

**PIV5-BASED VACCINES WITH MODIFIED ANTIGEN: METHODS OF
MAKING AND USING THE SAME
FIELD**

[0001] The invention is generally related to the field of vaccination, and more particularly to compositions and methods of using a PIV5-based viral vector to express modified proteins to enhance expression of the bacterial Antigen On the Surface of cells (AOS), and therefore enhance the immunogenicity of the vaccine candidates. The antigen may be a bacterial or viral antigen.

BACKGROUND

[0002] Parainfluenza virus type 5 (Parainfluenza virus 5, PIV5) belongs to the family Paramyxoviridae and the genus Rubulavirus which also includes mumps virus, and its genome is single negative strand RNA with a length of 15246 nt. The genome full-length structure of PIV5 is 3'-Leader-NP-V/P-M-F-SH-HN-L-Trailer-5', namely, from 3' end to 5' end, Leader sequence and Trailer sequence are removed, and Nucleocapsid Protein (NP), V protein/phosphorylated protein (P), Matrix protein (M), Fusion protein (F), Small hydrophobic protein (SH), Hemagglutinin-neuraminidase protein (HN) and polymerase protein (Large protein, L) are encoded in sequence. Wherein, the V protein and the SH protein are non-structural proteins.

[0003] PIV5 is an excellent viral vector for vaccine development, and research on PIV5 recombinant vaccines is underway. In recent years, researchers have continuously explored the feasibility of using PIV5 as a vaccine vector. A common approach is to insert a protective antigen gene from a virus or bacteria into PIV5, and to express the inserted foreign gene by replication and translation of PIV5 vector. Given that PIV5 can infect respiratory tract without causing any illness, researchers often take advantage of this and focus on controlling certain respiratory viral infections. Therefore, the deep research on the molecular biological characteristics, the replication mechanism and the like of the virus is beneficial to the comprehensive and thorough understanding of the PIV5, so that a foundation is laid for the research on the PIV5 as a genetic engineering vaccine vector and the gene function research of the virus.

[0004] A distinctive trait of PIV5 is its ability to infect most mammalian cell types through sialic acid receptors without causing cytopathic effect. This allows for active replication of PIV5 in the respiratory tract after intranasal immunization, leading to the induction of mucosal immunity via generation of antigen-specific IgA

antibodies and long-lived IgA plasma cells, in addition to systemic humoral and cell-mediated immune responses (Wang, D. *et al.*, *J Virol* **91** (2017); Xiao, P. *et al.*, *Front Immunol* **12**, 623996 (2021)). Live PIV5 (not replication deficient) has been a component of the kennel cough vaccine administered to dogs intranasally for over 50 years. Due to potential shedding of the vaccine up to 5 days post-administration (Chladek, D. W., *et al.*, *American journal of veterinary research* **42**, 266-270 (1981)), humans have been exposed to PIV5 since the vaccine came to market. However, no disease has been reported in exposed individuals, highlighting PIV5 excellent safety record. Previously, live PIV5-vectored vaccines for bacterial pathogens like *Mycobacterium tuberculosis* and *Burkholderia mallei* and *pseudomallei* have been tested and proven efficacious in a mouse model (Dyke, J. S. *et al.* *Virulence* **11**, 1024-1040 (2020); Lafontaine, E. R. *et al.*, *Vaccine X* **1**, 100002 (2019); Chen, Z. *et al.* *Vaccine* **33**, 7217-7224 (2015)). Furthermore, two PIV5-vectored vaccines for COVID-19 and respiratory syncytial virus (RSV) (CVXGA1 and BLB201, respectively) have undergone phase I/II clinical trials.

[0005] Provided herein are composition of PIV5-vectored vaccine expressing modified bacterial or viral proteins to enhance expression of the Antigen On the Surface of cells (AOS). Also provided are methods of using the same to enhance the immunogenicity of the vaccine candidates.

SUMMARY

[0006] In accordance with the purpose(s) of this invention, as embodied and broadly described herein, this invention, in one aspect, relates to a PIV5-based antigen expression vector comprising: a PIV5-based viral expression vector; and a gene expressing a antigen in mammalian cells, wherein the gene expressing a bacterial antigen is modified by replacing the signal peptide of the gene with a PIV5 HN protein N-terminal domain (NT) or a PIV5 HN protein N-terminal domain plus transmembrane domain (NTTM), or by adding the PIV5 HN protein N-terminal domain (NT) only or the PIV5 HN protein N-terminal domain plus transmembrane domain (NTTM) sequences directly upstream of the antigen without removing the signal peptide; and wherein the gene expressing the modified bacterial or bacterial antigen is placed between the SH and HN or HN and L genes of the PIV5 genome, or by replacing the SH gene. The antigen may be a viral antigen or a bacterial antigen.

