derived from two or more circulating strains of a Calicivirus and a second composite polypeptide derived from two or more circulating strains of a second Calicivirus. Preferably, the virus-like particle has antigenic properties of the two or more circulating strains of the first Calicivirus and the two or more circulating strains of the second Calicivirus.

[012] The present invention also provides an isolated polypeptide or fragment thereof having a composite amino acid sequence, wherein said composite amino acid sequence is derived from a consensus sequence representing the capsid proteins of two or more circulating strains of a non-enveloped virus, and wherein the polypeptide contains at least 1 different amino acid as compared to each of the capsid sequences of said two or more circulating strains. The non-enveloped virus may be a Calicivirus, such as a Sapovirus or Norovirus. Alternatively, the non-enveloped virus may be a Papillomavirus.

[013] The present invention contemplates vaccine formulations comprising one or more composite polypeptides or composite virus-like particles of the invention. Each of the composite virus-like particles comprises at least one polypeptide having a composite amino acid sequence derived from a consensus sequence representing the capsid proteins from two or more circulating strains of a non-enveloped virus. The non-enveloped virus may be a genogroup I or genogroup II Norovirus. In some embodiments, the vaccine formulation further comprises an adjuvant. In other embodiments, the vaccine formulation further comprises a delivery agent. In still other embodiments, the vaccine formulation further comprises a pharmaceutically acceptable carrier. The vaccine formulation may be a liquid formulation or a dry powder formulation.

[014] The invention also provides a method of inducing a protective immunity to a viral infection in a subject comprising administering to the subject a vaccine formulation disclosed herein. In one embodiment, the viral infection is a Norovirus infection. In another embodiment, the vaccine formulation confers protection from one or more symptoms of Norovirus infection.

[015] The present invention also contemplates a method of making a composite virus-like particle. In one embodiment, the method comprises aligning amino acid sequences of capsid proteins from two or more circulating strains of a non-enveloped virus; determining a consensus sequence from said aligned amino acid sequences; preparing a composite sequence based on said consensus sequence that contains at least 1 different amino acid as compared to each of the capsid sequences of said two or more circulating strains; and expressing said composite sequence in a host cell, thereby producing a virus-like particle. The non-enveloped virus may be a Calicivirus, Picornavirus, Astrovirus, Adenovirus, Reovirus, Polyomavirus, Papillomavirus, Parvovirus, and Hepatitis E virus.

BRIEF DESCRIPTION OF THE FIGURES

[016] Figure 1. Amino acid consensus sequence of VP1 proteins from genogroup II, genotype 4 Norovirus (SEQ ID NO: 2). The consensus sequence was determined from an alignment of Houston, Minerva, and Laurens strains.

[017] Figure 2. Nucleotide sequence encoding the composite VP1 protein from genogroup II, genotype 4 Norovirus (SEQ ID NO: 3).

[018] Figure 3. SDS-PAGE/Coomassie analysis of sucrose gradient purified composite VLPs.

[019] Figure 4. HPLC SEC chromatogram of readings at 220 nm (top) and 280 nm (bottom) of composite expression cell culture supernatant purified by sucrose gradient.

[020] Figure 5. SDS-PAGE/Silver-stain analysis of composite sequence VLPs purified by column chromatography.

[021] Figure 6. HPLC SEC chromatogram of readings at 280 nm of composite VLPs.

[022] Figure 7. Immunization with composite VLP (CVLP) elicits antigen-specific IgG. Groups of 7 mice were immunized (i.p.) with various concentrations of CVLP (indicated on the X axis) on days 0 and 7. Serum was collected on day 14 and CVLP-specific IgG was measured by ELISA. Horizontal lines indicate geometric means of each treatment group.

[023] Figure 8. Immunization with composite VLP/Norwalk VLP (CVLP/NVLP) combination elicits NVLP-specific IgG. Groups of 7 mice were immunized (i.p.) with various concentrations of NVLP alone (purple bars) or in combination with equal amounts of CVLP (black bars) on days 0 and 14. Serum was collected on day 21 and NVLP-specific IgG was measured by ELISA. Data is reported as the mean + standard error of the mean (SEM).

[024] Figure 9. Immunization with composite VLP/Norwalk VLP (CVLP/NVLP) combination elicits CVLP-specific IgG. Groups of 7 mice were immunized (i.p.) with various concentrations of either composite VLP alone (green bars) or in combination with equal amounts of NVLP (black bars) on days 0 and 14. Serum was collected on day 21 and CVLP-specific IgG was measured by ELISA. Data is reported as the mean + standard error of the mean (SEM).

[025] Figure 10. CVLP-specific IgG cross-reacts with other Norovirus isolates. Antibody titers measured 21 days after a single immunization with the either Composite VLPs or GII.4 2002 VLPs show that Composite VLPs elicit ~ 10 fold higher titers as compared to the GII.4 2002 VLPs. Antibody titers for animals immunized with all GII.4 VLPs show poor cross reactivity to GI.1 VLPs. Data are expressed as geometric mean + standard error of the mean (SEM).

[026] Figure 11. Rabbits were immunized IM on day 0 and 21 with equal amounts of Norwalk VLP (NVLP) and composite VLP (CVLP). Serum was collected on day 28 and VLP-specific IgG was evaluated. The resulting data was log transformed and evaluated by linear regression analysis. IgG titers are expressed as reciprocal dilutions and shown as geometric mean titers.

[027] Figure 12. Rabbits were immunized IM on day 0 and 21 with equal amounts of Norwalk VLP (NVLP) and composite VLP (CVLP). Spleens were collected on day 75 and unfractionated cells were stimulated in culture for 5 days with either NVLP or CVLP and the amount of thymidine incorporation was measured. The mean and SD are shown for each rabbit in the treatment groups indicated on the X axis. Data are expressed as mean + SD.

[028] Figure 13. Rabbits were immunized IM on day 0 and 21 with equal amounts of Norwalk VLP (NVLP) and composite VLP (CVLP). Spleens and mesenteric lymph nodes (LN) were collected on day 75 and analyzed for the presence of VLP-specific memory B-cells by ELISPOT. Individual responses are shown for NVLP and CVLP. Data are represented as the number of VLP-specific IgG secreting cells per million cells present.

[029] Figure 14. Rabbits were immunized IM on days 0, 14, and 21 with equal amounts of Norwalk VLP (NVLP) and composite VLP (CVLP) as indicated in the legend. Serum was collected on day 21 and 35 and NVLP-specific IgG and IgA was measured by ELISA. Results are displayed as geometric group means + SEM.

[030] Figure 15. Rabbits were immunized IM on days 0, 14, and 21 with equal amounts of Norwalk VLP (NVLP) and composite VLP (CVLP) as indicated in the legend. Serum was collected on day 21 and 35 and CVLP-specific IgG and IgA was measured by ELISA. Results are displayed as geometric group means + SEM.

[031] Figure 16. Rabbits were immunized IM on days 0, 14, and 21 with equal amounts of Norwalk VLP (NVLP) and composite VLP (CVLP). Spleens were collected on day 35 and unfractionated cells were stimulated *in vitro* for 5 days. Splenocytes were stimulated with various VLPs from the two genogroups as indicated in the graph legend. Results are displayed as geometric group means + SD.

[032] Figure 17. Mice were immunized IP on days 0 and 7 with equal amounts of Norwalk VLP (NVLP) and composite VLP (CVLP) as indicated on the X axis. Serum was collected on day 14 and analyzed for the presence of VLP-specific IgG by ELISA. Individual responses are

shown and titers are expressed as reciprocal dilutions. Horizontal bars represent geometric group means.

[033] **Figure 18.** Mice were immunized IP on days 0 and 7 with equal amounts of Norwalk VLP (NVLP) and composite VLP (CVLP) as indicated on the X axis. Serum was collected on day 14 and analyzed for the presence of antibodies capable of inhibiting hemagglutination of human red blood cells (type O positive). Individual responses are shown and titers are expressed as reciprocal dilutions. Horizontal bars represent geometric group means.

[034] **Figure 19.** Serum anti-VLP IgG in rabbits intranasally immunized on days 0 and 21 with 50μg of VLP vaccine formulation (Norwalk VLPs + composite GII.4 VLPs). Individual responses are shown and expressed in μg/mL from serum collected on day 35. Bars indicate the geometric group means.

[035] **Figure 20.** Amino acid consensus sequence of VP1 proteins from genogroup II Norovirus (SEQ ID NO: 7). The consensus sequence was determined from an alignment of GII.1 (Accession Number: AAL13001), GII.2 Snow Mountain (Accession Number: AAB61685), and GII.3 (Accession Number: AAL12998) strains. The “x” indicates positions in which the amino acid differed among all three strains.

[036] **Figure 21.** Amino acid consensus sequence of VP1 proteins from genogroup I Norovirus (SEQ ID NO: 12). The consensus sequence was determined from an alignment of Norwalk virus (Accession Number: M87661), Southampton (Accession Number: Q04542), and Chiba virus (Accession Number: BAB18267) strains. The “x” indicates positions in which the amino acid differed among all three strains.

[037] **Figure 22.** Amino acid consensus sequence of L1 proteins from Human Papillomavirus (SEQ ID NO: 17). The consensus sequence was determined from an alignment of HPV-11, HPV-16, and HPV-18 viral strains. The “x” indicates positions in which the amino acid differed among all three strains.

DETAILED DESCRIPTION OF THE INVENTION

[038] The present invention provides vaccine formulations comprising a polypeptide having a composite amino acid sequence, wherein the composite amino acid sequence is derived from capsid sequences of circulating strains of non-enveloped virus. Virus-like particles produced from such polypeptide sequences provide antigenic epitopes for several viral strains and can be

used to induce an immune response that is protective against viral infection from multiple strains. Accordingly, the present invention provides a virus-like particle comprising at least one polypeptide having a composite amino acid sequence. A “composite amino acid sequence” or “composite sequence”, as used herein, is a sequence derived from a consensus sequence of at least two viral protein sequences. In one embodiment, the viral protein sequences are capsid sequences. A composite amino acid sequence may be derived from a consensus sequence by selecting one of two or more amino acids at the variable positions in the consensus sequence.

[039] As used herein, a “consensus sequence” is a sequence containing one or more variable amino acids, and is determined by aligning and comparing the viral protein sequences of two or more viruses. A consensus sequence may also be determined by aligning and comparing the nucleotide sequences of two or more viruses. The consensus sequence may be determined from protein or nucleotide sequences of two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, or nine or more circulating strains of a non-enveloped virus.

[040] The polypeptide having a composite amino acid sequence may contain at least one different, at least two different, at least three different, at least four different, at least five different, at least six different, at least seven different, at least eight different, at least nine different, at least ten different, at least fifteen different, at least twenty different, at least twenty-five different, at least thirty different, at least thirty-five different, at least forty different, at least forty-five different, or at least fifty different amino acids as compared to each of the protein sequences of the two or more circulating strains used to determine the consensus sequence. In some embodiments, the polypeptide having a composite amino acid sequence may form a virus-like particle when expressed in a host cell.

[041] In one embodiment of the invention, the virus-like particle (VLP) comprises at least one polypeptide having a composite amino acid sequence, wherein said composite amino acid sequence is derived from a consensus sequence representing the capsid proteins of two or more circulating strains of a non-enveloped virus, and wherein the at least one polypeptide forms a virus-like particle when expressed in a host cell and contains at least 1 different amino acid as compared to each of the capsid sequences of said two or more circulating strains. Preferably, the virus-like particle has antigenic properties of the two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, or nine or more circulating strains of a non-enveloped virus. In some embodiments, the virus-like particle provides an increase in

antisera cross-reactivity to one or more, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, or nine or more circulating strains of the non-enveloped virus as compared to the antisera cross-reactivity obtained by immunizing with a virus-like particle containing only protein from one or more circulating strains. In one embodiment, the virus-like particle provides at least a two-fold increase in antisera cross-reactivity.

[042] In another embodiment, the virus-like particle comprises at least one polypeptide having a composite amino acid sequence derived from a consensus sequence representing the capsid proteins of two or more circulating strains of a non-enveloped virus, wherein the non-enveloped virus is selected from the group consisting of Calicivirus, Picornavirus, Astrovirus, Adenovirus, Reovirus, Polyomavirus, Papillomavirus, Parvovirus, and Hepatitis E virus. The invention also includes strains of non-enveloped viruses that have not yet been characterized or discovered at the time of filing. In some embodiments, among others, the non-enveloped virus is a Calicivirus. Caliciviruses are divided into four genera: Norovirus and Sapovirus, which cause infection in humans, and Lagovirus and Vesivirus, which are associated with veterinary infections. In preferred embodiments, the Calicivirus is a Sapovirus or Norovirus.

[043] The Norovirus genus is split primarily into two major genogroups (GI and GII). Two other genogroups (GIII and GIV) are proposed, but generally accepted. Representative of GIII is the bovine, Jena strain. GIV contains one virus, Alphatron, at this time. The GI and GII groups may be further segregated into clusters or genotypes based on genetic classification (Ando *et al.* (2000) J. Infectious Diseases, Vol. 181(Supp2):S336-S348; Lindell *et al.* (2005) J. Clin. Microbiol., Vol. 43(3): 1086–1092). As used herein, the term genetic clusters is used interchangeably with the term genotypes. Within genogroup I, there are 6 GI clusters (with prototype virus strain name): GI.1 (Norwalk); GI.2 (Southhampton); GI.3 (Desert Shield); GI.4 (Cruise Ship virus/Chiba); GI.5 (318/Musgrove); and GI.6 (Hesse). Within genogroup II, there are 9 GII clusters (with prototype virus strain name): GII.1 (Hawaii); GII.2 (Snow Mountain/Melksham); GII.3 (Toronto); GII.4 (Bristol/Lordsdale); GII.5 (290/Hillingdon); GII.6 (269/Seacroft); GII.7 (273/Leeds); GII.8 (539/Amsterdam); and GII.9 (378). The circulating Norovirus strains are classified through comparison to prototype strains belonging to these genetic clusters. The most prevalent circulating strains belong to genogroup II.

[044] Nucleic acid and protein sequences for a number of Norovirus isolates are known. Additional representative, non-limiting sequences, including sequences of ORF1, ORF2, ORF3, and their encoded polypeptides from Norovirus isolates are listed in the National Center for Biotechnology Information (NCBI) database. In one embodiment of the invention, the Norovirus may be a genogroup I or genogroup II Norovirus. Composite and consensus amino acid sequences may be determined from any of the known Norovirus strains. See, for example, GenBank entries: Norovirus genogroup 1 strain Hu/NoV/West Chester/2001/USA, GenBank Accession No. AY502016; Norovirus genogroup 2 strain Hu/NoV/Braddock Heights/1999/USA, GenBank Accession No. AY502015; Norovirus genogroup 2 strain Hu/NoV/Fayette/1999/USA, GenBank Accession No. AY502014; Norovirus genogroup 2 strain Hu/NoV/Fairfield/1999/USA, GenBank Accession No. AY502013; Norovirus genogroup 2 strain Hu/NoV/Sandusky/1999/USA, GenBank Accession No. AY502012; Norovirus genogroup 2 strain Hu/NoV/Canton/1999/USA, GenBank Accession No. AY502011; Norovirus genogroup 2 strain Hu/NoV/Tiffin/1999/USA, GenBank Accession No. AY502010; Norovirus genogroup 2 strain Hu/NoV/CS-E1/2002/USA, GenBank Accession No. AY50200; Norovirus genogroup 1 strain Hu/NoV/Wisconsin/2001/USA, GenBank Accession No. AY502008; Norovirus genogroup 1 strain Hu/NoV/CS-841/2001/USA, GenBank Accession No. AY502007; Norovirus genogroup 2 strain Hu/NoV/Hiram/2000/USA, GenBank Accession No. AY502006; Norovirus genogroup 2 strain Hu/NoV/Tontogany/1999/USA, GenBank Accession No. AY502005; Norwalk virus, complete genome, GenBank Accession No. NC.sub.--001959; Norovirus Hu/GI/Otofuke/1979/JP genomic RNA, complete genome, GenBank Accession No. AB187514; Norovirus Hu/Hokkaido/133/2003/JP, GenBank Accession No. AB212306; Norovirus Sydney 2212, GenBank Accession No. AY588132; Norwalk virus strain SN2000JA, GenBank Accession No. AB190457; Lordsdale virus complete genome, GenBank Accession No. X86557; Norwalk-like virus genomic RNA, Gifu'96, GenBank Accession No. AB045603; Norwalk virus strain Vietnam 026, complete genome, GenBank Accession No. AF504671; Norovirus Hu/GII.4/2004/N/L, GenBank Accession No. AY883096; Norovirus Hu/GII/Hokushin/03/JP, GenBank Accession No. AB195227; Norovirus Hu/GII/Kamo/03/JP, GenBank Accession No. AB195228; Norovirus Hu/GII/Sinsiro/97/JP, GenBank Accession No. AB195226; Norovirus Hu/GII/Ina/02/JP, GenBank Accession No. AB195225; Norovirus Hu/NLV/GII/Neustrelitz260/2000/DE, GenBank Accession No. AY772730; Norovirus

Hu/NLV/Dresden174/pUS-NorII/1997/GE, GenBank Accession No. AY741811; Norovirus Hu/NLV/Oxford/B2S16/2002/UK, GenBank Accession No. AY587989; Norovirus Hu/NLV/Oxford/B4S7/2002/UK, GenBank Accession No. AY587987; Norovirus Hu/NLV/Witney/B7S2/2003/UK, GenBank Accession No. AY588030; Norovirus Hu/NLV/Banbury/B9S23/2003/UK, GenBank Accession No. AY588029; Norovirus Hu/NLV/ChippingNorton/2003/UK, GenBank Accession No. AY588028; Norovirus Hu/NLV/Didcot/B9S2/2003/UK, GenBank Accession No. AY588027; Norovirus Hu/NLV/Oxford/B8S5/2002/UK, GenBank Accession No. AY588026; Norovirus Hu/NLV/Oxford/B6S4/2003/UK, GenBank Accession No. AY588025; Norovirus Hu/NLV/Oxford/B6S5/2003/UK, GenBank Accession No. AY588024; Norovirus Hu/NLV/Oxford/B5S23/2003/UK, GenBank Accession No. AY588023; Norovirus Hu/NLV/Oxford/B6S2/2003/UK, GenBank Accession No. AY588022; Norovirus Hu/NLV/Oxford/B6S6/2003/UK, GenBank Accession No. AY588021; Norwalk-like virus isolate Bo/Thirsk10/00/UK, GenBank Accession No. AY126468; Norwalk-like virus isolate Bo/Penrith55/00/UK, GenBank Accession No. AY126476; Norwalk-like virus isolate Bo/Aberystwyth24/00/UK, GenBank Accession No. AY126475; Norwalk-like virus isolate Bo/Dumfries/94/UK, GenBank Accession No. AY126474; Norovirus NLV/IF2036/2003/Iraq, GenBank Accession No. AY675555; Norovirus NLV/IF1998/2003/Iraq, GenBank Accession No. AY675554; Norovirus NLV/BUDS/2002/USA, GenBank Accession No. AY660568; Norovirus NLV/Paris Island/2003/USA, GenBank Accession No. AY652979; Snow Mountain virus, complete genome, GenBank Accession No. AY134748; Norwalk-like virus NLV/Fort Lauderdale/560/1998/US, GenBank Accession No. AF414426; Hu/Norovirus/hiroshima/1999/JP(9912-02F), GenBank Accession No. AB044366; Norwalk-like virus strain 11MSU-MW, GenBank Accession No. AY274820; Norwalk-like virus strain B-1SVD, GenBank Accession No. AY274819; Norovirus genogroup 2 strain Hu/NoV/Farmington Hills/2002/USA, GenBank Accession No. AY502023; Norovirus genogroup 2 strain Hu/NoV/CS-G4/2002/USA, GenBank Accession No. AY502022; Norovirus genogroup 2 strain Hu/NoV/CS-G2/2002/USA, GenBank Accession No. AY502021; Norovirus genogroup 2 strain Hu/NoV/CS-G12002/USA, GenBank Accession No. AY502020; Norovirus genogroup 2 strain Hu/NoV/Anchorage/2002/USA, GenBank Accession No. AY502019; Norovirus genogroup 2 strain Hu/NoV/CS-D1/2002/CAN, GenBank Accession No. AY502018; Norovirus genogroup 2

strain Hu/NoV/Germanton/2002/USA, GenBank Accession No. AY502017; Human calicivirus NLV/GII/Langen1061/2002/DE, complete genome, GenBank Accession No. AY485642; Murine norovirus 1 polyprotein, GenBank Accession No. AY228235; Norwalk virus, GenBank Accession No. AB067536; Human calicivirus NLV/Mex7076/1999, GenBank Accession No. AF542090; Human calicivirus NLV/Oberhausen 455/01/DE, GenBank Accession No. AF539440; Human calicivirus NLV/Herzberg 385/01/DE, GenBank Accession No. AF539439; Human calicivirus NLV/Boxer/2001/US, GenBank Accession No. AF538679; Norwalk-like virus genomic RNA, complete genome, GenBank Accession No. AB081723; Norwalk-like virus genomic RNA, complete genome, isolate:Saitama U201, GenBank Accession No. AB039782; Norwalk-like virus genomic RNA, complete genome, isolate:Saitama U18, GenBank Accession No. AB039781; Norwalk-like virus genomic RNA, complete genome, isolate:Saitama U25, GenBank Accession No. AB039780; Norwalk virus strain:U25GII, GenBank Accession No. AB067543; Norwalk virus strain:U201 GII, GenBank Accession No. AB067542; Norwalk-like viruses strain 416/97003156/1996/LA, GenBank Accession No. AF080559; Norwalk-like viruses strain 408/97003012/1996/FL, GenBank Accession No. AF080558; Norwalk-like virus NLV/Burwash Landing/331/1995/US, GenBank Accession No. AF414425; Norwalk-like virus NLV/Miami Beach/326/1995/US, GenBank Accession No. AF414424; Norwalk-like virus NLV/White River/290/1994/US, GenBank Accession No. AF414423; Norwalk-like virus NLV/New Orleans/306/1994/US, GenBank Accession No. AF414422; Norwalk-like virus NLV/Port Canaveral/301/1994/US, GenBank Accession No. AF414421; Norwalk-like virus NLV/Honolulu/314/1994/US, GenBank Accession No. AF414420; Norwalk-like virus NLV/Richmond/283/1994/US, GenBank Accession No. AF414419; Norwalk-like virus NLV/Westover/302/1994/US, GenBank Accession No. AF414418; Norwalk-like virus NLV/UK3-17/12700/1992/GB, GenBank Accession No. AF414417; Norwalk-like virus NLV/Miami/81/1986/US, GenBank Accession No. AF414416; Snow Mountain strain, GenBank Accession No. U70059; Desert Shield virus DSV395, GenBank Accession No. U04469; Norwalk virus, complete genome, GenBank Accession No. AF093797; Hawaii calicivirus, GenBank Accession No. U07611; Southampton virus, GenBank Accession No. L07418; Norwalk virus (SRSV-KY-89/89/J), GenBank Accession No. L23828; Norwalk virus (SRSV-SMA/76/US), GenBank Accession No. L23831; Camberwell virus, GenBank Accession No. U46500; Human calicivirus strain Melksham, GenBank Accession No. X81879; Human

calicivirus strain MX, GenBank Accession No. U22498; Minireovirus TV24, GenBank Accession No. U02030; and Norwalk-like virus NLV/Gwynedd/273/1994/US, GenBank Accession No. AF414409; sequences of all of which (as entered by the date of filing of this application) are herein incorporated by reference. Additional Norovirus sequences are disclosed in the following patent publications: WO 2005/030806, WO 2000/79280, JP2002020399, US2003129588, U.S. Pat. No. 6,572,862, WO 1994/05700, and WO 05/032457, all of which are herein incorporated by reference in their entireties. See also Green *et al.* (2000) J. Infect. Dis., Vol. 181(Suppl. 2):S322-330; Wang *et al.* (1994) J. Virol., Vol. 68:5982-5990; Chen *et al.* (2004) J. Virol., Vol. 78: 6469-6479; Chakravarty *et al.* (2005) J. Virol., Vol. 79: 554-568; Hansman *et al.* (2006) J. Gen. Virol., Vol. 87:909-919; Bull *et al.* (2006) J. Clin. Micro., Vol. 44(2):327-333; Siebenga, *et al.* (2007) J. Virol., Vol. 81(18):9932-9941, and Fankhauser *et al.* (1998) J. Infect. Dis., Vol. 178:1571-1578; for sequence comparisons and a discussion of genetic diversity and phylogenetic analysis of Noroviruses.

[045] Nucleic acid and protein sequences for a number of Sapovirus isolates are also known. Representative Sapovirus sequences, including sequences of ORF1 and ORF2, and their encoded polypeptides from Sapovirus isolates are listed in the National Center for Biotechnology Information (NCBI) database. See, for example, GenBank entries: Sapovirus Mc10, GenBank Accession No. NC.sub.--010624; Sapporo virus, GenBank Accession No. U65427; Sapovirus Mc10, GenBank Accession No. AY237420; Sapovirus SaKaeo-15/Thailand, GenBank Accession No. AY646855; Sapporo virus, GenBank Accession No. NC.sub.--006269; Sapovirus C12, GenBank Accession No. NC.sub.--006554; Sapovirus C12, GenBank Accession No. AY603425; Sapovirus Hu/Dresden/pJG-Sap01/DE, GenBank Accession No. AY694184; Human calicivirus SLV/cruise ship/2000/USA, GenBank Accession No. AY289804; Human calicivirus SLV/Arg39, GenBank Accession No. AY289803; Porcine enteric calicivirus strain LL14, GenBank Accession No. AY425671; Porcine enteric calicivirus, GenBank Accession No. NC.sub.--000940; Human calicivirus strain Mc37, GenBank Accession No. AY237415; Mink enteric calicivirus strain Canada 151A, GenBank Accession No. AY144337; Human calicivirus SLV/Hou7-1181, GenBank Accession No. AF435814; Human calicivirus SLV/Mex14917/2000, GenBank Accession No. AF435813; Human calicivirus SLV/Mex340/1990, GenBank Accession No. AF435812; Porcine enteric calicivirus, GenBank Accession No. AF182760; Sapporo virus-London/29845, GenBank Accession No. U95645; Sapporo virus-Manchester, GenBank

Accession No. X86560; Sapporo virus-Houston/86, GenBank Accession No. U95643; Sapporo virus-Houston/90, GenBank Accession No. U95644; and Human calicivirus strain HuCV/Potsdam/2000/DEU, GenBank Accession No. AF294739; sequences of all of which (as entered by the date of filing of this application) are herein incorporated by reference. See also Schuffenecker *et al.* (2001) Arch Virol., Vol. 146(11):2115-2132; Zintz *et al.* (2005) Infect. Genet. Evol., Vol. 5:281-290; Farkas *et al.* (2004) Arch. Virol., Vol. 149:1309-1323; for sequence comparisons and a discussion of genetic diversity and phylogenetic analysis of Sapoviruses.

[046] The composite and consensus amino acid sequences may be derived from capsid sequences of at least two Norovirus genogroup I or genogroup II strains. In one embodiment, the VLP comprises a polypeptide having a composite sequence derived from a consensus sequence of the capsid proteins from two or more genogroup II, genotype 4 Norovirus strains. Non-limiting examples of genogroup II, genotype 4 Norovirus strains include Houston strain, Minerva strain, Laurens strain, Bristol strain, Lordsdale strain, Farmington Hills strain, Hunter strain, Carlow strain, and the US95/96-US, 2006a, and 2006b strains.

[047] In another embodiment of the invention, the virus-like particle is comprised of at least one composite polypeptide wherein the sequence of the composite polypeptide is derived from the VP1 sequences of Houston, Minerva, and Laurens. In another embodiment, the composite sequence is SEQ ID NO: 1. In still another embodiment, composite sequences based on Houston, Minerva, and Laurens may be derived from the consensus sequence defined by SEQ ID NO: 2.

[048] In some embodiments, the consensus sequence may be determined from Norovirus strains from at least two different genotypes or at least two different genogroups. In one embodiment of the present invention the virus-like particle is comprised of at least one polypeptide having a composite amino acid sequence, wherein the composite amino acid sequence is derived from a consensus sequence of capsid proteins of Norovirus strains from at least two different genotypes within a genogroup. By way of example, the consensus sequence may be derived from the capsid sequences of genogroup II, genotype 2 and genogroup II, genotype 4 Norovirus strains. In another embodiment, the consensus sequence may be derived from the capsid sequences of three or more genotypes within a genogroup.

[049] In other embodiments, the consensus sequence may be determined from Norovirus strains from at least two different genogroups. One such embodiment, among others, would be a VLP comprising a polypeptide having a composite amino acid sequence, wherein said composite amino acid sequence is derived from a consensus sequence of capsid proteins of genogroup I, genotype 1 and genogroup II, genotype 4 Norovirus strains.

[050] The present invention also provides a virus-like particle (VLP) comprising a composite polypeptide derived from a consensus sequence of capsid proteins from two or more circulating strains of Norovirus and a capsid protein from a second Norovirus. The second Norovirus may be a genogroup I or genogroup II Norovirus. The capsid protein from the second Norovirus can be the major capsid protein,VP1, which is encoded by ORF 2, or the minor capsid protein, VP2, which is encoded by ORF 3, or combinations of VP1 and VP2. In one embodiment, the capsid protein from the second Norovirus is a VP1 protein from a genogroup I Norovirus.

[051] In another embodiment, the invention provides a VLP comprising a composite polypeptide derived from a consensus sequence representing the capsid proteins of two or more circulating strains of Calicivirus and a second polypeptide having a second composite amino acid sequence, wherein said second composite amino acid sequence is derived from a consensus sequence representing the capsid proteins of two or more circulating strains of a second Calicivirus. Preferably, the virus-like particle has antigenic properties of the two or more circulating strains of the first Calicivirus and the two or more circulating strains of the second Calicivirus.

[052] The second polypeptide contains at least one different, at least three different, at least five different, at least ten different, at least fifteen different, at least twenty different, at least twenty-five different, at least thirty different, at least thirty-five different, at least forty different, at least forty-five different, or at least fifty different amino acids as compared to each of the capsid sequences of said two or more circulating strains of the second Calicivirus. In some embodiments, the second polypeptide forms a virus-like particle when expressed in a host cell. In another embodiment, the second Calicivirus is a Norovirus. In another embodiment, the Norovirus is a genogroup I Norovirus. The genogroup I Norovirus may be any of the genogroup I strains disclosed herein. In one embodiment, the genogroup I Norovirus is selected from the group consisting of Norwalk virus, Southampton virus, Hesse virus, and Chiba virus.

[053] The present invention also encompasses isolated polypeptides or fragments thereof having the composite amino acid sequences defined here in, as well as nucleic acids or vectors encoding the same. In one embodiment, the isolated polypeptide or fragment thereof has a composite amino acid sequence, wherein said composite amino acid sequence is derived from a consensus sequence representing the capsid proteins of two or more circulating strains of a non-enveloped virus, and wherein the polypeptide contains at least 1 different amino acid as compared to each of the capsid sequences of said two or more circulating strains. In another embodiment, the composite sequence contains at least 3 different amino acids compared to the capsid sequence of one or more circulating strains of the non-enveloped virus. In another embodiment, the composite sequence contains 5-50 different amino acids compared to the capsid sequence of one or more circulating strains of the non-enveloped virus. In still another embodiment, the consensus sequence is SEQ ID NO: 2.

[054] The composite polypeptide may have a sequence derived from two or more circulating strains of any non-enveloped virus disclosed herein. In one embodiment, the non-enveloped virus is a Calicivirus. In another embodiment, the Calicivirus is a Norovirus or Sapovirus. In another embodiment, the Norovirus is a genogroup I or genogroup II Norovirus, or combinations thereof. In yet another embodiment, the isolated polypeptide has the amino acid sequence of SEQ ID NO: 1.

[055] In one embodiment, the present invention provides an isolated nucleic acid encoding the polypeptide having a composite amino acid sequence, wherein said composite amino acid sequence is derived from a consensus sequence representing the capsid proteins of two or more circulating strains of a non-enveloped virus, and wherein the polypeptide contains at least 1 different amino acid as compared to each of the capsid sequences of said two or more circulating strains. In another embodiment, the nucleic acid has the sequence of SEQ ID NO: 3. In another embodiment, the invention provides a vector comprising an isolated nucleic acid encoding a composite polypeptide. In yet another embodiment, the invention provides a host cell comprising a vector encoding a composite polypeptide.

[056] The antigenic molecules of the present invention (*e.g.* VLPs, polypeptides, and fragments thereof) can be prepared by isolation and purification from the organisms in which they occur naturally, or they may be prepared by recombinant techniques. Once coding sequences for the desired particle-forming polypeptides have been isolated or synthesized, they can be cloned into

any suitable vector or replicon for expression. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is within the skill of an ordinary artisan. The vector is then used to transform an appropriate host cell. Suitable recombinant expression systems include, but are not limited to, bacterial (*e.g. E. coli, Bacillus subtilis*, and *Streptococcus*), baculovirus/insect, vaccinia, Semliki Forest virus (SFV), Alphaviruses (such as, Sindbis, Venezuelan Equine Encephalitis (VEE)), mammalian (*e.g.* Chinese hamster ovary (CHO) cells, HEK-293 cells, HeLa cells, baby hamster kidney (BHK) cells, mouse myeloma (SB20), and monkey kidney cells (COS)), yeast (*e.g. S. cerevisiae, S. pombe, Pichia pastori* and other *Pichia* expression systems), plant, and Xenopus expression systems, as well as others known in the art. Particularly preferred expression systems are mammalian cell lines, bacteria, insect cells, and yeast expression systems.

[057] Each of the aforementioned antigens (*e.g.* VLPs, polypeptides, or fragments thereof) is preferably used in the substantially pure state. Depending on the expression system and host selected, VLPs are produced by growing host cells transformed by an expression vector under conditions whereby the particle-forming polypeptide is expressed and VLPs can be formed. The selection of the appropriate growth conditions is within the skill of the art.

[058] Preferably the VLP antigens are prepared from insect cells such as Sf9, High Five, TniPro, Aedes aegypti, Autographa californica, Bombyx mori, Drosophila melanogaster, Spodoptera frugiperda, and Trichoplusia ni. The procedures for producing VLPs in insect cell culture is well known in the art (see, for example, U.S. Patent No. 6,942,865, which is incorporated herein by reference in its entirety). Briefly, the recombinant baculoviruses carrying the composite capsid sequence are constructed from the sythetic cDNAs. The recombinant baculovirus are then used to infect insect cell cultures (*e.g.* Sf9 , High Five and TniPro cells) and composite VLPs can be isolated from the cell culture. A “composite VLP” is a VLP comprising at least one polypeptide having a composite amino acid sequence derived from a consensus sequence representing the capsid proteins of two or more circulating strains of a non-enveloped virus.

[059] If the VLPs are formed intracellularly, the cells are then disrupted, using chemical, physical or mechanical means, which lyse the cells yet keep the VLPs substantially intact. Such methods are known to those of skill in the art and are described in, *e.g.*, Protein Purification Applications: A Practical Approach, (E. L. V. Harris and S. Angal, Eds., 1990).

[060] The particles are then isolated (or substantially purified) using methods that preserve the integrity thereof, such as, by density gradient centrifugation, *e.g.*, sucrose gradients, PEG-precipitation, pelleting, and the like (see, e.g., Kirnbauer et al. J. Virol. (1993) 67:6929-6936), as well as standard purification techniques including, *e.g.*, ion exchange and gel filtration chromatography.

[061] General texts which describe molecular biological techniques, which are applicable to the present invention, such as cloning, mutation, and the like, include Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology volume 152 Academic Press, Inc., San Diego, Calif. (Berger); Sambrook *et al.*, Molecular Cloning--A Laboratory Manual (3rd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 2000 ("Sambrook") and Current Protocols in Molecular Biology, F. M. Ausubel *et al.*, eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., ("Ausubel"). These texts describe mutagenesis, the use of vectors, promoters and many other relevant topics related to, *e.g.*, the cloning and expression of capsid proteins of non-enveloped viruses, such as Calicivirus.

[062] In some embodiments, the antigenic molecules of the present invention (*e.g.* VLPs, polypeptides, and fragments thereof) are produced *in vivo* by administration of a vector comprising an isolated nucleic acid encoding a composite polypeptide. Suitable vectors include, but are not limited to, viral vectors, such as Vesicular Stomatitis Virus (VSV) vector, Equine Encephalitis Virus (EEV) vector, Poxvirus vector, Adenovirus vector, Adeno-Associated Virus (AAV), retrovirus vector, and expression plasmids, such as pFastBac1, pWINEO, pSV2CAT, pOG44, pXT1, pSG, pSVK3, pBPV, pMSG, and pSVL. Other suitable vectors will be readily apparent to the skilled artisan.

[063] The present invention also encompasses a vaccine formulation comprising the VLPs, polypeptides, or nucleic acids described herein. In one embodiment, the vaccine formulation comprises a composite VLP and a second virus-like particle, wherein said second virus-like particle comprises a capsid protein from a Norovirus. The second VLP may comprise a native capsid protein from a genogroup I or genogroup II Norovirus. The second VLP may comprise a full length Norovirus capsid protein such as VP1 and/or VP2 protein or certain VP1 or VP2 derivatives. Alternatively, the second VLP comprises a truncated capsid protein, such as a truncated VP1 protein. The truncation may be an N- or C-terminal truncation. Truncated capsid

proteins are suitably functional capsid protein derivatives. Functional capsid protein derivatives are capable of raising an immune response in the same way as the immune response is raised by a VLP consisting of the full length capsid protein. Vaccine formulations comprising mixtures of VLPs are described in WO 2008/042789, which is herein incorporated by reference in its entirety. Purely by way of example the vaccine formulation can contain VLPs from one or more strains of Norovirus genogroup I together with VLPs comprising a composite protein from one or more strains of Norovirus genogroup II. Preferably, the Norovirus VLP mixture is composed of the strains of Norwalk and genogroup II, genotype 4 Noroviruses. In another embodiment, the vaccine formulation comprises a composite VLP and a Norwalk VLP, wherein the composite VLP comprises a polypeptide having an amino acid sequence of SEQ ID NO: 1. In still another embodiment, the vaccine formulation comprises a first composite VLP and a second composite VLP, wherein said first and second composite VLPs comprise at least one polypeptide derived from different consensus sequences. For instance, a first composite VLP comprises a composite protein from one or more strains of Norovirus genogroup I and a second composite VLP comprises a composite protein from one or more strains of Norovirus genogroup II. In one embodiment, the first composite VLP comprises a composite protein from one or more strains of Norovirus genogroup I, genotype 1 (GI.1) and a second composite VLP comprises a composite protein from one or more strains of Norovirus genogroup II, genotype 4 (GII.4).

[064] In some embodiments, the vaccine formulation further comprises an adjuvant. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as Bordatella pertussis or Mycobacterium tuberculosis derived proteins. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Pifco Laboratories, Detroit, Mich.); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, N.J.); mineral salts, including aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate and salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; and Quil A.

[065] Suitable adjuvants also include, but are not limited to, toll-like receptor (TLR) agonists, monophosphoryl lipid A (MPL), synthetic lipid A, lipid A mimetics or analogs, aluminum salts, cytokines, saponins, muramyl dipeptide (MDP) derivatives, CpG oligos, lipopolysaccharide

(LPS) of gram-negative bacteria, polyphosphazenes, emulsions, virosomes, cochleates, poly(lactide-co-glycolides) (PLG) microparticles, poloxamer particles, microparticles, liposomes, oil-in-water emulsion, MF59, and squalene. In some embodiments, the adjuvants are bacterially-derived exotoxins. In other embodiments, adjuvants which stimulate a Th1 type response, such as 3DMPL or QS21, may be used. In certain embodiments, the adjuvant is a combination of MPL and aluminum hydroxide.

[066] In some embodiments, the adjuvant is monophosphoryl lipid A (MPL). MPL is a non-toxic derivative of lipid A from Salmonella, is a potent TLR-4 agonist that has been developed as a vaccine adjuvant (Evans *et al.* (2003) Expert Rev Vaccines, Vol. 2: 219-229). In pre-clinical murine studies intranasal MPL has been shown to enhance secretory, as well as systemic, humoral responses (Baldridge *et al.* (2000) Vaccine, Vol. 18: 2416-2425; Yang *et al.* (2002) Infect Immun., Vol. 70: 3557-3565). It has also been proven to be safe and effective as a vaccine adjuvant in clinical studies of greater than 120,000 patients (Baldrick *et al.* (2002) Regul Toxicol Pharmacol, Vol. 35: 398-413). MPL stimulates the induction of innate immunity through the TLR-4 receptor and is thus capable of eliciting nonspecific immune responses against a wide range of infectious pathogens, including both gram negative and gram positive bacteria, viruses, and parasites (Persing *et al.* (2002) Trends Microbiol, Vol. 10: S32-37). Inclusion of MPL in intranasal formulations should provide rapid induction of innate responses, eliciting nonspecific immune responses from viral challenge while enhancing the specific responses generated by the antigenic components of the vaccine. In some embodiments, MPL can be combined with one or more additional adjuvants. For instance, MPL can be combined with aluminum hydroxide to create a suitable adjuvant for intramuscular administration of a vaccine formulation.

[067] In other embodiments, the adjuvant is a naturally occurring oil, such as squalene. Squalene is a triterpenoid hydrocarbon oil ($C_{30}H_{50}$) produced by plants and is present in many foods. Squalene is also produced abundantly by human beings, for whom it serves as a precursor of cholesterol and steroid hormones. It is synthesized in the liver and the skin, transported in the blood by very-low-density lipoproteins (VLDL) and low-density lipoproteins (LDL), and secreted in large amounts by sebaceous glands.

[068] Since it is a natural component of the human body and is biodegradable, squalene has been used as a component of vaccine adjuvants. One of these squalene adjuvants is MF59, an oil-

in-water emulsion developed by Chiron. MF59 has been shown in various preclinical and clinical studies to significantly enhance the immune response to a wide variety of vaccine antigens. MF59 is a part of an influenza subunit vaccine, which has been licensed in various European countries since 1997. More than 20 million doses of this vaccine have been given, and it has been shown to have an excellent safety profile. The safety of vaccines with the MF59 adjuvant has also been shown by various investigational clinical studies using recombinant antigens from hepatitis B virus, hepatitis C virus, cytomegalovirus, herpes simplex virus, human immunodeficiency virus, uropathogenic *Escherichia coli*, etc., in various age groups, including 1- to 3-day-old newborns.

[069] The term "effective adjuvant amount" or “effective amount of adjuvant” will be well understood by those skilled in the art, and includes an amount of one or more adjuvants which is capable of stimulating the immune response to an administered antigen, *i.e.*, an amount that increases the immune response of an administered antigen composition, as measured in terms of the IgA levels in the nasal washings, serum IgG or IgM levels, or B and T-Cell proliferation. Suitably effective increases in immunoglobulin levels include by more than 5%, preferably by more than 25%, and in particular by more than 50%, as compared to the same antigen composition without any adjuvant.

[070] In another embodiment of the invention, the vaccine formulation may further comprise a delivery agent, which functions to enhance antigen uptake based upon, but not restricted to, increased fluid viscosity due to the single or combined effect of partial dehydration of host mucopolysaccharides, the physical properties of the delivery agent, or through ionic interactions between the delivery agent and host tissues at the site of exposure, which provides a depot effect. Alternatively, the delivery agent can increase antigen retention time at the site of delivery (*e.g.*, delay expulsion of the antigen). Such a delivery agent may be a bioadhesive agent. In some embodiments, the bioadhesive may be a mucoadhesive agent selected from the group consisting of glycosaminoglycans (*e.g.,* chondroitin sulfate, dermatan sulfate chondroitin, keratan sulfate, heparin, heparan sulfate, hyaluronan), carbohydrate polymers (*e.g.*, pectin, alginate, glycogen, amylase, amylopectin, cellulose, chitin, stachyose, unulin, dextrin, dextran) , cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrollidone, polysaccharides (including mucin, other mucopolysaccharides, and GelSite®, a natural acidic polysaccharide extracted from the aloe plant), polyions, cellulose derivatives (*e.g.*, hydroxypropyl

methylcellulose, carboxymethylcellulose), proteins (*e.g.* lectins, fimbrial proteins), and deoxyribonucleic acid. In one embodiment, the vaccine formulations comprise a polysaccharide such as chitosan, chitosan salt, chitosan base, or a natural polysaccharide (e.g. GelSite®).