[0007] In one embodiment, the PIV5-based viral expression vector comprises a PIV5-AVLP, PIV5-W3A, PIV5-CPI, or PIV5-CVB backbone. In another embodiment, the PIV5-based antigen expression vector has a heterologous nucleic acid sequence with at least 98% sequence identity to SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12.

[0008] In another embodiment, the modified antigen gene is expressed on the surface of the mammalian cell. In one other embodiment, the bacterial antigen is that of a gram negative bacteria, wherein the gram negative bacteria is selected from a group consisting of *Burkholderia mallei*, *Borrelia burgdorferi*, and *Chlamydia trachomatis*, and the viral antigen is that of a virus selected from a group consisting of coronaviruses, respiratory syncytial virus (RSV), influenza virus A, influenza B, influenza C, orthomyxoviruses, thogoto virus, and isavirus.

[0009] In one embodiment, the gene expressing a bacterial antigen is modified by replacing the N-terminus signal peptide of a *Burkholderia mallei* Pal protein with the N-terminus and transmembrane domains of PIV5 HN glycoprotein. In another embodiment the gene expressing a bacterial antigen is modified by adding the N-terminus and transmembrane domains of PIV5 HN glycoprotein directly upstream of the *Borrelia burgdorferi* OspA BPBPk protein. In yet another embodiment, the gene expressing a bacterial antigen is modified by replacing the signal peptide from *Chlamydia trachomatis* major outer membrane protein (MOMP) with the N-terminus alone, or the N-terminus and transmembrane domains of PIV5 HN glycoprotein.

[0010] In another aspect, the invention relates to a PIV5-based antigen vector composition comprising a PIV5-based viral expression vector; and a gene expressing an antigen in mammalian cells, wherein the PIV5-based viral expression vector comprises a PIV5-AVLP, PIV5-W3A, PIV5-CPI, or PIV5-CVB backbone; wherein the gene expressing a bacterial antigen is modified by replacing the signal peptide of the gene with a PIV5 HN protein N-terminal domain (NT) or a PIV5 HN protein N-terminal domain plus transmembrane domain (NTTM), or by adding the PIV5 HN protein N-terminal domain (NT) only or the PIV5 HN protein N-terminal domain plus transmembrane domain (NTTM) sequences directly upstream of the antigen without removing the signal peptide; and wherein the gene expressing the modified antigen is placed between the SH and HN or HN and L genes of the PIV5 genome, or by replacing the SH gene. The antigen may be a viral antigen or a bacterial antigen.

[0011] In some embodiments of the composition, the PIV5-based antigen vector has a nucleic acid sequence with at least 98% sequence identity to SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12. In other embodiments, wherein the composition is a vaccine against a gram negative bacterial infection, wherein the gram negative bacteria is selected from a group consisting of *Burkholderia mallei*, *Borrelia burgdorferi*, and *Chlamydia trachomatis*, and the viral antigen is that of a virus selected from a group consisting of coronaviruses, respiratory syncytial virus (RSV), influenza virus A, influenza B, influenza C, orthomyxoviruses, thogoto virus, and isavirus.

[0012] In yet another aspect, the invention relates to a method of inducing an immune response in a subject in need thereof comprising administering a PIV5-based antigen vaccine to the subject, wherein the immune response comprises a humoral immune response and/or a cellular immune response, and wherein the subject is susceptible to a gram negative bacterial infection, wherein the gram negative bacteria is selected from a group consisting of *Burkholderia mallei*, *Borrelia burgdorferi*, and *Chlamydia trachomatis*. In another embodiment, the subject is susceptible to a viral infection, wherein the virus is selected from a group consisting of coronaviruses, respiratory syncytial virus (RSV), influenza virus A, influenza B, influenza C, orthomyxoviruses, thogoto virus, and isavirus.

[0013] In some embodiments, the subject was previously vaccinated against the bacteria or viral infection, the method comprising administering the composition to the subject. In other embodiments the composition is administered intranasally, intramuscularly, topically, or orally.

[0014] Additional advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the invention. The advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

BRIEF DESCRIPTIONS OF THE DRAWINGS

[0015] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate (one) several embodiment(s) of the invention and together with the description, serve to explain the principles of the invention.

[0016] Figure 1 shows schematics of constructs of PIV5-based *Burkholderia mallei* and *pseudomallei* (Pal) cDNAs. PIV5-Pal contains native form of Pal, PIV5-NTTM-Pal contains the PIV5 NH N-terminal and transmembrane domain (NTTM) added to the N-terminus of Pal.

[0017] Figure 2 shows a Western blot analysis demonstrating expression of Pal protein in MHD11 (PIV5-Pal) and MHD39 (PIV5-NTTM-Pal) recombinant virus-infected cells as well as cell supernatant.