[071] Chitosan, a positively charged linear polysaccharide derived from chitin in the shells of crustaceans, is a bioadhesive for epithelial cells and their overlaying mucus layer. Formulation of antigens with chitosan increases their contact time with the nasal membrane, thus increasing uptake by virtue of a depot effect (Illum *et al.* (2001) Adv Drug Deliv Rev, Vol. 51: 81-96; Illum *et al.* (2003) J Control Release, Vol. 87: 187-198; Davis *et al.* (1999) Pharm Sci Technol Today, Vol. 2: 450-456; Bacon *et al.* (2000) Infect Immun., Vol. 68: 5764-5770; van der Lubben *et al.* (2001) Adv Drug Deliv Rev, Vol. 52: 139-144; van der Lubben *et al.* (2001) Eur J Pharm Sci, Vol. 14: 201-207; Lim *et al.* (2001) AAPS Pharm Sci Tech, Vol. 2: 20). Chitosan has been tested as a nasal delivery system for several vaccines, including influenza, pertussis and diphtheria, in both animal models and humans (Illum *et al.* (2001) Adv Drug Deliv Rev, Vol. 51: 81-96; Illum *et al.* (2003) J Control Release, Vol. 87: 187-198; Bacon *et al.* (2000) Infect Immun., Vol. 68: 5764-5770; Jabbal-Gill *et al.* (1998) Vaccine, Vol. 16: 2039-2046; Mills *et al.* (2003) A Infect Immun, Vol. 71: 726-732; McNeela *et al.* (2004) Vaccine, Vol. 22: 909-914). In these trials, chitosan was shown to enhance systemic immune responses to levels equivalent to parenteral vaccination. In addition, significant antigen-specific IgA levels were also measured in mucosal secretions. Thus, chitosan can greatly enhance a nasal vaccine's effectiveness. Moreover, due to its physical characteristics, chitosan is particularly well suited to intranasal vaccines formulated as powders (van der Lubben *et al.* (2001) Eur J Pharm Sci, Vol. 14: 201-207; Mikszta *et al.* (2005) J Infect Dis, Vol. 191: 278-288; Huang *et al.* (2004) Vaccine, Vol. 23: 794-801).

[072] In another embodiment of the invention, the vaccine formulation may further comprise a pharmaceutically acceptable carrier. A pharmaceutically acceptable carrier, including any suitable diluent or excipient, includes any pharmaceutical agent that does not itself induce the production of an immune response harmful to the subject receiving the vaccine formulation, and which may be administered without undue toxicity. As used herein, the term "pharmaceutically acceptable" means being approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopia, European Pharmacopia or other generally recognized pharmacopia for use in mammals, and more particularly in humans. Pharmaceutically acceptable

carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, sterile isotonic aqueous buffer, and combinations thereof. A thorough discussion of pharmaceutically acceptable carriers, diluents, and other excipients is presented in Remington's Pharmaceutical Sciences (Mack Pub. Co. N.J. current edition). The formulation should suit the mode of administration. In a preferred embodiment, the formulation is suitable for administration to humans, preferably the formulation is sterile, non-particulate and/or non-pyrogenic. The vaccine formulation, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

[073] In some embodiments of the present invention, among others, vaccine formulations comprise chitosan, a chitosan salt, or a chitosan base. The molecular weight of the chitosan may be between 10 kDa and 800 kDa, preferably between 100 kDa and 700 kDa and more preferably between 200 kDa and 600 kDa. The concentration of chitosan in the composition will typically be up to about 80% (w/w), for example, 5%, 10%, 30%, 50%, 70% or 80%. The chitosan is one which is preferably at least 75% deacetylated, for example 80-90%, more preferably 82-88% deacetylated, particular examples being 83%, 84%, 85%, 86% and 87% deacetylation.

[074] The compositions of the invention can be formulated for administration as vaccines or antigenic formulations. As used herein, the term "vaccine" refers to a formulation which contains VLPs or other antigens of the present invention as described above, which is in a form that is capable of being administered to a vertebrate and which induces a protective immune response sufficient to induce immunity to prevent and/or ameliorate an infection and/or to reduce at least one symptom of an infection and/or to enhance the efficacy of another dose of VLPs or antigen. As used herein, the term "antigenic formulation" or "antigenic composition" refers to a preparation which, when administered to a vertebrate, *e.g.* a mammal, will induce an immune response. As used herein, the term "immune response" refers to both the humoral immune response and the cell-mediated immune response. The humoral immune response involves the stimulation of the production of antibodies by B lymphocytes that, for example, neutralize infectious agents, block infectious agents from entering cells, block replication of said infectious agents, and/or protect host cells from infection and destruction. The cell-mediated immune response refers to an immune response that is mediated by T-lymphocytes and/or other cells, such as macrophages, against an infectious agent, exhibited by a vertebrate (*e.g.*, a human), that prevents or ameliorates infection or reduces at least one symptom thereof. In particular,

"protective immunity" or "protective immune response" refers to immunity or eliciting an immune response against an infectious agent, which is exhibited by a vertebrate (*e.g.*, a human), that prevents or ameliorates an infection or reduces at least one symptom thereof. Specifically, induction of a protective immune response from administration of the vaccine is evident by elimination or reduction of the presence of one or more symptoms of gastroenteritis or a reduction in the duration or severity of such symptoms. Clinical symptoms of gastroenteritis from Norovirus include nausea, diarrhea, loose stool, vomiting, fever, and general malaise. A protective immune response that reduces or eliminates disease symptoms will reduce or stop the spread of a Norovirus outbreak in a population. Vaccine preparation is generally described in Vaccine Design ("The subunit and adjuvant approach" (eds Powell M. F. & Newman M. J.) (1995) Plenum Press New York). The compositions of the present invention can be formulated, for example, for administration to a subject by mucosal or parenteral (*e.g.* intramuscular, intravenous, subcutaneous, intradermal, subdermal, or transdermal) routes of administration. Such mucosal administration could be, but is not limited to, through gastro-intestinal, intranasal, oral, or vaginal delivery. In one embodiment, the vaccine formulation is in the form of a nasal spray, nasal drops or dry powder. In another embodiment, the vaccine formulation is in a form suitable for intramuscular administration.

[075] Vaccine formulations of the invention may be liquid formulations or dry powder formulations. Where the composition is intended for delivery to the respiratory (*e.g.* nasal) mucosa, typically it is formulated as an aqueous solution for administration as an aerosol or nasal drops, or alternatively, as a dry powder, *e.g.* for rapid deposition within the nasal passage. Compositions for administration as nasal drops may contain one or more excipients of the type usually included in such compositions, for example preservatives, viscosity adjusting agents, tonicity adjusting agents, buffering agents, and the like. Viscosity agents can be microcrystalline cellulose, chitosan, starches, polysaccharides, and the like. Compositions for administration as dry powder may also contain one or more excipients usually included in such compositions, for example, mucoadhesive agents, bulking agents, and agents to deliver appropriate powder flow and size characteristics. Bulking and powder flow and size agents may include mannitol, sucrose, trehalose, and xylitol.

[076] In one embodiment, the vaccine formulation contains one or more composite VLPs as the immunogen, an adjuvant such as MPL®, squalene, or MF59®, a biopolymer such as chitosan or

GelSite® to promote adhesion to mucosal surfaces, and bulking agents such as mannitol and sucrose.

[077] For example, a vaccine may be formulated as 10 mg of a dry powder containing one or more composite VPLs as discussed herein, such as the GII.4 composite VPL, MPL® adjuvant, chitosan mucoadhesive, and mannitol and sucrose as bulking agents and to provide proper flow characteristics. The formulation may comprise about 7.0 mg (25 to 90% w/w range) chitosan, about 1.5 mg mannitol (0 to 50% w/w range), about 1.5 mg sucrose (0 to 50% w/w range), about 25 µg MPL® (0.1 to 5% w/w range), and about 100 µg composite VLP antigen (0.05 to 5% w/w range).

[078] Composite VLPs/antigens may be present in a concentration of from about 0.01% (w/w) to about 80% (w/w). In one embodiment, VLPs can be formulated at dosages of about 5 µg, about 15 µg, about 25 µg , about 50 µg, about 100 µg, about 200 µg, about 500 µg, and about 1 mg per 10 mg dry powder formulation (0.05, 0.15, 0.25, 0.5, 1.0, 2.0, 5.0, and 10.0% w/w) for administration into both nostrils (10 mg per nostril) or about 10 µg, about 30 µg, about 50 µg, about 100 µg, about 200 µg, about 400 µg, about 1 mg, and about 2 mgs (0.1, 0.3, 0.5, 1.0, 2.0, 4.0, 10.0 and 20.0% w/w) per 20 mg dry powder formulation for administration into one nostril. The formulation may be given in one or both nostrils during each administration. There may be a booster administration 1 to 12 weeks after the first administration to improve the immune response. The content of each VLP/antigen in the vaccine and antigenic formulations may be in the range of 1 µg to 100 mg, preferably in the range 1-1000 µg, more preferably 5-500 µg, most typically in the range 10-200 µg. Total VLP/antigen administered at each dose can be either about 10 µg, about 30 µg, about 200 µg, about 250 µg, about 400 µg, about 500 µg, or about 1000 µg. The total vaccine dose can be administered into one nostril or can be split in half for administration to both nostrils. Dry powder characteristics are such that less than 10% of the particles are less than 10 µm in diameter. Mean particle sizes range from 10 to 500 µm in diameter.

[079] In another embodiment of the invention, the dry powder formulation may be in combination with one or more devices for administering one or more doses of the formulation. Such a device may be a single-use nasal administrative device. In another embodiment, one or more doses are unit doses.

[080] In some embodiments, the antigenic and vaccine formulations are liquid formulations for subsequent administration to a subject. A liquid formulation intended for intranasal administration would comprise composite VLP/antigen(s), adjuvant, and a delivery agent such as chitosan. Liquid formulations for parenteral (*e.g.*, subcutaneous, intradermal, or intramuscular (i.m.)) administration would comprise composite VLP/antigen(s), adjuvant, and a buffer, without a delivery agent (*e.g.*, chitosan).

[081] Preferably the antigenic and vaccine formulations hereinbefore described are lyophilized and stored anhydrous until they are ready to be used, at which point they are reconstituted with diluent. Alternatively, different components of the composition may be stored separately in a kit (any or all components being lyophilized). The components may remain in lyophilized form for dry formulation or be reconstituted for liquid formulations, and either mixed prior to use or administered separately to the patient. For dry powder administration, the vaccine or antigenic formulation may be preloaded into an intranasal delivery device and stored until use. Preferably, such intranasal delivery device would protect and ensure the stability of its contents.

[082] The invention also encompasses compositions comprising one or more of the immunogenic nucleic acids, polypeptides, and/or VLPs, described herein. Different polypeptides, including composite polypeptides and capsid polypeptides or fragments thereof may be mixed together in a single formulation. Within such combinations, an antigen of the immunogenic composition may be present in more than one polypeptide, or multiple epitope polypeptide.

[083] The immunogenic compositions may comprise a mixture of composite polypeptides and nucleic acids encoding composite polypeptides, which in turn may be delivered using the same or different vehicles. Antigens may be administered individually or in combination, in *e.g.*, prophylactic (*i.e.*, to prevent infection) or therapeutic (to treat infection) immunogenic compositions. The immunogenic composition may be given more than once (*e.g.*, a "prime" administration followed by one or more "boosts") to achieve the desired effects. The same composition can be administered in one or more priming and one or more boosting steps. Alternatively, different compositions can be used for priming and boosting.

[084] The present invention also contemplates a method of inducing protective immunity to a viral infection in a subject comprising administering any of the vaccine formulations described herein. In one embodiment, the viral infection is a Norovirus infection. In another embodiment, the vaccine formulation confers protection from one or more symptoms of Norovirus infection.

[085] The present invention also provides a method for making a VLP comprising a composite polypeptide. In one embodiment, the method comprises aligning amino acid sequences of capsid proteins from two or more circulating strains of a non-enveloped virus; determining a consensus sequence from said aligned amino acid sequences; preparing a composite sequence based on said consensus sequence that contains at least one different amino acid as compared to each of the capsid sequences of said two or more circulating strains; and expressing said composite sequence in a host cell, thereby producing a virus-like particle. In another embodiment, the composite sequence contains at least three different amino acids as compared to each of the capsid sequences of said two or more circulating strains. In another embodiment, the composite sequence contains at least five different amino acids as compared to each of the capsid sequences of said two or more circulating strains. In yet another embodiment, the composite sequence contains at least nine different amino acids as compared to each of the capsid sequences of said two or more circulating strains. In some embodiments, the consensus sequence may be determined from aligning nucleotide sequences of capsid proteins from two or more circulating strains of a non-enveloped virus; and preparing a composite nucleotide sequence based on said consensus sequence. Non-limiting examples of a non-enveloped virus suitable for use in the method are Calicivirus, Picornavirus, Astrovirus, Adenovirus, Reovirus, Polyomavirus, Papillomavirus, Parvovirus, and Hepatitis E virus. In some embodiments, the non-enveloped virus is a Calicivirus. The Calicivirus may be a Norovirus or Sapovirus. In another embodiment, the Norovirus is a genogroup I or genogroup II Norovirus.

[086] The invention will now be illustrated in greater detail by reference to the specific embodiments described in the following examples. The examples are intended to be purely illustrative of the invention and are not intended to limit its scope in any way.

EXAMPLES

Example 1. Design of a Norovirus GII.4 Consensus Gene

[087] A consensus amino acid sequence for the major capsid protein (VP1) of genogroup II, genotype 4 (GII.4) Norovirus was determined by homology comparison of two recently circulating GII.4 Strains, Minerva, AKA 2006-a; and Laurens, AKA 2006-b, with a GII.4 Houston strain obtained in 2002. The alignment of the three different Norovirus GII.4 isolates is

shown below. The consensus sequence (SEQ ID NO: 2) determined from the homology comparison of the three GII.4 strains is shown in Figure 1.

[088] A composite sequence was derived from the consensus sequence by selecting amino acids from the Minerva sequence in variable positions of the consensus sequence where all three strains differed. The chosen amino acids were present in antigenic regions near to but not including the proposed carbohydrate binding domain. The composite GII.4 sequence was used for the production of a synthetic gene encoding a composite GII.4 Norovirus VP1 protein (SEQ ID NO: 1). The GII.4 composite VP1 amino acid sequence (GII.4 Comp) is shown in the alignment below as SEQ ID NO: 1 with the amino acid sequences of the VP1 proteins from Houston, Minerva, and Laurens virus (SEQ ID NOs: 4, 5, and 6, respectively). The DNA sequence encoding the GII.4 composite VP1 (SEQ ID NO: 3) is shown in Figure 2.

```
    Houston MKMASSDASPSDGSTANLVPEVNNEVMALEPVVGAAIAAPVAGQQNVIDPWIR 53
    Minerva MKMASSDANPSDGSTANLVPEVNNEVMALEPVVGAAIAAPVAGQQNVIDPWIR 53
    Laurens MKMASNDANPSDGSAANLVPEVNNEVMALEPVVGAAIAAPVAGQQNVIDPWIR 53
GII.4 Comp MKMASSDANPSDGSTANLVPEVNNEVMALEPVVGAAIAAPVAGQQNVIDPWIR 53

    Houston NNFVQAPGGEFTVSPRNAPGEILWSAPLGPDLNPYLSHLARMYNGYAGGFEVQ 106
    Minerva NNFVQAPGGEFTVSPRNAPGEILWSAPLGPDLNPYLSHLARMYNGYAGGFEVQ 106
    Laurens NNFVQAPGGEFTVSPRNAPGEILWSAPLGPDLNPYLSHLARMYNGYAGGFEVQ 106
GII.4 Comp NNFVQAPGGEFTVSPRNAPGEILWSAPLGPDLNPYLSHLARMYNGYAGGFEVQ 106

    Houston VILAGNAFTAGKIIFAAVPPNFPTEGLSPSQVTMFPHIIVDVRQLEPVLIPLP 159
    Minerva VILAGNAFTAGKIIFAAVPPNFPTEGLSPSQVTMFPHIIVDVRQLEPVLIPLP 159
    Laurens VILAGNAFTAGKIIFAAVPPNFPTEGLSPSQVTMFPHIIVDVRQLEPVLIPLP 159
GII.4 Comp VILAGNAFTAGKIIFAAVPPNFPTEGLSPSQVTMFPHIIVDVRQLEPVLIPLP 159

    Houston DVRNNFYHYNQSNDPTIKLIAMLYTPLRANNAGDDVFTVSCRVLTRPSPDFDF 212
    Minerva DVRNNFYHYNQSNDPTIKLIAMLYTPLRANNAGDDVFTVSCRVLTRPSPDFDF 212
    Laurens DVRNNFYHYNQSNDSTIKLIAMLYTPLRANNAGEDVFTVSCRVLTRPSPDFDF 212
GII.4 Comp DVRNNFYHYNQSNDPTIKLIAMLYTPLRANNAGDDVFTVSCRVLTRPSPDFDF 212

    Houston IFLVPPTVESRTKPFTVPILTVEEMTNSRFPIPLEKLFTGPSGAFVVQPQNGR 265
    Minerva IFLVPPTVESRTKPFSVPILTVEEMTNSRFPIPLEKLFTGPSSAFVVQPQNGR 265
    Laurens IFLVPPTVESRTKPFTVPILTVEEMTNSRFPIPLEKLFTGPSGAFVVQPQNGR 265
GII.4 Comp IFLVPPTVESRTKPFTVPILTVEEMTNSRFPIPLEKLFTGPSGAFVVQPQNGR 265

    Houston CTTDGVLLGTTQLSPVNICTFRGDVTHIAGTHDYTMNLASQNWNNYDPTEEIP 318
    Minerva CTTDGVLLGTTQLSPVNICTFRGDVTHIAGTQEYTMNLASQNWNNYDPTEEIP 318
    Laurens CTTDGVLLGTTQLSPVNICTFRGDVTHIAGSRNYTMNLASLNWNNYDPTEEIP 318
GII.4 Comp CTTDGVLLGTTQLSPVNICTFRGDVTHIAGTQEYTMNLASQNWNNYDPTEEIP 318

    Houston APLGTPDFVGKIQGVLTQTTRGDGSTRGHKATVSTGSVHFTPKLGSVQFTTDT 371
    Minerva APLGTPDFVGKIQGVLTQTTRRDGSTRGHKATVSTGSVHFTPKLGRIQFSTDT 371
    Laurens APLGTPDFVGKIQGVLTQTTKGDGSTRGHKATVYTGSAPFTPKLGSVQFSTDT 371
GII.4 Comp APLGTPDFVGKIQGVLTQTTRGDGSTRGHKATVSTGSVHFTPKLGSVQFSTDT 371


    Houston NNDLETGQNTKFTPVGVVQDGNSAHQNEPQQWVLPNYSGRTGHNVHLAPAVAP 424
    Minerva SNDFETGQNTRFTPVGVVQDGSTTHQNEPQQWVLPDYSGRDSHNVHLAPAVAP 424
    Laurens ENDFETHQNTKFTPVGVIQDGSTTHRNEPQQWVLPSYSGRNVHNVHLAPAVAP 424
GII.4 Comp SNDFETGQNTKFTPVGVVQDGSTTHQNEPQQWVLPDYSGRDSHNVHLAPAVAP 424
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   Houston TFPGEQLLFFRSTMPGCSGYPNMNLDCLLPQEWVLHFYQEAAPAQSDVALLRF 477
   Minerva SFPGEQLLFFRSTMPGCSGYPNMNLDCLLPQEWVQHFYQEAAPAQSDVALLRF 477
   Laurens TFPGEQLLFFRSTMPGCSGYPNMDLDCLLPQEWVQHFYQEAAPAQSDVALLRF 477
GII.4 Comp TFPGEQLLFFRSTMPGCSGYPNMNLDCLLPQEWVQHFYQEAAPAQSDVALLRF 477

   Houston VNPDTGRVLFECKLHKSGYVTVAHTGPHDLVIPPNGYFRFDSWVNQFYTLAPM 530
   Minerva VNPDTGRVLFECKLHKSGYVTVAHTGQHDLVIPPNGYFRFDSWVNQFYTLAPM 530
   Laurens VNPDTGRVLFECKLHKSGYVTVAHTGQHDLVIPPNGYFRFDSWVNQFYTLAPM 530
GII.4 Comp VNPDTGRVLFECKLHKSGYVTVAHTGQHDLVIPPNGYFRFDSWVNQFYTLAPM 530

   Houston GNGAGRRRA (SEQ ID NO: 4)                             539
   Minerva GNGTGRRRA (SEQ ID NO: 5)                             539
   Laurens GNGTGRRRA (SEQ ID NO: 6)                             539
GII.4 Comp GNGTGRRRA (SEQ ID NO: 1)                             539
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Example 2. Purification of Composite VLPs

[089] Synthetic gene construct of Norovirus GII.4 composite sequence for capsid domains described in Example 1 was cloned into recombinant Baculovirus. Infection of insect cells demonstrated high yield of production of VLP. A 40 mL aliquot of a P2 pFastBac recombinant baculovirus stock for the composite VLP VP1 gene was processed with a sucrose gradient to verify the expression and assembly of composite VLPs. The conditioned media was first layered onto a 30% sucrose cushion and then centrifuged at 140 K x g to pellet the VLP. The pellet was resuspended, layered onto a sucrose gradient and then centrifuged at 140 K x g. A visible white layer was observed within the gradient after centrifugation. 500 µL fractions from the gradient were collected and then analyzed by SDS-PAGE / Coomassie gel (Figure 3). The expected banding pattern for composite VLP at ~56 kDa was observed within the sucrose gradient fractions.

[090] Using a high pressure liquid chromatography system with a running buffer of 20 mM Tris 150 mM NaCl pH 7 at a flow rate of 0.5 mL/minute, a 50 µL aliquot of the composite expression cell culture supernatant was loaded on to a Superose-6 size exclusion column. An intact VLP peak was observed at ~15.3 minutes at 280 nm and 220 nm confirming integrity of the composite VLPs (Figure 4).

[091] Composite VLPs were also purified from conditioned media using column chromatography. Conditioned media was processed by cation exchange chromatography. The cation exchange elution fraction was further purified by hydrophobic interaction chromatography (HIC). The HIC elution fraction was concentrated and buffer exchanged by tangential flow filtration. The final product was sterile filtered and stored at 4°C. 500 ng of the purified composite VLPs (CM3 lot) was analyzed by silver-stained SDS-PAGE (Figure 5).

[092] Using a high pressure liquid chromatography system with a running buffer of 20 mM Tris 150 mM NaCl pH 7 at a flow rate of 1.0 mL/minute, a 50 µL aliquot of the purified CM3 composite VLPs was loaded on to a Superose-6 size exclusion column. An intact VLP peak was observed at ~7.5 minutes at 280 nm confirming integrity of the composite VLPs (Figure 6).

Example 3. Composite Immunogenicity

[093] Female C57BL/6 mice approximately 8-10 weeks of age were immunized intraperitoneally with decreasing concentrations of composite VLP (CVLP) starting with 50µg and decreasing 2 fold to 0.19 µg. The CVLP contained a polypeptide having the sequence of SEQ ID NO: 1 as described in Example 1. A group of animals immunized with PBS alone was included as a negative control. Serum samples were collected and analyzed for the presence of CVLP-specific IgG by ELISA (Figure 7). The results from this experiment indicate that the linear range of the dose curve is between approximately 6 µg and 0.2 µg. Doses above 6.25 µg of CVLP do not appear to enhance immune responses in a dose-dependent manner. The EC_{50} value (defined as the effective dose yielding a 50% response) was calculated to be approximately 1.0µg/mL using Softmax Pro software (Molecular Devices Corporation, Sunnyvale, CA).

Example 4. Multiple Antigen Effect of Composite VLPs

[094] Female C57BL/6 mice (8-10 weeks of age) were immunized intraperitoneally with varying doses of either Norwalk VLP alone (NVLP), composite VLP (CVLP) alone or in combination. A group of animals immunized with PBS alone was included as a negative control. Serum samples were collected and analyzed for the presence of antigen-specific IgG by ELISA (Figures 8 and 9) . The results indicate that immunizing with the combination of the CVLP and NVLP enhances the immune response such that a higher IgG level is achieved with a lower dose of antigen. For example, immunizing with 1µg of each NVLP and CVLP elicits a more robust immune response then administering with either VLP alone. The antibodies from animals immunized with CVLP did not cross-react with NVLP and vise versa (data not shown).

Example 5. Composite VLPs Elicit Cross-Reactivite Antibodies

[095] Female C57/BL6 mice, approximately 10-12 weeks of age, were immunized intraperitoneally with either 30 µg Houston VLPs or composite VLPs formulated with MPL (20

μg) as an adjuvant. The composite VLPs contained a polypeptide having the sequence of SEQ ID NO: 1 as described in Example 1. The mice were bled on day 21 following immunization and the sera were assayed in an antigen-specific ELISA to determine antibody titers for composite, Houston, Laurens, and Norwalk VLPs. The data are shown in Figure 10. Immunization with composite VLP induces a broader response across more serotypes as evidenced by the greater response to the Laurens strain while maintaining response to the Houston strain. Immunization with Houston VLPs also induces cross-reactive antibodies against composite and Laurens but the magnitude of the response is not as great as that observed with immunization with the composite VLPs. There was no detectable response to Norwalk VLP, which is a GI.1 Norovirus.

Example 6. Efficacy of Bivalent Vaccine in Rabbits

[096] A study was performed to evaluate the efficacy of a bivalent Norovirus vaccine comprising Norovirus GII.4 composite VLPs (CVLPS) as described in Example 2 and Norwalk VLPs (NVLPs, GI.1). Rabbits were intramuscularly immunized with this bivalent vaccine on days 0 and 21. VLP doses ranged from 20μg to 0.002μg of each type of VLP and each vaccine formulation contained 25μg MPL and 250μg AlOH. Serum was collected from each rabbit on day 28 and VLP-specific IgG was evaluated. Spleens and mesenteric lymph nodes were collected on day 75 and evaluated for the presence antigen-specific cellular immunity.

[097] Serum IgG titers were measured by ELISA using microtiter plates coated with either NVLP or CVLP as a capture. Titers are expressed as reciprocal dilutions (Figure 11). Antigen-specific T-cell responsiveness was evaluated by tritiated thymidine incorporation after a 5-day *in vitro* stimulation with 5μg of either NVLP or CVLP (Figure 12). Memory B-cells were evaluated by VLP-specific ELISPOT and results are expressed as antibody-secreting cells per million cells (Figure 13).

[098] The results of this study demonstrate that the IM bivalent norovirus vaccine formulated with the adjuvants MPL and AlOH elicits high VLP-specific IgG responses, responsive T-cells and memory B-cells capable of responding to stimulation with both NVLP and CVLP.

Example 7. High-dose Bivalent Vaccination in Rabbits

[099] This example outlines experiments designed to determine if high doses of the composite and Norwalk VLPs in the bivalent vaccine would lead to any adverse events. Rabbits were

intramuscularly immunized with the bivlaent vaccine (see Example 6) on days 0, 14, and 21. VLP doses ranged from 150μg to 5μg of each VLP (Norwalk and composite) and each formulation contained 50μg MPL and 500μg AlOH. The general health, coat condition, and injection site of the immunized rabbits were monitored every 12 hours for the first 72 hours and then daily thereafter. Serum was collected from each rabbit on day 21 and day 35 and Norwalk VLP (NVLP)-specific (Figure 14) and composite VLP (CVLP)-specific (Figure 15) IgG and IgA were evaluated. Spleens were also harvested on day 35 and evaluated for the presence of antigen-specific cellular immunity (Figure 16).

[0100] Serum IgG titers were measured by ELISA using microtiter plates coated with either NVLP or CVLP as a capture. Titers are expressed as reciprocal dilutions. Antigen-specific T-cell responsiveness was evaluated by tritiated thymidine incorporation after a 5-day *in vitro* stimulation with the indicated antigens (*e.g.* composite VLPs, GII.4 (2002) VLPs, GII.4 (2006 VLPs, and Norwalk VLPs).

[0101] The results from this study shows that the Norovirus bivalent vaccine is safe at the tested doses as evidenced by the fact that all rabbits appeared healthy throughout the study duration and no injection site reactions were observed. The immune responses measured from vaccinated rabbits confirm that the bivalent Norovirus vaccine is effective for eliciting both VLP-specific antibodies as well as VLP-responsive T-cells.

Example 8. Mouse Potency Assay for Norovirus Vaccine Efficacy

[0102] This example outlines the development of a mouse potency assay to evaluate the potency of the bivalent Norovirus vaccine. Mice were immunized IP on day 0 and 7 with equal concentrations ranging from 0.002μg to 30μg of Norwalk VLP (NVLP) and composite VLP (CVLP). Serum was collected from each mouse on day 14 and VLP-specific IgG was evaluated (Figure 17). The neutralizing activity of the antibodies was also measured by hemagglutination inhibition assay (HAI) using Type O positive human red blood cells (Figure 18). Only Norwalk-specific HAI titers could be assessed because the GII.4 genotypes do not hemagglutinate red blood cells.

[0103] Serum IgG titers were measured by ELISA using microtiter plates coated with either NVLP or CVLP as a capture. Titers are expressed as reciprocal dilutions. HAI titers were measured by using a standard hemagglutination assay.

[0104] The results from this study indicate that vaccination with the bivalent Norovirus vaccine elicits potent and functional IgG titers such that they are capable of inhibiting hemagglutination of human red blood cells. These results are of particular importance because they demonstrate that the antibodies elicited in response to the vaccination have functionality, which may lead to neutralization of the actual virus during an infection.

Example 9. Chitosan Formulations of a Norovirus Bivalent Vaccine

[0105] A study was performed in rabbits with the bivalent Norovirus VLP vaccine to evaluate the role of chitosan in this vaccine formulation. The formulation contained equal amounts of a Norwalk VP1 VLP and a composite GII.4 VLP (see Example 2). Rabbits were intranasally immunized with dry powder formulations on days 0 and 21. VLP doses ranged from 150µg to 5µg of each type of VLP and each formulation contained 50µg MPL. Chitosan concentration was varied for each dose range (7 mg, 0.7 mg and 0 mg) to determine its role in immunogenicity. Serum was collected from each rabbit and VLP-specific IgG was evaluated (Figure 19).

[0106] Serum IgG titers were measured by ELISA using microtiter plates coated with VLP as a capture. Serial dilutions of a proprietary in-house rabbit anti-VLP serum were used to generate standard curves. Titers are expressed in Units anti-VLP/mL (one Unit is approximately equal to 1 µg).

[0107] Results from these experiments indicate that chitosan at the highest dose (7 mg) is required to achieve maximum immunogenicity. The IgG data for the 50µg dose is shown in Figure 19 and results are represented as U/ml. The IgA antibody response is shown below in Table 1.

Table 1. Antigen-Specific IgA Responses.

VLP (µg)	50	50	50
Chitosan (mg)	7	0.7	0
Geometric Mean IgA Titers (95% CI)	**770** (474, 1253)	**67** (32, 142)	**83** (38, 179)

Example 10. Design of a Norovirus GII Consensus Gene

[0108] The methods of the present invention may also be used to generate capsid consensus sequences amongst Norovirus GII isolates from different GII genotypes, GII.1, GII.2, GII.3. The following alignment was generated from VP1 sequences from three different Norovirus GII isolates. The consensus sequence (SEQ ID NO: 7) determined from the homology comparison of the three GII strains is shown in Figure 20.

[0109] A composite sequence is derived from the consensus sequence by selecting amino acids from a sequence of one of the strains for variable positions of the consensus sequence where two or more strains differ. Preferably the sequence from which amino acids are selected is a recently circulating strain, or a strain that is more commonly associated with disease or more commonly occurring amongst the strains being evaluated. In this Example, amino acids were selected from the Snow Mountain sequence at variable positions of the consensus sequence at which all three strains differed to generate a composite VP1 GII sequence. The composite GII sequence is used for production of a synthetic gene encoding a composite GII VP1 protein for induction of cross-immunity amongst GII Norovirus isolates.

[0110] The composite GII VP1 amino acid sequence (Composite) is shown in the alignment below as SEQ ID NO: 11 with the amino acid sequences of the VP1 proteins from GII.1 (Accession Number: AAL13001), GII.2 Snow Mountain (Accession Number: AAB61685), and GII.3 virus (Accession Number: AAL12998) (SEQ ID NOs: 8, 9, and 10, respectively).

```
 Composite MKMASNDAAPSNDGAAGLVPESNNEVMALEPVAGAAIAAPLTGQTNIIDPWIR 53
     GII.1 MKMASNDAAPSNDGAAGLVPEVNNETMALEPVAGASIAAPLTGQNNVIDPWIR 53
GII.2 Snow MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIR 53
     GII.3 MKMASNDAAPSNDGAAGLVPEINNEAMALEPVAGAAIAAPLTGQQNIIDPWIM 53

 Composite ANFVQAPNGEFTVSPRNSPGEVLLNLELGPELNPYLAHLARMYNGYAGGMEVQ 106
     GII.1 MNFVQAPNGEFTVSPRNSPGEILLNLELGPELNPFLAHLSRMYNGYAGGVEVQ 106
GII.2 Snow ANFVQAPNGEFTVSPRNAPGEVLLNLELGPELNPYLAHLARMYNGYAGGMEVQ 106
     GII.3 NNFVQAPGGEFTVSPRNSPGEVLLNLELGPEINPYLAHLARMYNGYAGGFEVQ 106

 Composite VMLAGNAFTAGKLVFAAIPPHFPIENLSPQQITMFPHVIIDVRTLEPVLLPLP 159
     GII.1 VLLAGNAFTAGKLVFAAIPPHFPIGNLSPGQIAMFPHVIIDVRTLEPVLLPLP 159
GII.2 Snow VMLAGNAFTAGKLVFAAVPPHFPVENLSPQQITMFPHVIIDVRTLEPVLLPLP 159
     GII.3 AVLAGNAFTAGKVIFAAIPPNFPIDNLSAAQITMCPHVIVDVRQLEPINLPMP 159

 Composite DVRNNFFHYNQKDDPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDF 212
     GII.1 DVRNNFFHYNQEPEPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDF 212
GII.2 Snow DVRNNFFHYNQKDDPKMRIVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDF 212
     GII.3 DVRNNFFHYNQGSDSRLRLIAMLYTPLRANNSGDDVFTVSCRVLTRPSPDFSF 212

 Composite NYLVPPTVESKTKPFTLPILTIGELSNSRFPVPIDQLYTSPNEVIVVQCQNGR 265
     GII.1 NYLVPPTVESKTKPFTLPILTIGELSNSRFPVPIDELYTSPNEGLVVQPQNGR 265
GII.2 Snow TYLVPPTVESKTKPFTLPILTLGELSNSRFPVSIDQMYTSPNEVISVQCQNGR 265
```

```
     GII.3 NFLVPPTVESKTKLFTLPILTISEMSNSRFPVPIDSLHTSPTENIVVQCQNGR 265

 Composite CTLDGELQGTTQLQPSGICAFRGEVTR--AHLSDQDN-------DHRWNIQIT 318
     GII.1 STLDGELLGTTQLVPSNICSLRG---RINAHLSD--N-------QHRWNMQVT 306
GII.2 Snow CTLDGELQGTTQLQVSGICAFKGEVT---AHL--QDN-------DHLYNITIT 306
     GII.3 VTLDGELMGTTQLLPSQICAFRGTLTRSTSRASDQADTPTPRLFNHRWHIQLD 318

 Composite NLNGTPFDPSEDIPAPLGTPDFQGRVFGVISQRNPDNT-------NRAHDAVV 371
     GII.1 NANGTPFDPTEDVPAPLGTPDFLANIYGVTSQRNPDNT-------CRAHDGIL 352
GII.2 Snow NLNGSPFDPSEDIPAPLGVPDFQGRVFGVITQRDKQNAAGQSQPANRGHDAVV 359
     GII.3 NLNGTPYDPAEDIPAPLGTPDFRGKVFGVASQRNPDST-------TRAHEAKV 364

 Composite PTYSAQFTPKLGSVQIGTWETDDFDVNQPTKFTPV--GLNDTEHFNQWVLPRY 424
     GII.1 ATWSPKFTPKLGSVVLGTWEDRDFDINQPTRFTPV--GLYDTDHFNQWVLPYY 403
GII.2 Snow PTYTAQYTPKLGQVQIGTWQTDDLKVNQPVKFTPV--GLNDTEHFNQWVVPRY 410
     GII.3 DTTSDRFTPKLGSLEIIT-ESGDFDTNQSTKFTPVGIGVDNEAEFQQWSLPNY 416

 Composite SGALTLNMNLAPSVAPVFPGEQLLFFRSYLPLKGGYSNGAIDCLLPQEWVQHF 477
     GII.1 SGALTLNMNLAPSVAPLFPGEQLLFFRSHVPLKGGTSNGAIDCLLPQEWVQHF 456
GII.2 Snow AGALNLNTNLAPSVAPVFPGERLLFFRSYLPLKGGYGNPAIDCLLPQEWVQHF 463
     GII.3 SGQFTHNMNLAPAVAPNFPGEQLLFFRSQLPSSGGRSNGVLDCLVPQEWVQHF 469

 Composite YQESAPSMTEVALVRYINPDTGRVLFEAKLHRAGFMTVASNGSAPIVVPPNGY 530
     GII.1 YQESAPSSTDVALIRYTNPDTGRVLFEAKLHRQGFITVANSGSRPIVVPPNGY 509
GII.2 Snow YQEAAPSMSEVALVRYINPDTGRALFEAKLHRAGFMTVSSNTSAPVVVPANGY 516
     GII.3 YQESAPAQTQVALVRYVNPDTGRVLFEAKLHKLGFMTIAKNGDSPITVPPNGY 522

 Composite FRFDSWVNQFYSLAPMGTGNGRRRI (SEQ ID NO: 11)           555
     GII.1 FRFDSWVNQFYSLAPMGTGNGRRRV (SEQ ID NO: 8)            534
GII.2 Snow FRFDSWVNQFYSLAPMGTGNGRRRI (SEQ ID NO: 9)            541
     GII.3 FRFESWVNPFYTLAPMGTGNGRRRI (SEQ ID NO: 10)           547
```

Example 11. Design of a Norovirus GI Consensus Gene

[0111] The methods of the present invention may also be used to generate capsid consensus sequences amongst Norovirus GI isolates. The following alignment was generated from VP1 sequences from three different Norovirus GI isolates. The consensus GI sequence (SEQ ID NO: 12) determined from the homology comparison of the three GI strains is shown in Figure 21.

[0112] A composite sequence is derived from the consensus sequence by selecting amino acids from a sequence of one of the strains for variable positions of the consensus sequence where two or more strains differ. Preferably the sequence from which amino acids are selected is a recently circulating strain, or a strain that is more commonly associated with disease or more commonly occurring amongst the strains being evaluated. In this Example, amino acids were selected from the Southampton sequence at variable positions of the consensus sequence at which all three strains differed to generate a composite VP1 GI sequence. The composite GI sequence is used for production of a synthetic gene encoding a composite GI VP1 protein for induction of cross-immunity amongst GI Norovirus isolates.

[0113] The composite GI VP1 amino acid sequence (Composite) is shown in the alignment below as SEQ ID NO: 16 with the amino acid sequences of the VP1 proteins from Norwalk virus (Accession Number: M87661), Southampton (Accession Number: Q04542), and Chiba virus (Accession Number: BAB18267) (SEQ ID NOs: 13, 14, and 15, respectively).

```
 Composite MMMASKDATQSADGASGAGQLVPEVNTADPLPMDPVAGSSTAVATAGQVNMID 53
Norwalk VP MMMASKDATSSVDGASGAGQLVPEVNASDPLAMDPVAGSSTAVATAGQVNPID 53
Southampto MMMASKDAPQSADGASGAGQLVPEVNTADPLPMEPVAGPTTAVATAGQVNMID 53
Chiba VP1  MMMASKDATPSADGATGAGQLVPEVNTADPIPIDPVAGSSTALATAGQVNLID 53

 Composite PWIINNFVQAPQGEFTISPNNTPGDVLFDLQLGPHLNPFLSHLSQMYNGWVGN 106
Norwalk VP PWIINNFVQAPQGEFTISPNNTPGDVLFDLSLGPHLNPFLLHLSQMYNGWVGN 106
Southampto PWIVNNFVQSPQGEFTISPNNTPGDILFDLQLGPHLNPFLSHLSQMYNGWVGN 106
Chiba VP1  PWIINNFVQAPQGEFTISPNNTPGDVLFDLQLGPHLNPFLSHLSQMYNGWVGN 106

 Composite MRVRILLAGNAFTAGKIIVCCVPPGFTSSSLTIAQATLFPHVIADVRTLDPIE 159
Norwalk VP MRVRIMLAGNAFTAGKIIVSCIPPGFGSHNLTIAQATLFPHVIADVRTLDPIE 159
Southampto MRVRILLAGNAFSAGKIIVCCVPPGFTSSSLTIAQATLFPHVIADVRTLEPIE 159
Chiba VP1  MRVRVVLAGNAFTAGKVIICCVPPGFQSRTLSIAQATLFPHVIADVRTLDPVE 159

 Composite VPLEDVRNVLYHNND-NQPTMRLVCMLYTPLRTGGGSGNSDSFVVAGRVLTCP 212
Norwalk VP VPLEDVRNVLFHNNDRNQQTMRLVCMLYTPLRTGGGTG--DSFVVAGRVMTCP 210
Southampto MPLEDVRNVLYHTND-NQPTMRLVCMLYTPLRTGGGSGNSDSFVVAGRVLTAP 211
Chiba VP1  VPLEDVRNVLYHNND-TQPTMRLLCMLYTPLRTGGASGGTDSFVVAGRVLTCP 211

 Composite SPDFNFLFLVPPTVEQKTRPFTVPNIPLQTLSNSRFPSPIQGMILSPDASQVV 265
Norwalk VP SPDFNFLFLVPPTVEQKTRPFTLPNLPLSSLSNSRAPLPISSIGISPDNVQSV 263
Southampto SSDFSFLFLVPPTIEQKTRAFTVPNIPLQTLSNSRFPSLIQGMILSPDASQVV 264
Chiba VP1  GPDFNFLFLVPPTVEQKTRPFTVPNIPLKYLSNSRIPNPIEGMSLSPDQTQNV 264

 Composite QFQNGRCTIDGQLLGTTPVSxSQLFKVRGKITSGARVLNLTELDGKPFMAFDS 318
Norwalk VP QFQNGRCTLDGRLVGTTPVSLSHVAKIRG--TSNGTVINLTELDGTPFHPFEG 314
Southampto QFQNGRCLIDGQLLGTTPATSGQLFRVRGKINQGARTLNLTEVDGKPFMAFDS 317
Chiba VP1  QFQNGRCTIDGQPLGTTPVSVSQLCKFRGRITSGQRVLNLTELDGSPFMAFAA 317

 Composite PAPVGFPDLGKCDWHIRMSKTPNSSGQGDPMRSVSVQTNVQGFVPHLGSIQFD 371
Norwalk VP PAPIGFPDLGGCDWHINMTQFGHSS-QTQ----YDVDTTPDTFVPHLGSIQAN 362
Southampto PAPVGFPDFGKCDWHMRISKTPNNTGSGDPMRSVSVQTNVQGFVPHLGSIQFD 370
Chiba VP1  PAPAGFPDLGSCDWHIEMSKIPNSSTQNNPIVTNSVKPNSQQFVPHLSSITLD 370

 Composite EVFS-PTGDYIGTIEWISPPSTPPGTDINLWKIPDYGSSLSEAANLAPPVYPP 424
Norwalk VP GIGS---GNYVGVLSWISPPSHPSGSQVDLWKIPNYGSSITEATHLAPSVYPP 412
Southampto EVFNHPTGDYIGTIEWISQPSTPPGTDINLWEIPDYGSSLSQAANLAPPVFPP 423
Chiba VP1  ENVS-SGGDYIGTIQWTSPPSDSGGANTNFWKIPDYGSSLAEASQLAPAVYPP 422

 Composite GFGEVLVYFMSAFPGPNNRGAPNDVPCLLPQEYITHFVSEQAPTMGEAALLHY 477
Norwalk VP GFGEVLVFFMSKMPGP----GAYN-LPCLLPQEYISHLASEQAPTVGEAALLHY 461
Southampto GFGEALVYFVSAFPGPNNRSAPNDVPCLLPQEYITHFVSEQAPTMGDAALLHY 476
Chiba VP1  GFNEVIVYFMASIPGPNQSGSPNLVPCLLPQEYITHFISEQAPIQGEAALLHY 475

 Composite VDPDTNRNLGEFKLYPGGYLTCVPNGVSAGPQQLPLNGVFVFVSWVSRFYQLK 530
Norwalk VP VDPDTGRNLGEFKAYPDGFLTCVPNGASSGPQQLPINGVFVFVSWVSRFYQLK 514
Southampto VDPDTNRNLGEFKLYPGGYLTCVPNGVGAGPQQLPLNGVFLFVSWVSRFYQLK 529
Chiba VP1  VDPDTNRNLGEFKLYPGGYLTCVPNSSSTGPQQLPLDGVFVFASWVSRFYQLK 528

 Composite PVGTASTARGRLGVRR  (SEQ ID NO: 16)                     546
Norwalk VP PVGTASSARGRLGLRR  (SEQ ID NO: 13)                     530
Southampto PVGTASTARGRLGVRR  (SEQ ID NO: 14)                     545
Chiba VP1  PVGTAGPARGRLGVRR  (SEQ ID NO: 15)                     544
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Example 12. Design of a Human Papillomavirus Consensus Gene for L1

[0114] The methods of the present invention may also be used to generate consensus sequences amongst other non-enveloped viruses. The following alignment was generated from three Human Papillomavirus (HPV): HPV-11, HPV-16, and HPV-18. The consensus L1 capsid protein sequence (SEQ ID NO: 17) determined from the homology comparison of the three HPV strains is shown in Figure 22.

[0115] A composite sequence is derived from the consensus sequence by selecting amino acids from a sequence of one of the strains for variable positions of the consensus sequence where two or more strains differ. Preferably the sequence from which amino acids are selected is a recently circulating strain, or a strain that is more commonly associated with disease or more commonly occurring amongst the strains being evaluated. In this Example, amino acids were selected from the HPV-18 sequence at variable positions of the consensus sequence at which all three strains differed to generate a composite L1 HPV sequence. The composite HPV sequence is used for production of a synthetic gene encoding a composite L1 polypeptide for induction of cross-immunity amongst a variety of HPV strains.