[0018] Figures 3A-3B show PIV5 AVLP-based *Burkholderia mallei* and *pseudomallei* (Pal) constructs and immunogenicity. Figure 3A is a schematic of constructs of PIV5 AVLP-based *Burkholderia mallei* and *pseudomallei* (Pal) cDNAs. PIV5 AVLP-Pal contains native form of Pal, PIV5 AVLP-NTTM-Pal contains the PIV5 HN N-terminal and transmembrane domain (NTTM) added to the N-terminus of Pal. Figure 3B is a data plot showing the anti-Pal serum IgG antibody response from AVLP-immunized mice. Five, six-to-eight-week-old Balb/c female mice were immunized with 3.8×10^4 AP of MHD30 or GOB10 AVLPs, or just PBS control. Animals were bled at 14 days post-immunization and then boosted at 21 days post-immunization with the same dose of each construct. At 28-days post-immunization, mice were humanely euthanized, and blood was collected. The serum samples were used to perform an ELISA to quantify anti-Pal antibodies. GOD10 (AVLP-NTTM-Pal) elicited anti-Pal antibodies while MHD30 (AVLP-Pal) did not.

[0019] Figures 4A-4B show schematics of pCAGGS and PIV5-based Lyme disease (OspA/BPBPk) constructs cDNAs. Figure 4A shows that pMHD22 contains the human codon-optimized OspA BPBPk gene, pMHD24 contains the human codon-optimized OspA BPBPk gene with the PIV5 HN N-terminal and transmembrane domain (NTTM) added to the N-terminus. Figure 4B shows that pMHD35 is PIV5 (W3A) containing the human codon-optimized OspA BPBPk gene with the PIV5 HN N-terminal and transmembrane domain (NTTM) added to the N-terminus and the chimeric gene was inserted between the SH and HN genes, pMHD36 is PIV5 (W3A) containing the human codon-optimized OspA B31 gene with the PIV5 HN N-terminal and transmembrane domain (NTTM) added to the N-terminus inserted between the SH and HN genes, pMHD106 is CPI containing the human codon-optimized OspA BPBPk gene with the PIV5 HN N-terminal and transmembrane domain (NTTM) added to the N-terminus inserted between the SH and HN genes, and pMHD116 is CVB containing the human codon-optimized OspA BPBPk gene with the PIV5 HN

N-terminal and transmembrane domain (NTTM) added to the N-terminus in place of the SH gene.

[0020] Figures 5A-5B shows Western blot analysis (FIG. 5A) and bar graph (FIG. 5B) of OspA expression in cell lysate of pMHD22 and pMHD24 transfected cells.

[0021] Figures 6A-6C are images of immunofluorescence analysis of OspA surface expression in cells that were either transfected with pMHD22 (FIG. 6B) and pMHD24 (FIG. 6C) constructs. The images are shown at 20X.

[0022] Figure 7 is an image of a Western blot showing the detection of OspA expression. Vero cells were mock-infected or infected with PIV5 vector control, PIV5-AB31, or PIV5-ABPBK at an MOI of 1. Forty-eight hours after infection the cells were lysed, and the lysates resolved on an SDS-PAGE gel and immunoblotted with anti-OspA and anti-PIV5 NP antibodies.

[0023] Figure 8 shows a plot of Anti-OspA serum IgG data. Mice received two doses of alum alone or alum + 20 µg of rOspAB31 protein subcutaneously, or 10⁶ PFU of PIV5 vector control, PIV5-ABPBK (MHD35), or PIV5-AB31 (MHD36) intranasally at 21 days interval. Blood collected at day 17 and months 3, 6, or 12 post-prime were used. Anti-OspAB31 IgG antibody titers were quantified by ELISA. The LOD is indicated by the dotted line. Statistical significance was calculated for each vaccine group by nonparametric test with Dunn's multiple comparisons in comparison to pre-boost. (*p≤0.05, **p<0.01, ***p<0.001, ****p<0.0001).

[0024] Figures 9A-9C are data plots showing the neutralization of *Borrelia burgdorferi* with serum from animals vaccinated with PIV5-ABPBK or PIV5-AB31. Serum from vaccinated mice was collected before tick challenge at 4 months after prime vaccination on D117 (FIG. 9A), at 9 months after prime vaccination on D270 (FIG. 9B) and at 18 months after prime vaccination on D533 (FIG. 9C). Serum from the 4-month experiment was pooled per group (n=5 mice/group) because the volume per individual was insufficient. For subsequent experiments (9-month and 18-month), serum from n=3-6 mice per group were individually tested. Statistical significance was calculated for each vaccine group by nonparametric test with Dunn's multiple comparisons in comparison to day 0. (*p≤0.05, **p<0.01, ****p<0.0001). Bb, *Borrelia burgdorferi*.