[0116] The composite HPV L1 amino acid sequence (Composite) is shown in the alignment below as SEQ ID NO: 21 with the amino acid sequences of the L1 proteins from HPV-11, HPV-16, and HPV-18 virus (SEQ ID NOs: 18, 19, and 20, respectively).

```
Composite MCLYTRVLILHYHLLPLYGPLYHPRPLPLHSILVYMVHIIICGHYIILFLRNV 53
 HPV16 L1                             MQVTFIYIL-VITC------YENDV 18
 HPV18 L1 MCLYTRVLILHYHLLPLYGPLYHPRPLPLHSILVYMVHIIICGHYIILFLRNV 53

Composite NVFPIFLQMALWRPSDNTVYLPPP-PVSKVVNTDDYVTRTNIFYHAGSSRLLA 106
 HPV11 L1           MWRPSDSTVYVPPPNPVSKVVATDAYVKRTNIFYHASSSRLLA 43
 HPV16 L1 NVYHIFFQMSLWLPSEATVYLPPV-PVSKVVSTDEYVARTNIYYHAGTSRLLA 70
 HPV18 L1 NVFPIFLQMALWRPSDNTVYLPPP-SVARVVNTDDYVTPTSIFYHAGSSRLLT 105

Composite VGHPYFRIKKGGGNKQDVPKVSGYQYRVFRVQLPDPNKFGLPDTSIYNPETQR 159
 HPV11 L1 VGHPYYSIKK--VNKTVVPKVSGYQYRVFKVVLPDPNKFALPDSSLFDPTTQR 94
 HPV16 L1 VGHPYFPIKKPNNNKILVPKVSGLQYRVFRIHLPDPNKFGFPDTSFYNPDTQR 123
 HPV18 L1 VGNPYFRVPAGGGNKQDIPKVSAYQYRVFRVQLPDPNKFGLPDTSIYNPETQR 158

Composite LVWACAGVEVGRGQPLGVGLSGHPLLNKLDDTENSHAYTSNVGEDNRDNVSMD 212
 HPV11 L1 LVWACTGLEVGRGQPLGVGVSGHPLLNKYDDVENSGGYGGNPGQDNRVNVGMD 147
 HPV16 L1 LVWACVGVEVGRGQPLGVGISGHPLLNKLDDTENASAYAANAGVDNRECISMD 176
 HPV18 L1 LVWACAGVEIGRGQPLGVGLSGHPFYNKLDDTESSHAATSNVSEDVRDNVSVD 211

Composite YKQTQLCILGCAPPIGEHWGKGTACKNRPVSQGDCPPLELINTVIQDGDMVDT 265
 HPV11 L1 YKQTQLCMVGCAPPLGEHWGKGTQCSNTSVQNGDCPPLELITSVIQDGDMVDT 200
 HPV16 L1 YKQTQLCLIGCKPPIGEHWGKGSPCTNVAVNPGDCPPLELINTVIQDGDMVDT 229
 HPV18 L1 YKQTQLCILGCAPAIGEHWAKGTACKSRPLSQGDCPPLELKNTVLEDGDMVDT 264
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Composite GFGAMDFSTLQDNKSEVPLDICQSICKYPDYLQMSADPYGDSLFFYLRREQMF 318
 HPV11 L1 GFGAMNFADLQTNKSDVPLDICGTVCKYPDYLQMAADPYGDRLFFYLRKEQMF 253
 HPV16 L1 GFGAMDFTTLQANKSEVPLDICTSICKYPDYIKMVSEPYGDSLFFYLRREQMF 282
 HPV18 L1 GYGAMDFSTLQDTKCEVPLDICQSICKYPDYLQMSADPYGDSMFFCLRREQLF 317

Composite ARHFFNRAGTVGETVPDDLYIKGTGMPASPASSVYSPTPSGSIVTSDAQLFNK 371
 HPV11 L1 ARHFFNRAGTVGEPVPDDLLVKGGNNRSSVASSIYVHTPSGSLVSSEAQLFNK 306
 HPV16 L1 VRHLFNRAGAVGENVPDDLYIKGSGSTANLASSNYFPTPSGSMVTSDAQIFNK 335
 HPV18 L1 ARHFWNRAGTMGDTVPQSLYIKGTGMPASPGSCVYSPSPSGSIVTSDSQLFNK 370

Composite PYWLQKAQGHNNGICWGNQLFVTVVDTTRSTNMTLCAS-QSPSPGTYDNTKFK 424
 HPV11 L1 PYWLQKAQGHNNGICWGNHLFVTVVDTTRSTNMTLCAS-VSKSA-TYTNSDYK 357
 HPV16 L1 PYWLQRAQGHNNGICWGNQLFVTVVDTTRSTNMSLCAA-ISTSETTYKNTNFK 387
 HPV18 L1 PYWLHKAQGHNNGVCWHNQLFVTVVDTTPSTNLTICASTQSPVPGQYDATKFK 423

Composite EYSRHVEEYDLQFIFQLCTITLTADVMSYIHSMNSSILEDWNFGLPPPPTGTL 477
 HPV11 L1 EYMRHVEEFDLQFIFQLCSITLSAEVMAYIHTMNPSVLEDWNFGLSPPPNGTL 410
 HPV16 L1 EYLRHGEEYDLQFIFQLCKITLTADVMTYIHSMNSTILEDWNFGLQPPPGGTL 440
 HPV18 L1 QYSRHVEEYDLQFIFQLCTITLTADVMSYIHSMNSSILEDWNFGVPPPPTTSL 476

Composite EDTYRFVQSQAITCQKDTPPAEKKDPYKKLKFWEVNLKEKFSLDLDQFPLGRK 530
 HPV11 L1 EDTYRYVQSQAITCQKPTPEKEKQDPYKDMSFWEVNLKEKFSSELDQFPLGRK 463
 HPV16 L1 EDTYRFVTSQAIACQKHTPPAPKEDPLKKYTFWEVNLKEKFSADLDQFPLGRK 493
 HPV18 L1 VDTYRFVQSVAITCQKDAAPAENKDPYDKLKFWNVDLKEKFSLDLDQYPLGRK 529

Composite FLLQAGLRRKPTI-GGRKRSAPSASTSSTPAKRKRVKAR (SEQ ID NO: 21) 569
 HPV11 L1 FLLQSGYRGRTSARTGIKR--PAVSKPSTAPKRKRTKTK (SEQ ID NO: 18) 500
 HPV16 L1 FLLQAGLKAKPKFTLGKRKATPTTSSTSTTAKRKKRKL  (SEQ ID NO: 19) 531
 HPV18 L1 FLVQAGLRRKPTI-GPRKRSAPSATTSSKPAKRVRVRAR (SEQ ID NO: 20) 567
```

Example 13. Dose Escalation Safety Study of Composite VLP Vaccine Formulation in Humans

[0117] A double-blind, controlled, dose-escalation phase 1 study of the safety and immunogenicity of a Norovirus vaccine is conducted. The vaccine consists of composite Norovirus virus-like particles (VLPs) in a dry powder matrix designed for intranasal administration. The composite VLPs contain a polypeptide having the amino acid sequence of SEQ ID NO: 1. Vaccinees include healthy adult volunteers who are H type 1 antigen secretors. The rationale for enrollment of H type 1 antigen secretors is that H type 1 antigen secretors are susceptible to Norovirus infections while non-secretors are resistant. As a control, 2 additional volunteers at each dosage level receive matrix alone. The dry powder matrix includes 25 µg MPL® adjuvant, 7 mg chitosan, 1.5 mg mannitol, and 1.5 mg sucrose. Volunteers are dosed on days 0 and 21 and are required to keep a 7-day diary of symptoms after each dose. Blood for serology, antibody secreting cells (ASC), and stool and saliva samples for mucosal antibody evaluation are collected.

[0118] The components of the vaccine are listed in Table 2. The vaccine is packaged in an intranasal delivery device. Single administrations of the composite VLP vaccine are packaged in a single dose Bespak (Milton Keynes, UK) UniDose DP dry powder intranasal delivery device.

Each device delivers 10 mg of the dry powder vaccine formulation. Each dose of vaccine consists of two delivery devices, one in each nostril. The total vaccine dose is 20 mg of dry power. The formulation of Adjuvant/Excipient is the same as the composite VLP vaccine except that no composite VLP antigen is included in the formulation. The formulation of the Adjuvant/Excipient (also referred to as dry powder matrix) is summarized in Table 3.

Table 2. Composite VLP Vaccine Composition

Component	Molecular class	Quantity per 10 mg dry powder	% of Final Formulation
Composite VLP	Recombinant protein	2.5, 7.5 or 25, 50 µg	0.025, 0.075, 0.25, or 0.50%
Monophosphoryl Lipid A	Phospholipid	25 µg	0.25%
Chitosan	Polysaccharide	7.0 mg	70%
Mannitol	Sugar	1.5 mg	15%*
Sucrose	Sugar	1.5 mg	15%

Table 3. Adjuvant/Excipient (dry powder matrix)

Component	Molecular class	Quantity per 10 mg dry powder	% of Final Formulation
Monophosphoryl Lipid A	Phospholipid	25 µg	0.25%
Chitosan	Polysaccharide	7.0 mg	70%
Mannitol	Sugar	1.5 mg	15%
Sucrose	Sugar	1.5 mg	15%

[0119] Specifically, the dose escalation of the vaccine is conducted as follows: After appropriate screening for good health, a group of 3 volunteers is randomized to receive either 5 µg of the composite VLP vaccine plus dry powder matrix (n=2) or dry powder matrix alone (n=1) by the intranasal route. These 3 volunteers are followed for safety for 21 days, and the Independent Safety Monitor (ISM) reviews their safety data. After approval of the ISM, these individuals receive their second dose of vaccine or matrix on day 21, and 4 additional volunteers are randomized to receive either 5 µg VLP protein plus dry powder matrix (n=3) or matrix alone (n=1) by the intranasal route. The ISM reviews the safety data from this second group and after approval of the ISM, the second intranasal dose is given 21 days after the first dose. Volunteers keep a 7-day diary of symptoms after each dose. After the ISM determines that escalation to the next higher dose is acceptable, another group of 7 volunteers is randomized to receive either the composite VLP vaccine containing 15 µg VLP protein (n=5) or dry powder matrix alone (n=2) by the intranasal route at day 0 and day 21. Again, 7-day symptom diaries are recorded and reviewed by the ISM before the second dose at day 21. Finally, after review of the safety data from the first two dosage cohorts, the ISM determines if dose escalation is acceptable and a final group of 7 volunteers is randomized to receive either the composite VLP vaccine containing 50 µg VLP protein (n=5) or dry powder matrix alone (n=2) by the intranasal route on day 0 and day 21. Again, the ISM reviews seven-day symptom diaries and other safety data before the second dose at day 21.

[0120] The volunteers keep a daily diary of symptoms (including local symptoms such as: nasal discharge, nasal pain/discomfort, nasal congestion, runny nose, nasal itching, nose bleed, headache and systemic symptoms such as: daily oral temperature, myalgia, nausea, vomiting, abdominal cramps, diarrhea, and loss of appetite) for 7 days after receiving the composite VLP vaccine or dry powder matrix alone. Interim medical histories are obtained at each follow-up visit (days 7±1, 21±2, 28±2, 56±2 and 180±14); volunteers are queried about interim illness, medications, and doctor's visits. Volunteers are asked to report all serious or severe adverse events including events that are not solicited during follow up visits. Volunteers have CBC and serum creatinine, glucose, AST, and ALT assessed on days 7 and 28 (7 days after each immunization) and, if abnormal, the abnormal laboratory test is followed until the test becomes normal or stabilizes.

[0121] Blood is collected before immunization and on days 7±1, 21±2, 28±2, 56±2, and 180±14 to measure serum antibodies to the composite VLP vaccine by enzyme-linked immunosorbent assays (ELISA). Before and on day 7 after administration of each dose of vaccine or dry powder matrix alone peripheral blood lymphocytes are collected to detect antibody secreting cells by ELISPOT assay. Before and on days 21±2, 56±2 and 180±14 after vaccination, whole blood is obtained to separate cells and freeze for future studies of cell mediated immunity, including cytokine production in response to composite VLP antigen, and lymphoproliferation. Whole stool samples are collected before immunization and on days 7±1, 21±2, 28±2, 56±2, and day 180±14 for anti-composite VLP sIgA screening. Saliva is collected with a commercially available device (Salivette, Sarstedt, Newton, NC) before immunization and on days 7±1, 21±2, 28±2, 56±2, and if positive for mucosal antibodies at day 56, a day 180±14 sample is collected and screened for anti-composite VLP sIgA. Finally blood from volunteers receiving the highest dose of composite VLPs (50 µg, third cohort described above) is screened for memory B-cells on days 0, 21, 56 and 180.

[0122] The following methods are used to analyze the blood, stool, and saliva samples collected from immunized individuals or individuals receiving the dry powder matrix alone:

A. Serum Antibody Measurements By ELISA

[0123] Twenty mL of blood are collected before and at multiple time points after vaccination for measurement of antibodies to the composite VLP by ELISA, using purified recombinant composite VLPs as target antigen to screen the coded specimens. Briefly, composite VLPs in carbonate coating buffer pH 9.6 are used to coat microtiter plates. Coated plates are washed, blocked, and incubated with serial two-fold dilutions of test serum followed by washing and incubation with enzyme-conjugated secondary antibody reagents specific for human IgG, IgM, and IgA. Appropriate substrate solutions are added, color developed, plates read, and the IgG, IgM, and IgA endpoint titers are determined in comparison to a reference standard curve for each antibody class. A positive response is defined as a 4-fold rise in titer after vaccination.

B. Antibody Secreting Cell Assays

[0124] Peripheral blood mononuclear cells (PMBCs) are collected from thirty mL of heparinized blood for ASC assays to detect cells secreting antibodies to composite VLPs. These assays are

performed on days 0, 7±1, 21±2, and 28±2 after administration of the composite VLP vaccine or dry powder matrix alone. A positive response is defined as a post-vaccination ASC count per 10^6 PBMCs that is at least 3 standard deviations (SD) above the mean pre-vaccination count for all subjects (in the log metric) and at least 8 ASC spots, which corresponds to the mean of medium-stimulated negative control wells (2 spots) plus 3 SD as determined in similar assays.

C. Measurement of Composite VLP-Specific Memory B-Cells

[0125] Heparinized blood is collected from cohort 3 (30 mL days 0 and 21, 50 mL days 56 and 180) to measure memory B cells on days 0, 21, 56 and 180 after vaccination using an ELISpot assay preceded by an *in vitro* antigen stimulation. A similar assay was successfully used to measure frequency of memory B cells elicited by Norwalk VLP formulations in rabbits (See WO 2008/042789, herein incorporated by reference in its entirety). Peripheral blood mononuclear cells ($5x10^6$ cells/mL, 1 mL/well in 24-well plates) are incubated for 4 days with composite VLP antigen (2-10 µg/mL) to allow for clonal expansion of antigen-specific memory B cells and differentiation into antibody secreting cells. Controls include cells incubated in the same conditions in the absence of antigen and/or cells incubated with an unrelated antigen. Following stimulation, cells are washed, counted and transferred to ELISpot plates coated with composite VLP. To determine frequency of VLP-specific memory B cells per total Ig-secreting B lymphocytes, expanded B cells are also added to wells coated with anti-human IgG and anti-human IgA antibodies. Bound antibodies are revealed with HRP-labeled anti-human IgG or anti-human IgA followed by True Blue substrate. Conjugates to IgA and IgG subclasses (IgA1, IgA2 and IgG1-4) may also be used to determine antigen-specific subclass responses which may be related with distinct effector mechanisms and locations of immune priming. Spots are counted with an ELISpot reader. The expanded cell populations for each volunteer are examined by flow cytometry to confirm their memory B cell phenotype, i.e. CD19+, CD27+, IgG+, IgM+, CD38+, IgD -.

D. Cellular Immune Responses

[0126] Heparinized blood (50 mL cohorts 1 and 2, 25 mL cohort 3) is collected as coded specimens and the PBMCs isolated and cryopreserved in liquid nitrogen for possible future evaluation of cell-mediated immune (CMI) responses to composite VLP antigen. Assays that

may be performed include PBMC proliferative and cytokine responses to composite VLP antigen and can be determined by measuring interferon (IFN)-γ and interleukin (IL)-4 levels according to established techniques.

E. Collections Of Stool And Saliva For Anti-Composite VLP sIgA

[0127] Anti-composite VLP IgA is measured in stool and saliva samples. Saliva specimens are treated with protease inhibitors (i.e. AEBSF, leupeptin, bestatin, and aprotinin) (Sigma, St. Louis, MO), stored at –70 °C, and assayed using a modification of a previously described assay (Mills *et al.* (2003) Infect. Immun. 71: 726-732). Stool is collected on multiple days after vaccination and specimens stored at –70 °C until analysis. The specimens are thawed, and protease inhibitor buffer added to prepare a 10 % w/v stool suspension. Stool supernatants are assayed for composite VLP-specific mucosal IgA by ELISA, as described below.

[0128] Approximately 2-3 mL of whole saliva is collected before and at multiple time points after vaccination. Saliva is collected by a commercially available device (Salivette, Sarstedt, Newton, NC), in which a Salivette swab is chewed or placed under the tongue for 30-45 seconds until saturated with saliva. Saliva is collected from the swab by centrifugation.

F. Measurement Of Anti-Composite VLP In Stool And Saliva

[0129] ELISAs, utilizing plates coated with either anti-human IgA antibody reagents or target composite VLP antigen coatings, are performed to determine total IgA and to titer the specific anti-VLP IgA responses for each specimen. Total or specific IgA are revealed with HRP-labeled anti-human IgA as described above. An internal total IgA standard curve is included to quantify the IgA content. Response is defined as a 4-fold rise in specific antibody.

Example 14. Safety and Immunogenicity Study of Two Dosages of Intranasal Composite VLP Vaccine in Humans

[0130] A randomized, double blind study in healthy adults is conducted to compare the safety and immunogenicity of two dosage levels of a composite Norovirus virus-like particle (VLP) vaccine with adjuvant/excipients and placebo controls (empty device). The vaccine consists of composite Norovirus virus-like particles (VLPs) in a dry powder matrix designed for intranasal administration as described in Example 13. Vaccinees include healthy adult volunteers who are

H type 1 antigen secretors. The human volunteers are randomly assigned to one of four groups and each group receives one of the following treatments: a 50 μg dose of the composite VLP vaccine, a 100 μg dose of the composite VLP vaccine, the adjuvant/excipient, or placebo. Volunteers are dosed on days 0 and 21 and are required to keep a 7-day diary of symptoms after each dose. Blood for serology, antibody secreting cells (ASC), and stool and saliva samples for mucosal antibody evaluation are collected.

[0131] The components of the vaccine are listed in Table 2 in Example 13. The vaccine is packaged in an intranasal delivery device. Single administrations of the composite VLP vaccine are packaged in a single dose Bespak (Milton Keynes, UK) UniDose DP dry powder intranasal delivery device. Each device delivers 10 mg of the dry powder vaccine formulation. Each dose of vaccine consists of two delivery devices, one in each nostril. The total vaccine dose is 20 mg of dry power. Therefore, the 50 μg vaccine dose consists of two devices that each deliver 10 mg of dry powder formulation, wherein each 10 mg of dry powder formulation consists of 25 μg of composite VLP, 25 μg MPL® adjuvant, 7 mg chitosan, 1.5 mg mannitol, and 1.5 mg sucrose. Similarly, the 100 μg vaccine dose consists of two devices that each deliver 10 mg of dry powder formulation, wherein each 10 mg of dry powder formulation consists of 50 μg of composite VLP, 25 μg MPL® adjuvant, 7 mg chitosan, 1.5 mg mannitol, and 1.5 mg sucrose. The formulation of Adjuvant/Excipient is the same as the composite VLP vaccine except that no composite VLP antigen is included in the formulation. The formulation of the Adjuvant/Excipient (also referred to as dry powder matrix) is summarized in Table 3 in Example 13. The placebo group receives two empty devices.

[0132] The volunteers keep a daily diary of symptoms (including local symptoms such as: nasal discharge, nasal pain/discomfort, nasal congestion, runny nose, nasal itching, nose bleed, headache and systemic symptoms such as: daily oral temperature, myalgia, nausea, vomiting, abdominal cramps, diarrhea, and loss of appetite) for 7 days after receiving either one of two doses of the composite VLP vaccine, dry powder matrix alone, or the placebo. Interim medical histories are obtained at each follow-up visit (days 7+1, 21+2, 28+2, 56+2 and 180+14); volunteers are queried about interim illness, medications, and doctor's visits. Volunteers are asked to report all serious or severe adverse events including events that are not solicited during follow up visits. Volunteers have CBC and serum creatinine, glucose, AST, and ALT assessed

on days 7 and 28 (7 days after each immunization) and, if abnormal, the abnormal laboratory test is followed until the test becomes normal or stabilizes.

[0133] Blood is collected before immunization and on days 7+1, 21+2, 28+2, 56+2, and 180+14 to measure serum antibodies to the composite VLP vaccine by enzyme-linked immunosorbent assays (ELISA). Before and on day 7 after administration of each dose of vaccine, dry powder matrix alone, or placebo, peripheral blood lymphocytes are collected to detect antibody secreting cells by ELISPOT assay. Before and on days 21+2, 56+2 and 180+14 after vaccination, whole blood is obtained to separate cells and freeze for future studies of cell mediated immunity, including cytokine production in response to composite VLP antigen, and lymphoproliferation. Whole stool samples are collected before immunization and on days 7+1, 21+2, 28+2, 56+2, and day 180+14 for anti-composite VLP sIgA screening. Saliva is collected with a commercially available device (Salivette, Sarstedt, Newton, NC) before immunization and on days 7+1, 21+2, 28+2, 56+2, and if positive for mucosal antibodies at day 56, a day 180+14 sample is collected and screened for anti-composite VLP sIgA. Blood is also screened for memory B-cells on days 0, 21, 56 and 180.

[0134] Methods used to analyze the blood, stool, and saliva samples collected from immunized individuals, or individuals receiving the dry powder matrix alone or placebo are described in detail in Example 13.

<u>Example 15. Experimental Human Challenge Study with Infectious Norovirus Following Vaccination with Composite Norovirus VLP Vaccine</u>

[0135] A multi-site, randomized, double-blind, placebo-controlled Phase 1-2 challenge study is conducted in 80 human volunteers immunized with the composite Norovirus VLP vaccine. Eligible subjects include those 18-50 years of age, in good health, who express the H type-1 oligosaccharide (as measured by positive salivary secretor status) and who are other than Type B or AB blood type. Subjects who are non H type-1 secretors or who have Type B or AB blood are reported to be more resistant to infection with Norwalk virus and are excluded from the study. At least 80% of volunteers are expected to be eligible based on these two criteria.

[0136] Following screening, eligible volunteers who meet all acceptance criteria are randomized (1:1) into one of two equal sized cohorts with approximately 40 volunteers in each cohort. Cohort 1 is immunized with composite VLP and cohort 2 receives placebo. Volunteers are

immunized with 10 mg composite VLP vaccine in each nostril (20 mg total dry powder) or placebo. Each 10 mg of composite VLP vaccine contains 50 µg of Composite VLP, 7 mg chitosan, 25 µg MPL®, 1.5 mg of sucrose and approximately 1.5 mg of mannitol. Thus, each volunteer in cohort 1 receives a total dosage of 100 µg of composite VLP antigen at each immunization. Volunteers receive vaccine or placebo on study days 0 and 21.

[0137] The safety of the composite virus VLP vaccine compared to placebo is assessed. Volunteers keep a diary for 7 days following each immunization with the vaccine or placebo to document the severity and duration of adverse events. Serious adverse events (SAEs) and the occurrence of any significant new medical conditions is followed for 6 months after the last dose of vaccine or placebo and for 4 months after the challenge with infectious virus.

[0138] All volunteers are challenged with infectious Norovirus between 21 to 42 days after the second dose of vaccine or placebo (between study days 42 and 56). Each volunteer receives at or > than the 50% Human Infectious Dose (HID 50), *i.e.* the amount of infectious virus that is expected to cause disease in at least 50% of volunteers in the placebo group. The HID 50 is between about 48 and about 480 viral equivalents of the challenge virus strain. The challenge Norovirus is mixed with sterile water and given orally. The inoculation is preceded by ingestion of 500 mg sodium bicarbonate in water, to prevent breakdown of the virus by stomach acid and pepsin. A second ingestion of sodium bicarbonate solution (500 mg sodium bicarbonate in water) is taken 5 minutes after oral inoculation of the infectious virus. The volunteers remain at the challenge facility for at least 4 days and at least 18 hours after symptoms/signs of acute gastroenteritis (vomiting, diarrhea, loose stool, abdominal pain, nausea, and fever) are absent.

[0139] Several metrics are monitored to determine the efficacy of the composite VLP vaccine in preventing or reducing symptoms/signs of acute gastroenteritis induced by the viral challenge. All volunteers record their clinical symptoms of acute gastroenteritis and these symptoms are documented by the research staff at the study sites. Disease symptoms/signs from cohort 1 receiving the vaccine are compared to cohort 2 placebo recipients.

[0140] Sera and stool samples are routinely collected from all volunteers prior to immunization with the vaccine or placebo, and after challenge. Serum samples are analyzed by ELISA for IgA and IgG, titers against the challenge VLPs. The challenge virus antigen and challenge virus RNA are tested in stool samples by ELISA and PCR, respectively, which indicate the presence of virus, the amount of virus shed from the intestines, and the duration of viral shedding.

Subjects who become ill after challenge, are subject to additional laboratory studies including serum chemistries, BUN, creatinine, and liver function tests until symptoms/signs resolve.

[0141] Results from the vaccine group (cohort 1) and the placebo group (cohort 2) are compared to assess the protective efficacy of the vaccine against Norovirus disease overall (primary endpoint), and/or its efficacy in ameliorating the symptoms/signs (severity and # of days of illness) and/or the reduction of the presence, the amount and/or the duration of virus shedding (secondary endpoints).

[0142] The present invention is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein, will become apparent to those skilled in the art from the foregoing description and accompanying drawings using no more than routine experimentation. Such modifications and equivalents are intended to fall within the scope of the appended claims.

[0143] All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

[0144] Citation or discussion of a reference herein shall not be construed as an admission that such is prior art to the present invention.

Claims:

1. A virus-like particle comprising at least one polypeptide having a composite amino acid sequence, wherein said composite amino acid sequence is derived from a consensus sequence representing the capsid proteins of two or more circulating strains of a non-enveloped virus, and wherein the at least one polypeptide forms a virus-like particle when expressed in a host cell and contains at least 1 different amino acid as compared to each of the capsid sequences of said two or more circulating strains.

2. The virus-like particle of claim 1, wherein the virus-like particle has antigenic properties of the two or more circulating strains of the non-enveloped virus.

3. The virus-like particle of claim 1, wherein the virus-like particle provides at least a two-fold increase in antisera cross-reactivity to one or more circulating strains of the non-enveloped virus as compared to the antisera cross-reactivity obtained by immunizing with a virus-like particle containing only protein from said one or more circulating strains.

4. The virus-like particle of claim 1, wherein the composite sequence contains at least 3 different amino acids compared to the capsid sequence of one or more circulating strains of the non-enveloped virus.

5. The virus-like particle of claim 1, wherein the composite sequence contains at least 5 different amino acids compared to the capsid sequence of one or more circulating strains of the non-enveloped virus.

6. The virus-like particle of claim 1, wherein the composite sequence contains at least 9 different amino acids compared to the capsid sequence of one or more circulating strains of the non-enveloped virus.

7. The virus-like particle of claim 1, wherein the consensus sequence is SEQ ID NO: 2.

8. The virus-like particle of claim 1, wherein the non-enveloped virus is selected from the group consisting of Calicivirus, Picornavirus, Astrovirus, Adenovirus, Reovirus, Polyomavirus, Papillomavirus, Parvovirus, and Hepatitis E virus.

9. The virus-like particle of claim 8, wherein the non-enveloped virus is a Calicivirus.

10. The virus-like particle of claim 9, wherein the Calicivirus is a Norovirus or Sapovirus.

11. The virus-like particle of claim 10, wherein the Norovirus is a genogroup I Norovirus.

12. The virus-like particle of claim 10, wherein the Norovirus is a genogroup II Norovirus.

13. The virus-like particle of claim 12, wherein the consensus sequence is derived from genogroup II, genotype 4 Norovirus strains.

14. The virus-like particle of claim 13, wherein the genogroup II, genotype 4 Norovirus strains are selected from Houston strain, Minerva strain, and Laurens strain.

15. The virus-like particle of claim 14, wherein the composite sequence is SEQ ID NO: 1.

16. The virus-like particle of claim 10, wherein the consensus sequence is derived from Norovirus strains from at least two different genotypes within a genogroup.

17. The virus-like particle of claim 16, wherein the consensus sequence is derived from genogroup II, genotype 2 and genogroup II, genotype 4 Norovirus strains.

18. The virus-like particle of claim 10, wherein the consensus sequence is derived from Norovirus strains from at least two different genogroups.

19. The virus-like particle of claim 18, wherein the consensus sequence is derived from genogroup I, genotype 1 and genogroup II, genotype 4 Norovirus strains.

20. The virus-like particle of claim 10, further comprising a capsid protein from a second Norovirus.

21. The virus-like particle of claim 20, wherein the second Norovirus is a genogroup I or genogroup II Norovirus.

22. The virus-like particle of claim 21, wherein the capsid protein from the second Norovirus is a VP1 protein from a genogroup I Norovirus.

23. The virus-like particle of claim 9, further comprising a second polypeptide having a second composite amino acid sequence, wherein said second composite amino acid sequence is derived from a consensus sequence representing the capsid proteins of two or more circulating strains of a second Calicivirus and contains at least 1 different amino acid as compared to each of the capsid sequences of said two or more circulating strains of the second Calicivirus.

24. The virus-like particle of claim 23, wherein the composite sequence contains 5-50 different amino acids compared to the capsid sequence of one or more circulating strains of the second Calicivirus.

25. The virus-like particle of claim 23, wherein the second Calicivirus is a Norovirus.

26. The virus-like particle of claim 25, wherein the Norovirus is a genogroup I Norovirus.

27. The virus-like particle of claim 26, wherein the genogroup I Norovirus is selected from the group consisting of Norwalk virus, Southampton virus, Hesse virus, and Chiba virus.

28. The virus-like particle of claim 23, wherein the virus-like particle has antigenic properties of the two or more circulating strains of the first Calicivirus and the two or more circulating strains of the second Calicivirus.

29. An isolated polypeptide or fragment thereof having a composite amino acid sequence, wherein said composite amino acid sequence is derived from a consensus sequence representing the capsid proteins of two or more circulating strains of a non-enveloped virus, and wherein the polypeptide contains at least 1 different amino acid as compared to each of the capsid sequences of said two or more circulating strains.

30. The isolated polypeptide of claim 29, wherein the composite sequence contains at least 3 different amino acids compared to the capsid sequence of one or more circulating strains of the non-enveloped virus.

31. The isolated polypeptide of claim 29, wherein the composite sequence contains 5-50 different amino acids compared to the capsid sequence of one or more circulating strains of the non-enveloped virus.

32. The isolated polypeptide of claim 29, wherein the consensus sequence is SEQ ID NO: 2.

33. The isolated polypeptide of claim 29, wherein said non-enveloped virus is a Calicivirus.

34. The isolated polypeptide of claim 33, wherein said Calicivirus is a Norovirus or Sapovirus.

35. The isolated polypeptide of claim 34, wherein said Norovirus is a genogroup I or genogroup II Norovirus, or combinations thereof.

36. The isolated polypeptide of claim 35, wherein the isolated polypeptide has the amino acid sequence of SEQ ID NO: 1.

37. An isolated nucleic acid encoding the polypeptide of claim 29.

38. The isolated nucleic acid of claim 37, wherein the nucleic acid has the sequence of SEQ ID NO: 3.

39. A vector comprising the isolated nucleic acid of claim 37.

40. A host cell comprising the vector of claim 39.

41. A vaccine formulation comprising the virus-like particle of claim 1, claim 20, or claim 23.

42. A vaccine formulation comprising the virus-like particle of claim 1 and a second virus-like particle, wherein said second virus-like particle comprises a capsid protein from a Norovirus.

43. The vaccine formulation of claim 42, wherein said Norovirus is a genogroup I or genogroup II Norovirus.

44. The vaccine formulation of claim 41 further comprising an adjuvant.

45. The vaccine formulation of claim 44, wherein the adjuvant is selected from the group consisting of toll-like receptor (TLR) agonists, monophosphoryl lipid A (MPL), synthetic lipid A, lipid A mimetics or analogs, aluminum salts, cytokines, saponins, muramyl dipeptide (MDP) derivatives, CpG oligos, lipopolysaccharide (LPS) of gram-negative bacteria, polyphosphazenes, emulsions, virosomes, cochleates, poly(lactide-co-glycolides) (PLG) microparticles, poloxamer particles, microparticles, liposomes, oil-in-water emulsion, MF59, and squalene.

46. The vaccine formulation of claim 44, further comprising a delivery agent.

47. The vaccine formulation of claim 46, wherein the delivery agent is a mucoadhesive.

48. The vaccine formulation of claim 47, wherein the mucoadhesive is selected from the group consisting of glycosaminoglycans (e.g., chondroitin sulfate, dermatan sulfate chondroitin,

keratan sulfate, heparin, heparan sulfate, hyaluronan), carbohydrate polymers (e.g., pectin, alginate, glycogen, amylase, amylopectin, cellulose, chitin, stachyose, unulin, dextrin, dextran) , cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrollidone, polysaccharides (including mucin, other mucopolysaccharides, and GelSite®, a natural acidic polysaccharide extracted from the aloe plant), polyions, cellulose derivatives (e.g., hydroxypropyl methylcellulose, carboxymethylcellulose), proteins (e.g. lectins, fimbrial proteins), and deoxyribonucleic acid.

49. The vaccine formulation of claim 48, wherein the mucoadhesive is a polysaccharide.

50. The vaccine formulation of claim 49, wherein said polysaccharide is chitosan, chitosan salt, or chitosan base.

51. The vaccine formulation of claim 44, wherein the vaccine formulation is a liquid formulation.

52. The vaccine formulation of claim 44, wherein the vaccine formulation is a dry powder formulation.

53. The dry powder formulation of claim 52 in combination with one or more devices for administering one or more doses of said formulation.

54. The dry powder formulation of claim 53, wherein said one or more doses are unit doses.

55. The dry powder formulation of claim 53, wherein the device is a single-use nasal administration device.

56. The vaccine formulation of claim 41, wherein said formulation is administered to a subject by a route selected from the group consisting of mucosal, intramuscular, intravenous, subcutaneous, intradermal, subdermal, and transdermal routes of administration.

57. The vaccine formulation of claim 56, wherein said mucosal administration is intranasal, oral, or vaginal.

58. The vaccine formulation of claim 57, wherein the formulation is in the form of a nasal spray, nasal drops or dry powder.

59. A vaccine formulation comprising the vector of claim 39.

60. A method of inducing a protective immunity to a viral infection in a subject comprising administering to the subject the vaccine formulation of claim 41.

61. The method of claim 60, wherein the viral infection is a Norovirus infection.

62. The method of claim 61, wherein said vaccine formulation confers protection from one or more symptoms of Norovirus infection.

63. A method of making a virus-like particle comprising expressing the polypeptide of claim 29 in a host cell; growing the cell in conditions in which virus-like particles are formed; and isolating the virus-like particles.

64. The method of claim 63, wherein the non-enveloped virus is a Calicivirus.

65. The method of claim 64, wherein said Calicivirus is a Norovirus or Sapovirus.

66. The method of claim 65, wherein said Norovirus is a genogroup I or genogroup II Norovirus.

67. The method of claim 66, wherein the polypeptide has the amino acid sequence of SEQ ID NO: 1.

68. The method of claim 63, wherein the consensus sequence is SEQ ID NO: 2.

69. A method of making a virus-like particle comprising:

aligning amino acid sequences of capsid proteins from two or more circulating strains of a non-enveloped virus;

determining a consensus sequence from said aligned amino acid sequences;

preparing a composite sequence based on said consensus sequence that contains at least 1 different amino acid as compared to each of the capsid sequences of said two or more circulating strains; and

expressing said composite sequence in a host cell, thereby producing a virus-like particle.

70. The method of claim 69, wherein the non-enveloped virus is selected from the group consisting of Calicivirus, Picornavirus, Astrovirus, Adenovirus, Reovirus, Polyomavirus, Papillomavirus, Parvovirus, and Hepatitis E virus.

71. The method of claim 70, wherein the non-enveloped virus is a Calicivirus.

72. The method of claim 71, wherein the Calicivirus is a Norovirus or Sapovirus.

73. The method of claim 72, wherein the Norovirus is a genogroup I or genogroup II Norovirus.

FIGURE 1

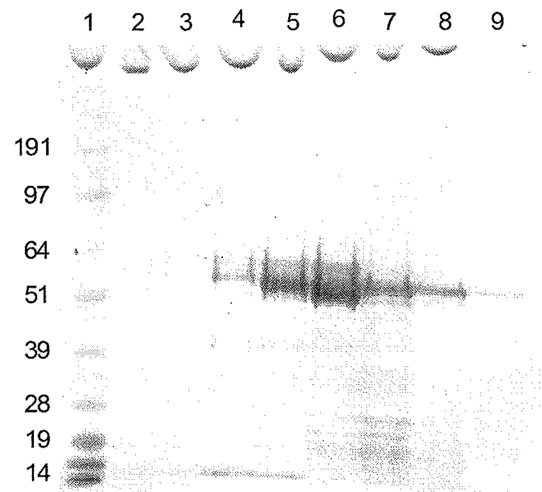
MKMAS-X_1-DA-X_2-PSDGS-X_3-ANLVPEVNNEVMALEPVVGAAIAAPVAGQQNVIDPWIRNNFVQAPGG
EFTVSPRNAPGEILWSAPLGPDLNPYLSHLARMYNGYAGGFEVQVILAGNAFTAGKIIFAAVPPNFPTEG
LSPSQVTMFPHIIVDVRQLEPVLIPLPDVRNNFYHYNQSND-X_4-TIKLIAMLYTPLRANNAG-X_5-DVFT
VSCRVLTRPSPDFDFIFLVPPTVESRTKPF-X_6-VPILTVEEMTNSRFPIPLEKLFTGPS-X_7-AFVVQPQ
NGRCTTDGVLLGTTQLSPVNICTFRGDVTHIAG-X_8-X_9-X_{10}-YTMNLAS-X_{11}-NWNNYDPTEEIPAPLGT
PDFVGKIQGVLTQTT-X_{12}-X_{13}-DGSTRGHKATV-X_{14}-TGS-X_{15}-X_{16}-FTPKLG-X_{17}-X_{18}-QF-X_{19}-TD
T-X_{20}-ND-X_{21}-ET-X_{22}-QNT-X_{23}-FTPVGV-X_{24}-QDG-X_{25}-X_{26}-X_{27}-H-X_{28}-NEPQQWVLP-X_{29}-YSG
R-X_{30}-X_{31}-HNVHLAPAVAP-X_{32}-FPGEQLLFFRSTMPGCSGYPNM-X_{33}-LDCLLPQEWV-X_{34}-HFYQEA
APAQSDVALLRFVNPDTGRVLFECKLHKSGYVTVAHTG-X_{35}-HDLVIPPNGYFRFDSWVNQFYTLAPMGN
G-X_{36}-GRRRA,

wherein X_1 = S or N; X_2 = S or N; X_3 = T or A; X_4 = S or P; X_5 = D or E; X_6 = S or T; X_7 = G or S; X_8 = S or T; X_9 = Q, R, or H; X_{10} = E, D, or N; X_{11} = Q or L; X_{12} = R or K; X_{13} = G or R; X_{14} = S or Y; X_{15} = V or A; X_{16} = P or H; X_{17} = S or R; X_{18} = V or I; X_{19} = S or T; X_{20} = S, E, or N; X_{21} = F or L; X_{22} = G or H; X_{23} = R or K; X_{24} = V or I; X_{25} = S or N; X_{26} = S or T; X_{27} = A or T; X_{28} = Q or R; X_{29} = D, S, or N; X_{30} = D, N, or T; X_{31} = S, V, or G; X_{32} = S or T; X_{33} = N or D; X_{34} = Q or L; X_{35} = Q or P; and X_{36} = T or A. **(SEQ ID NO: 2)**

FIGURE 2

TTAATTAAGCGGCCGCCCCCTTCACCATGAAGATGGCTTCCTCCGACGCTAACCCCTCCG
ACGGTTCCACCGCTAACCTGGTGCCCGAGGTGAACAACGAGGTGATGGCTCTCGAGCCCG
TGGTGGGCGCTGCTATCGCTGCTCCCGTGGCTGGCCAGCAGAACGTGATCGACCCCTGGA
TCCGTAACAACTTCGTGCAGGCTCCCGGTGGCGAGTTCACCGTGTCCCCCCGTAACGCTC
CCGGCGAGATCCTGTGGTCCGCTCCCCTGGGTCCCGACCTGAACCCCTACCTGTCCCACC
TGGCTCGTATGTACAACGGTTACGCTGGCGGTTTCGAGGTGCAGGTGATCCTGGCTGGTA
ACGCTTTCACCGCTGGCAAGATCATCTTCGCTGCTGTGCCCCCCAACTTCCCCACCGAGG
GCCTGAGCCCCTCCCAGGTGACCATGTTCCCCCACATCATCGTGGACGTGCGCCAGCTCG
AGCCTGTGCTGATCCCCCTGCCCGACGTGCGCAACAACTTCTACCACTACAACCAGTCCA
ACGACCCCACCATCAAGCTGATCGCTATGCTGTACACCCCCCTGCGTGCTAACAACGCTG
GTGACGACGTGTTCACTGTGTCCTGCCGTGTGCTGACCCGTCCCTCCCCCGACTTCGACT
TCATCTTCCTGGTGCCCCCTACCGTGGAGTCCCGTACCAAGCCCTTCACCGTGCCCATCC
TGACCGTGGAGGAGATGACCAACTCCCGTTTCCCCATCCCCCTCGAGAAGCTGTTCACCG
GTCCCTCCGGTGCTTTCGTGGTGCAGCCCCAGAACGGTCGTTGCACCACCGACGGTGTCC
TGCTGGGCACCACTCAGCTGTCCCCCGTGAACATCTGCACCTTCCGTGGTGACGTGACCC
ACATCGCTGGCACCCAAGAGTACACCATGAACCTGGCCTCCCAGAACTGGAACAACTACG
ACCCTACCGAGGAGATCCCCGCTCCTCTGGGCACCCCTGACTTCGTGGGCAAGATCCAGG
GTGTCCTGACCCAGACCACCCGCGGTGACGGCTCCACCCGTGGTCACAAGGCTACCGTGT
CCACCGGTTCCGTGCACTTCACCCCCAAGCTGGGTTCCGTCCAGTTCTCCACCGACACCT
CCAACGACTTCGAGACTGGCCAGAACACCAAGTTCACCCCCGTGGGTGTGGTGCAGGACG
GTTCTACCACCCACCAGAACGAGCCCCAGCAGTGGGTGCTGCCTGACTACTCCGGTCGTG
ACTCCCACAACGTGCACCTGGCTCCCGCTGTGGCTCCCACCTTCCCCGGCGAGCAGCTGC
TGTTCTTCCGTTCCACCATGCCCGGTTGCTCCGGTTACCCCAACATGAACCTCGACTGCC
TGCTGCCTCAGGAGTGGGTCCAGCACTTCTACCAGGAGGCTGCTCCCGCTCAGTCCGACG
TGGCTCTGCTGCGTTTCGTGAACCCCGACACCGGTCGTGTGCTGTTCGAGTGCAAGCTGC
ACAAGTCCGGTTACGTGACCGTGGCTCACACCGGCCAGCACGACCTGGTGATCCCTCCCA
ACGGTTACTTCCGTTTCGACTCCTGGGTGAACCAGTTCTACACCCTGGCTCCCATGGGTA
ACGGCACCGGTCGTCGTCGTGCTCTGTAATGGCTGGAGCTTTCTTTGCTGGATTGGCATC
TGATGTCCTTGGCTCTGGACTTGGTTCCCTAATCAATGCTGGGGCTGGGGCCATCAACCA
AAAAGTTGAATTTGAAAATAACAGAAAATTGCAACAAGCTTGGCGCGCC (SEQ ID NO: 3)

FIGURE 3



Lane 1	Molecular weight marker
Lane 2	Sucrose gradient fraction 1
Lane 3	Sucrose gradient fraction 2
Lane 4	Sucrose gradient fraction 3
Lane 5	Sucrose gradient fraction 4
Lane 6	Sucrose gradient fraction 5
Lane 7	Sucrose gradient fraction 6
Lane 8	Sucrose gradient fraction 7
Lane 9	Sucrose gradient fraction 8

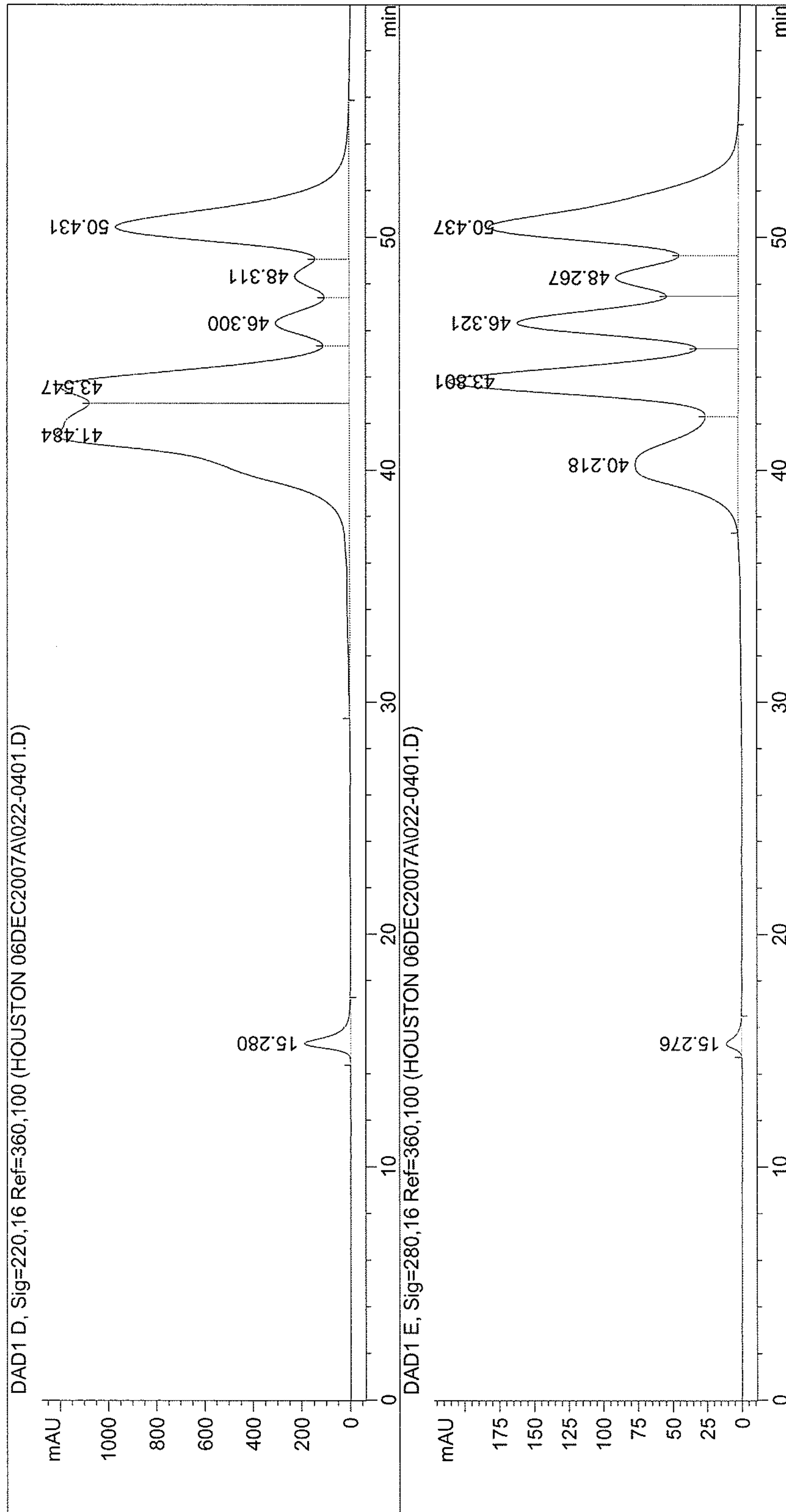
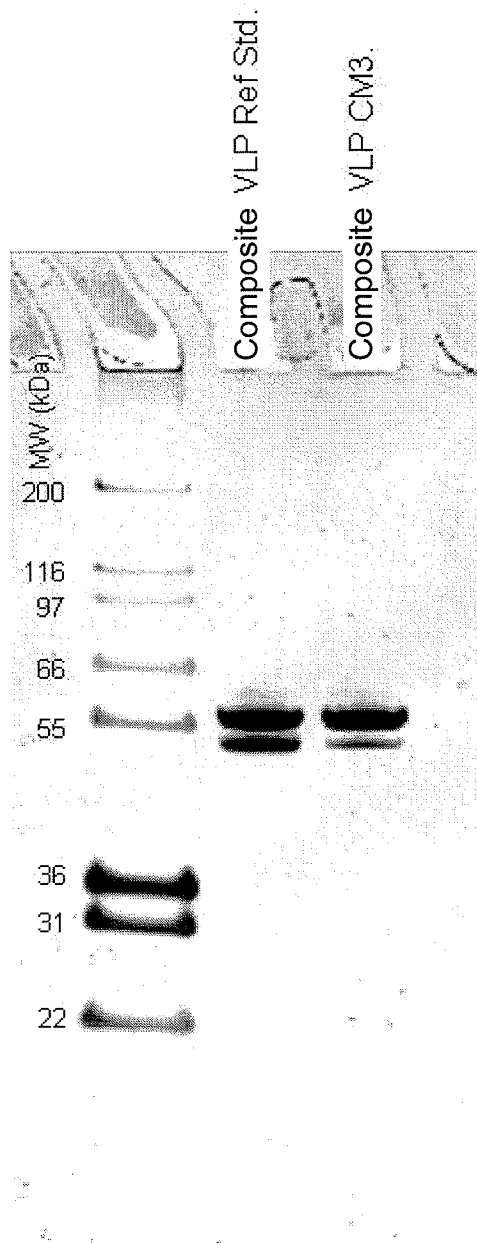


FIGURE 4

FIGURE 5



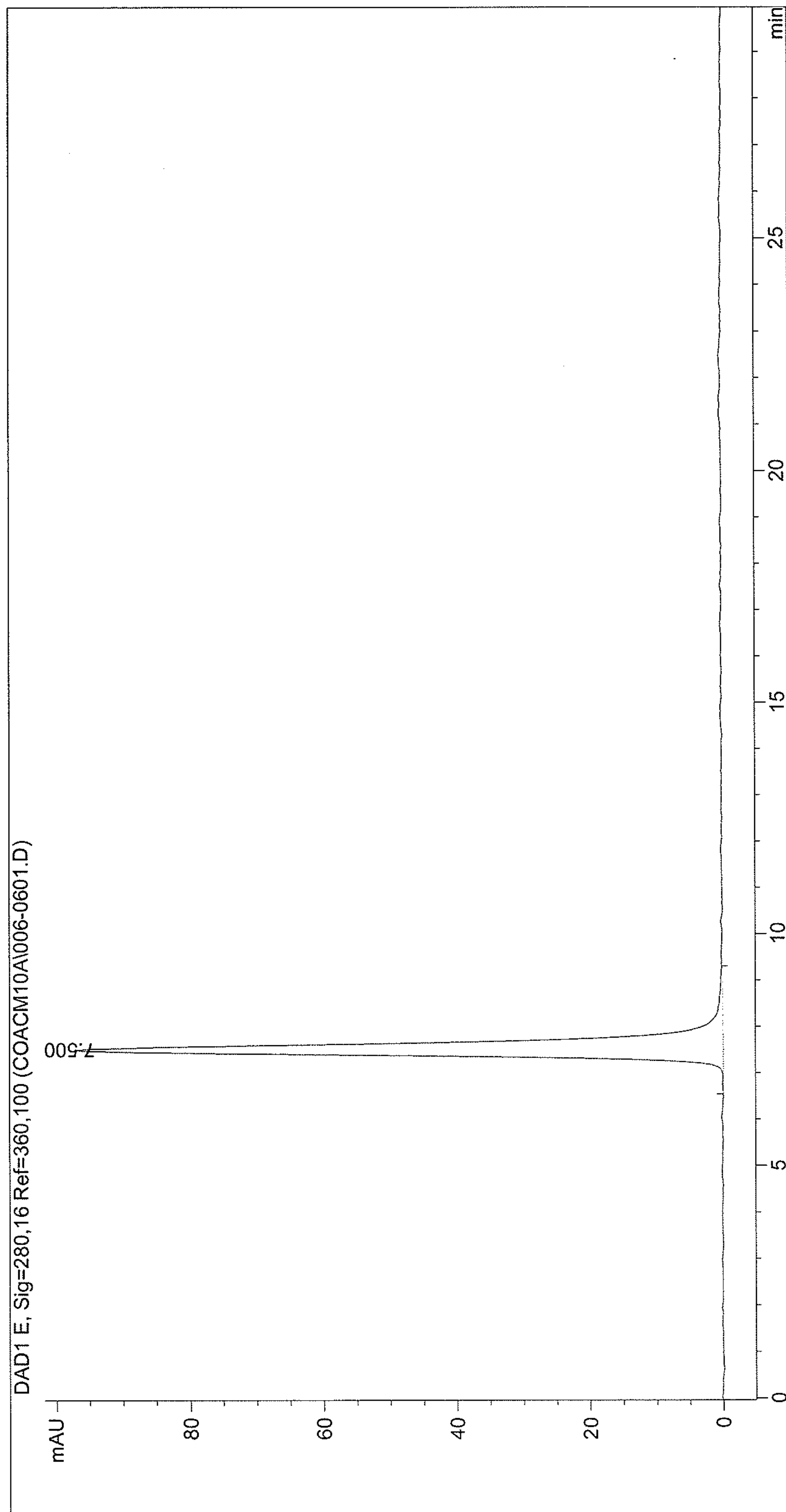


FIGURE 6

FIGURE 7

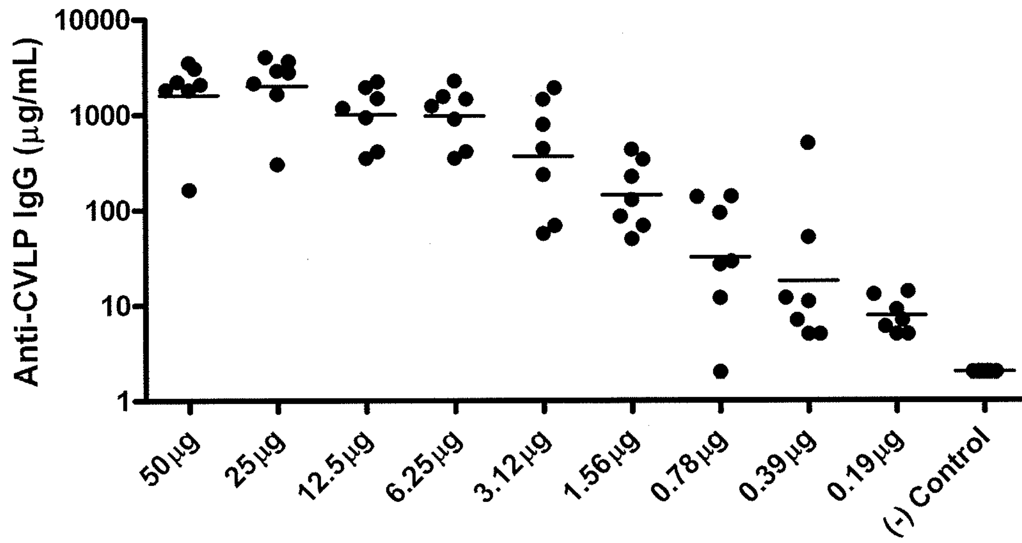


FIGURE 8

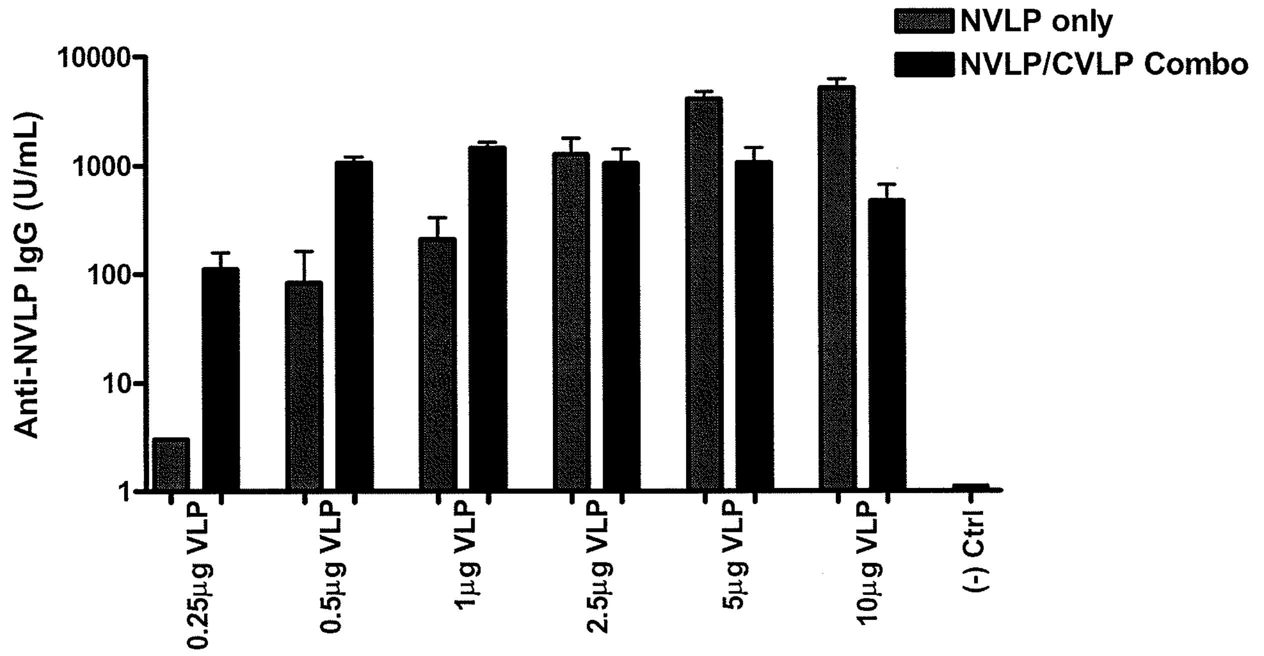


FIGURE 9

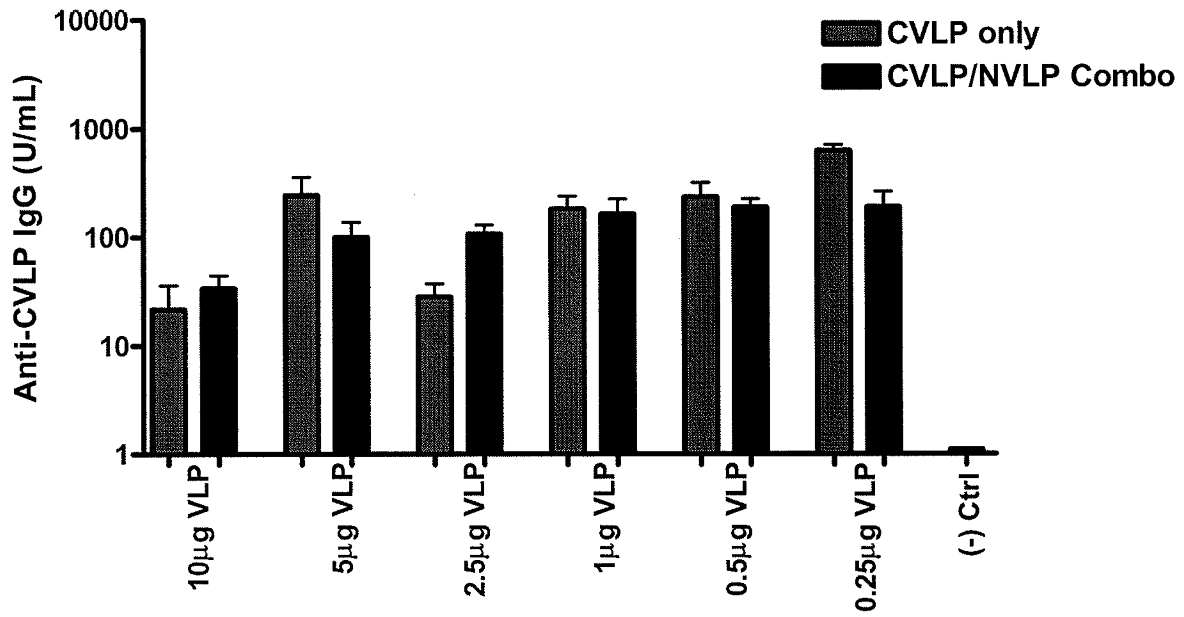


FIGURE 10

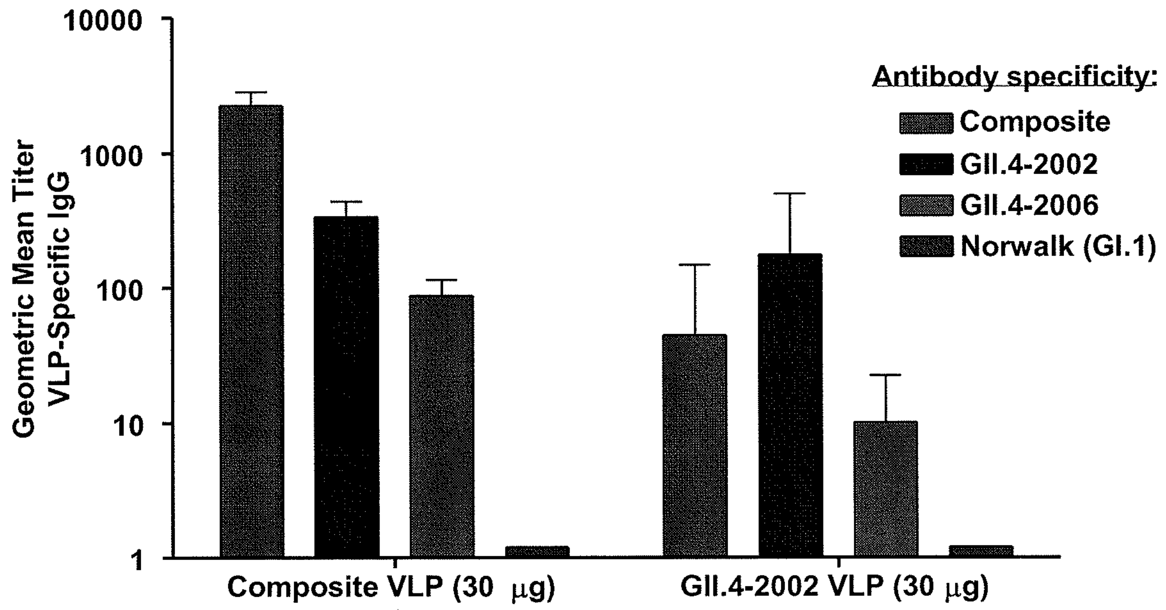


FIGURE 11

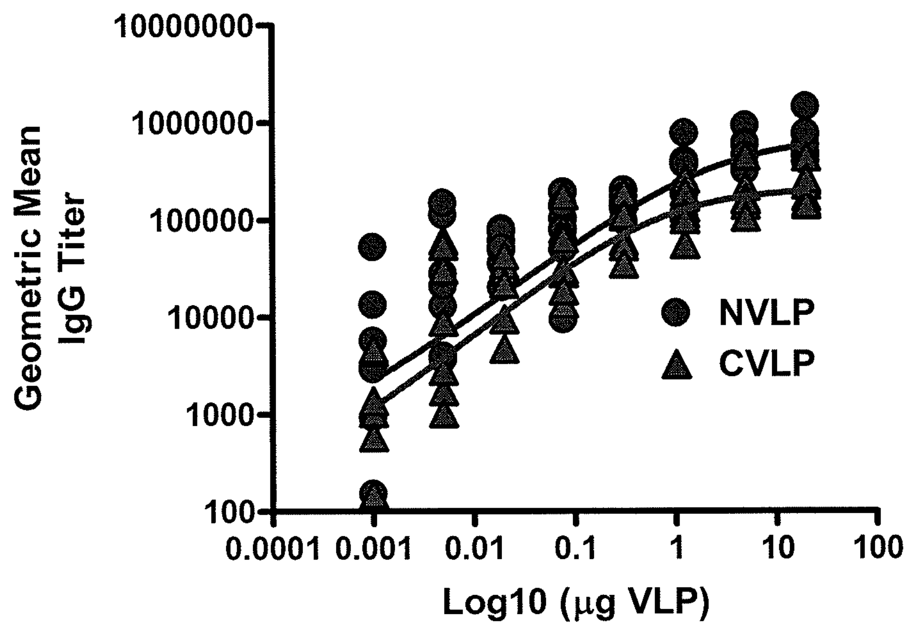


FIGURE 12

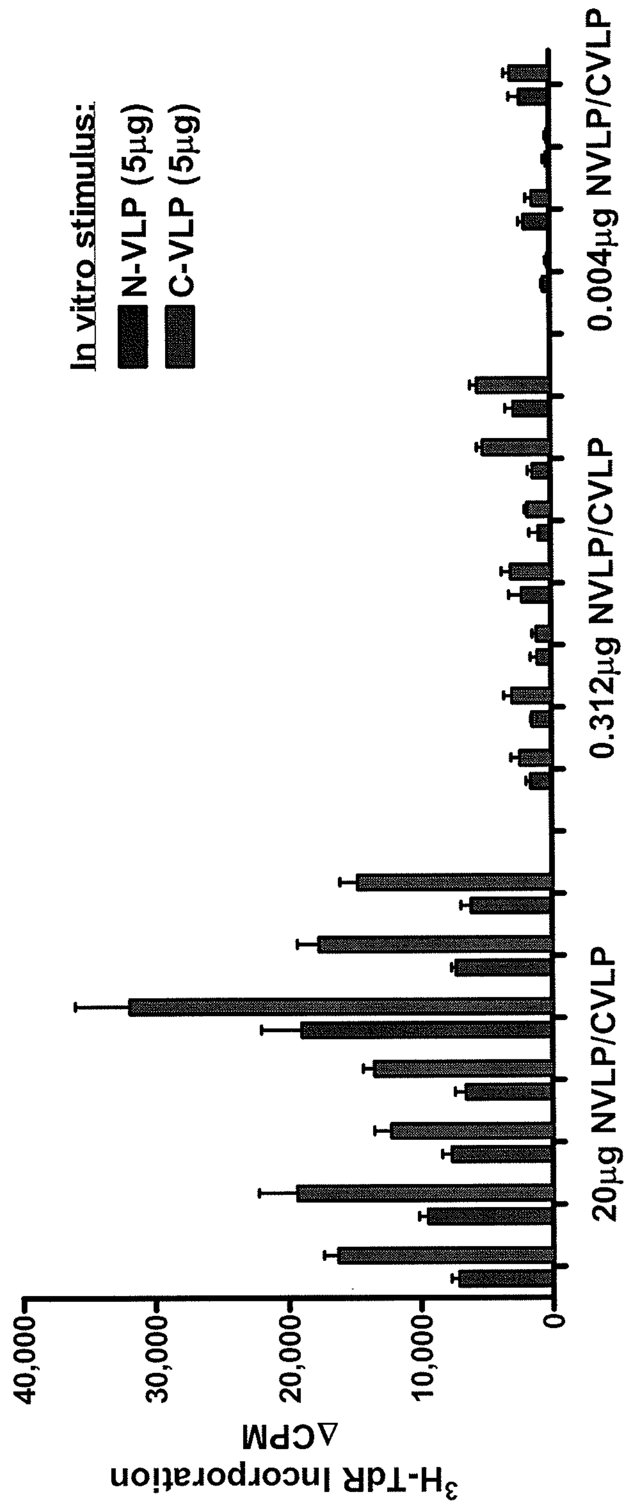


FIGURE 13

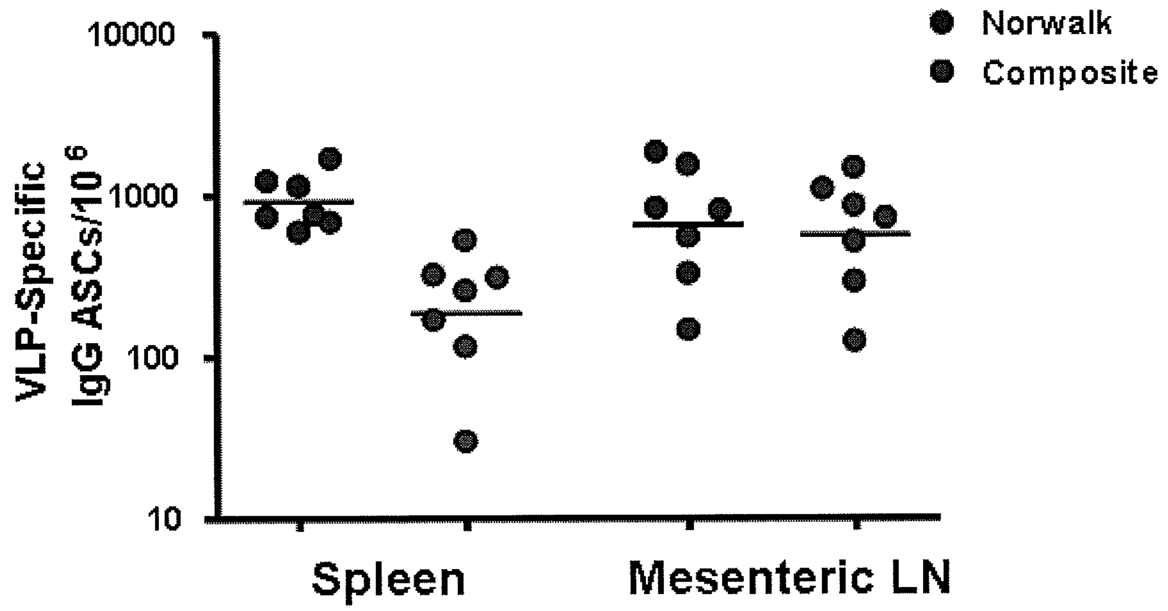


FIGURE 14

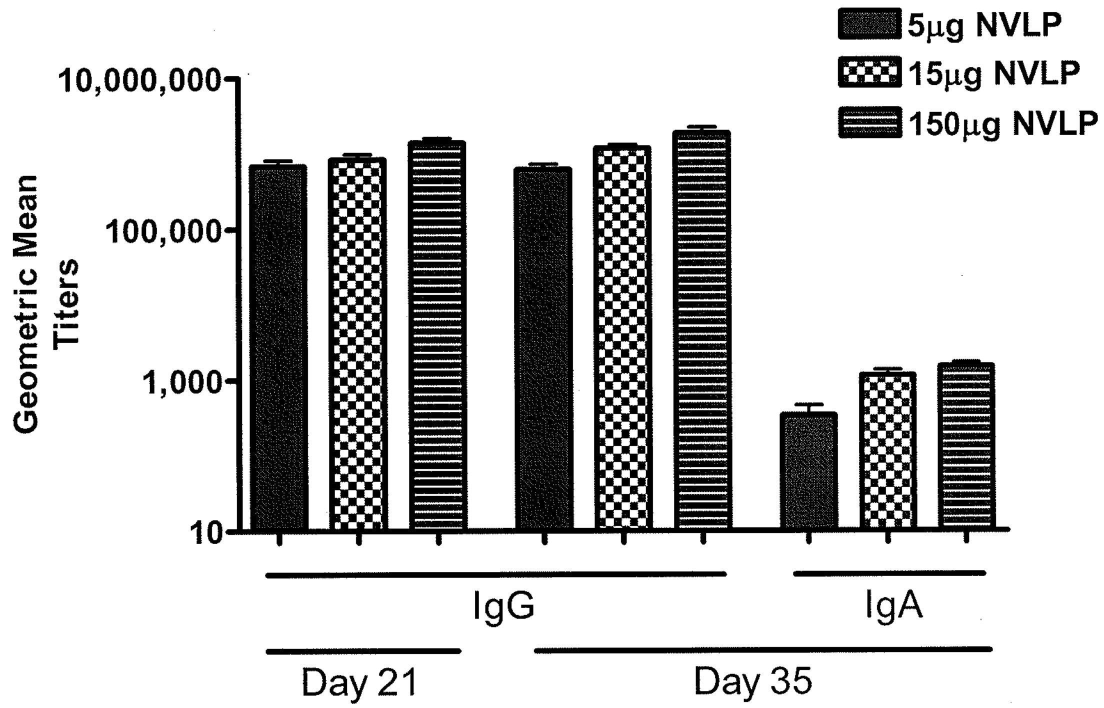


FIGURE 15

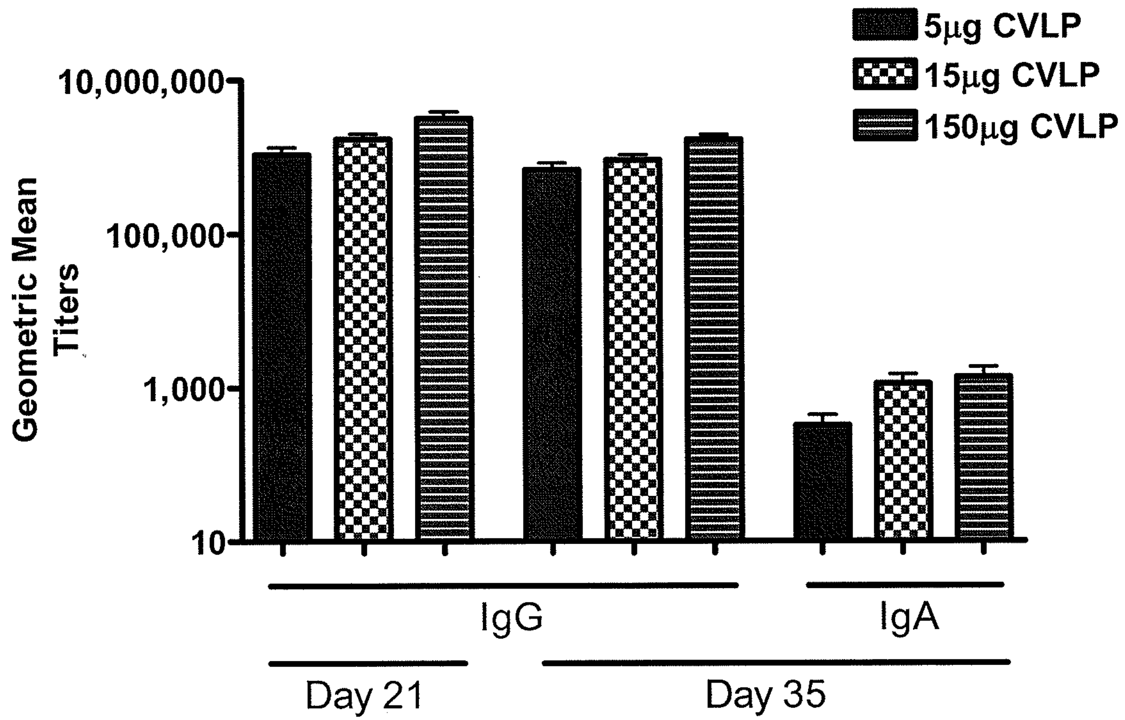


FIGURE 16

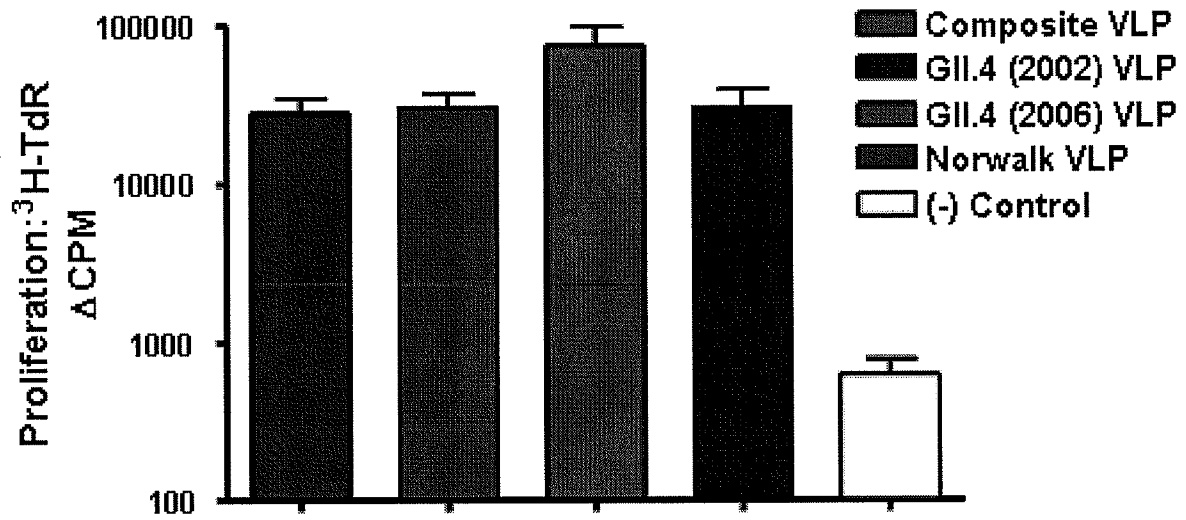


FIGURE 17

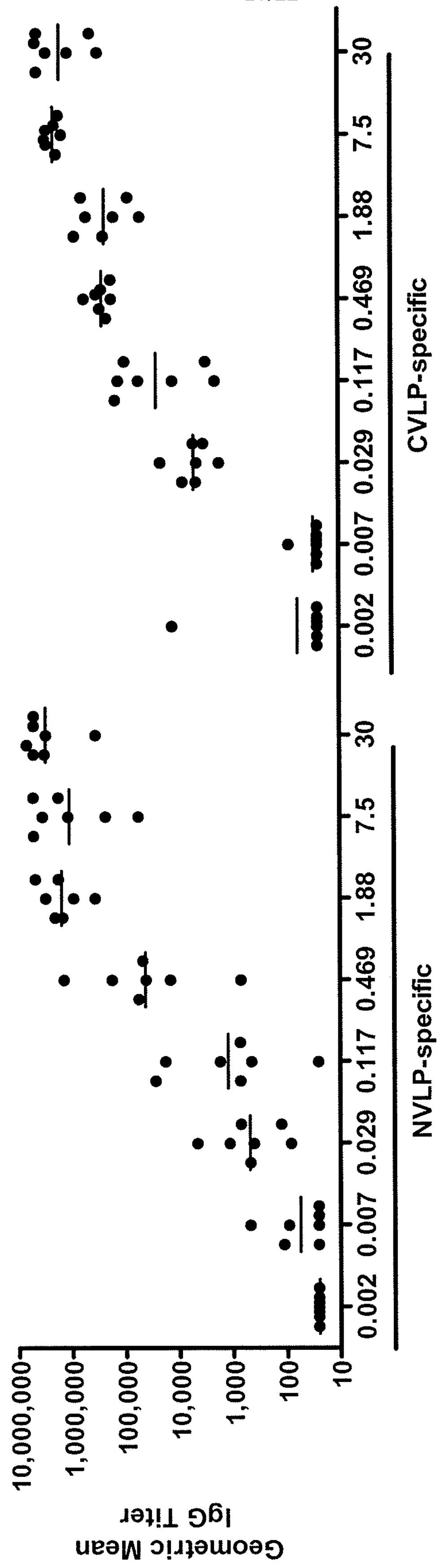


FIGURE 18

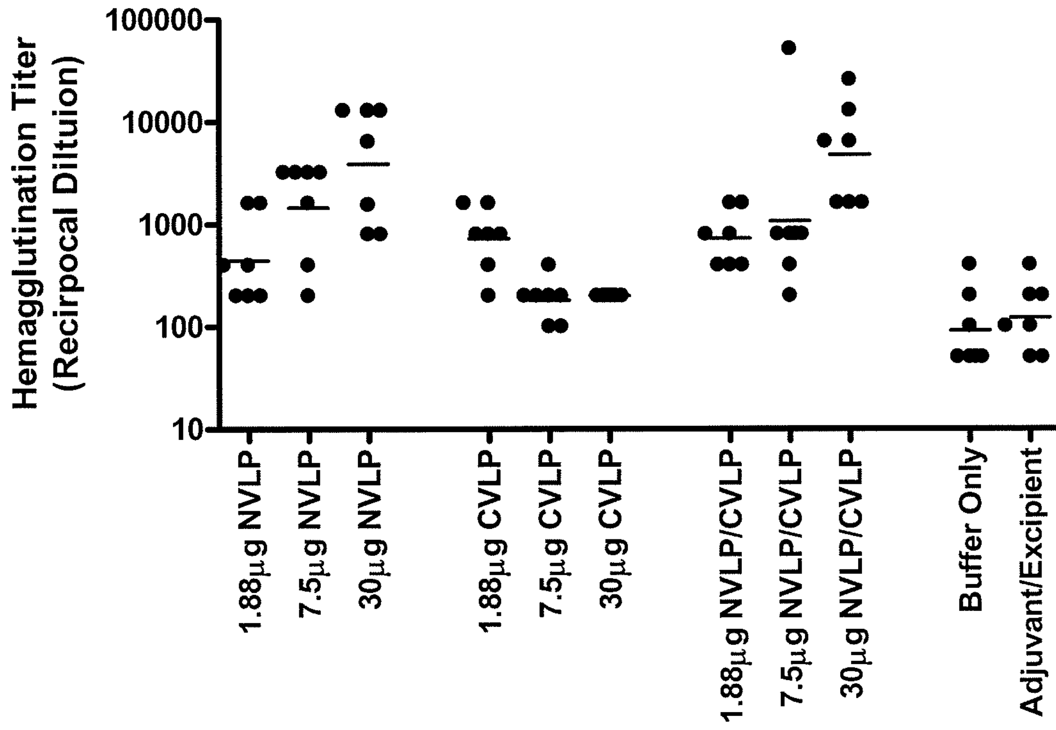


FIGURE 19

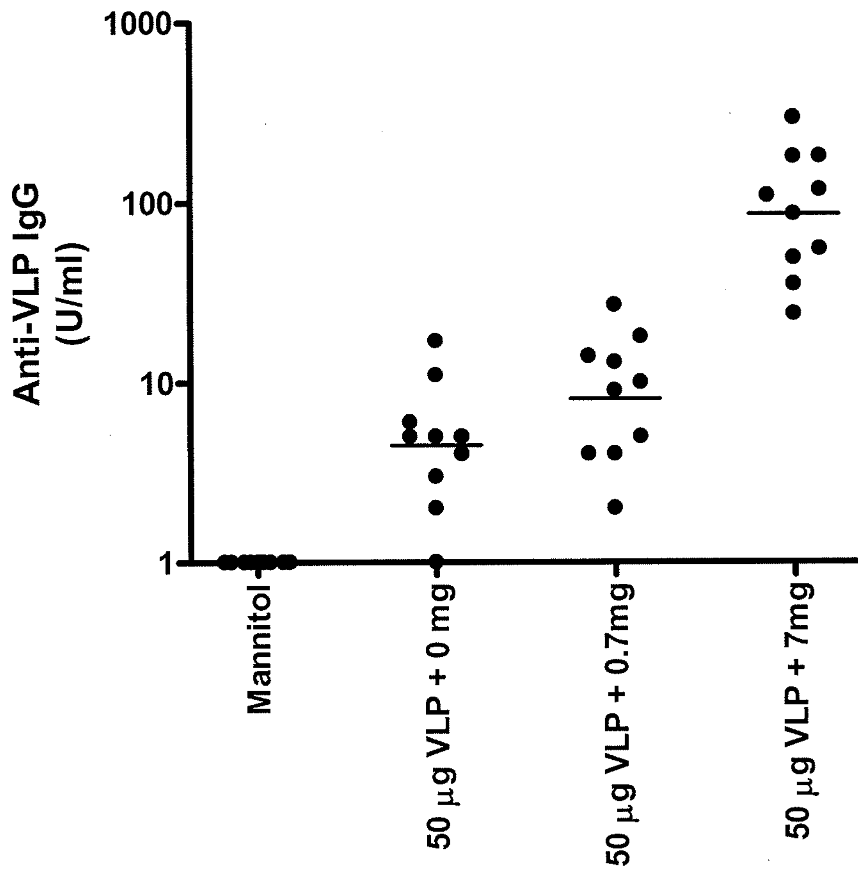


FIGURE 20

MKMASNDAAPSNDGAAGLVPExNNExMALEPVAGAAIAAPLTGQxNIIDPWIRxNFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPYLAHLARMYNGYAGGxEVQVxLAGNAFTAGKLVFAAIPPHFPIxNLSPxQITMFPHVIIDVRTLEPV
LLPLPDVRNNFFHYNQxxDPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFNYLVPPTVESKTKPFTLPI
LTIGELSNSRFPVPIDxLYTSPNExIVVQCQNGRxTLDGELxGTTQLxPSxICAFRGxxTRxxAHLSDQxN------
-xHRWNIQxTNLNGTPFDPxEDIPAPLGTPDFxGxVFGVxSQRNPDNT-------xRAHDAxVxTxSxxFTPKLGSV
xIGTWExxDFDxNQPTKFTPV—GLxDTxHFNQWVLPxYSGALTLNMNLAPSVAPxFPGEQLLFFRSxLPLKGGxSNG
AIDCLLPQEWVQHFYQESAPSxTxVALVRYxNPDTGRVLFEAKLHRxGFMTVAxNGSxPIVVPPNGYFRFDSWVNQF
YSLAPMGTGNGRRRI **(SEQ ID NO: 7)**

FIGURE 21

MMMASKDATxSADGASGAGQLVPEVNTADPLPMDPVAGSSTAVATAGQVNxIDPWIINNFVQAPQGEFTISPNNTP
GDVLFDLQLGPHLNPFLSHLSQMYNGWVGNMRVRIxLAGNAFTAGKIIVCCVPPGFxSxxLTIAQATLFPHVIADVR
TLDPIEVPLEDVRNVLYHNND-NQPTMRLVCMLYTPLRTGGGSGxxDSFVVAGRVLTCPSPDFNFLFLVPPTVEQKT
RPFTVPNIPLxxLSNSRxPxPIxGMxLSPDxxQxVQFQNGRCTIDGQLLGTTPVSxSQLxKxRGxITSGxRVLNLTE
LDGxPFMAFxxPAPxGFPDLGxCDWHIxMSKxPNSSxQxxPxxxxSVxTNxQxFVPHLGSIQxDExxS-xxGDYIGT
IxWISPPSxPxGxxxNLWKIPDYGSSLxEAxxLAPxVYPPGFGEVLVYFMSxxPGPNxxGAPNxVPCLLPQEYITHF
xSEQAPTxGEAALLHYVDPDTNRNLGEFKLYPGGYLTCVPNGxSxGPQQLPLNGVFVFVSWVSRFYQLKPVGTASxA
RGRLGVRR **(SEQ ID NO: 12)**

FIGURE 22

MCLYTRVLILHYHLLPLYGPLYHPRPLPxxxxxxYxxxxIxCxxxxxxxxxxVNVxxIFxQMxLWRPSDxTVYLPPP
-PVSKVVxTDxYVxRTNIFYHAGSSRLLAVGHPYFxIKKxxxNKxxVPKVSGYQYRVFRVxLPDPNKFGLPDTSxYN
PxTQRLVWACxGVEVGRGQPLGVGxSGHPLLNKLDDTENSxAYxxNxGxDNRxNVSMDYKQTQLCxxGCAPPIGEHW
GKGTxCxNxxVxxGDCPPLELINTVIQDGDMVDTGFGAMDFxTLQxNKSEVPLDICxSICKYPDYLQMxADPYGDSL
FFYLRREQMFARHFFNRAGTVGExVPDDLYIKGxGxxASxASSxYxPTPSGSxVTSDAQLFNKPYWLQKAQGHNNGI
CWGNQLFVTVVDTTRSTNMTLCAS-xSxSxxTYxNTxFKEYxRHVEEYDLQFIFQLCxITLTADVMxYIHSMNSSIL
EDWNFGLxPPPxGTLEDTYRFVQSQAITCQKxTPPAEKxDPYKKxxFWEVNLKEKFSxDLDQFPLGRKFLLQAGLRx
KPxxxxGxKRxxPxxSxxSTxAKRKRxKxx **(SEQ ID NO: 17)**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/53249

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 7/01; C07K 14/00; C07H 21/00 (2009.01)
USPC - -435/235.1, 530/350, 536/23.72
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC-435/235.1, 530/350, 536/23.72
IPC- C12N 7/01; C07K 14/00; C07H 21/00 (2009.01)

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWest, Google Scholar: amino acid$2, differn$4, capsid$2, antigenic, virus-like, host cell$2, non-enveloped, picornavirus, composite, polypeptide$2, protein$2, express$4, strain$2, antigenic, Calicivirus, Picornavirus, Astrovirus, Adenovirus, Reovirus, Polyomavirus, Papillomavirus, Parvovirus, Hepatitis E virus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2007/0207526 A1 (COIT et al.) 06 September 2007 (06.09.2007); abstract; para [0008], [0011], [0200], [0225], [0236], [0247], [0251], [0263], [0281], [0285], [0286], [0311], [0337], [0399], [0407], [0408], [0419], [0436], [0443], [0607], SEQ ID NO: 13	1-6, 8-14, 16-31, 33-35, 37, 39, 40-66
Y		69-73
A		7, 15, 32, 36, 38, 67, 68
Y	US 5,953,727 A (MASLYN et al.) 14 September 1999 (14.09.1999); col 11, ln 27-40	69-73
A	US 7,067,638 A (TAKEDA et al.) 27 Jun 2006 (27.06.2006); SEQ ID NO: 5	7, 15, 32, 36, 67, 68

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
05 December 2009 (05.12.2009)

Date of mailing of the international search report
14 DEC 2009

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