[0025] Figures 10A-10F are data plots showing the protection of PIV5-ABPBK or PIV5-AB31-vaccinated mice against a multi-strain *Borrelia burgdorferi* tick

challenge. Bladder, heart, and joint tissues were collected from all vaccinated mice euthanized at 4 months, 9 months and 15 months post prime vaccination and processed for Bb DNA purification and *flaB* qPCR. Bladder and heart tissues were also cultured in BSK-H media for analysis of *B. burgdorferi* viability and samples were collected for confirmation by PCR. Representative graphs are shown for Bb load in tissues (FIGs. 10A-10C) and viability of Bb in tissues (FIGs. 10D-10F). Bb, *Borrelia burgdorferi*.

[0026] Figure 11 is a schematic describing the pCAGGS *Chlamydia* (MOMP) construct cDNAs. pCVL168 contains the codon-optimized MOMP gene, pCVL169 contains the human codon-optimized MOMP gene with the signal peptide deleted and replaced with that of CD5, pCVL170 contains the human codon-optimized MOMP gene with the PIV5 HN N-terminal and transmembrane domain (NTTM) added to the N-terminus, pCVL171 contains the human codon-optimized MOMP gene with the signal peptide deleted and replaced with the PIV5 HN N-terminal and transmembrane domain (NTTM), pCVL172 contains the human codon-optimized MOMP gene with the signal peptide deleted and replaced with the PIV5 HN N-terminal domain (NT).

[0027] Figure 12 is a schematic describing the PIV5-based *Chlamydia* (MOMP) vaccine virus construct cDNAs. pMHD175 is CPI containing the human codon-optimized MOMP gene with the signal peptide deleted and replaced with that of CD5 in between the SH and HN genes, pMHD177 is CPI containing the human codon-optimized MOMP gene with the signal peptide deleted and replaced with the PIV5 HN N-terminal domain (NT) in between the SH and HN genes, pMHD181 is CVB containing the human codon-optimized MOMP gene with the signal peptide deleted and replaced with that of CD5 in place of SH, pMHD182 is CVB containing the human codon-optimized MOMP gene with the signal peptide deleted and replaced with the PIV5 HN N-terminal domain (NT) in place of SH.

[0028] Figures 13A-13F are images of immunofluorescence analysis of MOMP total expression in cells that were transfected with pCVL168-pCVL172 constructs.

[0029] Figure 14 is an image of a Western blot analysis of MOMP expression in cell lysate of cells transfected with pCVL168-pCVL172 constructs.

DETAILED DESCRIPTION

[0030] The present invention may be understood more readily by reference to the following detailed description of preferred embodiments of the invention and the

Examples included therein and to the Figures and their previous and following description.

I. Definitions

[0031] To facilitate an understanding of the principles and features of the various embodiments of the disclosure, various illustrative embodiments are explained herein. Although exemplary embodiments of the disclosure are explained in detail, it is to be understood that other embodiments are contemplated. Accordingly, it is not intended that the disclosure is limited in its scope to the details of construction and arrangement of components set forth in the description or examples. The disclosure is capable of other embodiments and of being practiced or carried out in various ways.

[0032] In describing the exemplary embodiments, specific terminology will be resorted to for the sake of clarity. As used in the specification and the appended claims, the singular forms “a,” “an” and “the” include plural references unless the context clearly dictates otherwise. For example, reference to a component is intended also to include composition of a plurality of components. References to a composition containing “a” constituent is intended to include other constituents in addition to the one named.

[0033] Ranges may be expressed herein as from “about” or “approximately” or “substantially” one particular value and/or to “about” or “approximately” or “substantially” another particular value. When such a range is expressed, other exemplary embodiments include from the one particular value and/or to the other particular value.

[0034] Similarly, as used herein, “substantially free” of something, or “substantially pure”, and like characterizations, can include both being “at least substantially free” of something, or “at least substantially pure”, and being “completely free” of something, or “completely pure.”

[0035] The terms “patient”, “individual”, “subject”, and “animal” are used interchangeably herein and refer to mammals, including, without limitation, human and veterinary animals (e.g., cats, dogs, cows, horses, sheep, pigs, etc.) and experimental animal models. In a preferred embodiment, the subject is a human.

[0036] As described herein, the term “vaccinating” designates typically the sequential administration of one or more antigens to a subject, to produce and/or

enhance an immune response against the antigen(s). The sequential administration includes a priming immunization followed by one or several boosting immunizations.

[0037] Within the context of the present invention, the term “pathogen” refers to any agent that can cause a pathological condition. Examples of “pathogens” include, without limitation, cells (e.g., bacteria cells, diseased mammal cells, cancer mammal cells), fungus, parasites, viruses, prions or toxins. Preferred pathogens are infectious pathogens. In a particular embodiment, the infectious pathogen is a virus, such as coronaviruses, respiratory syncytial virus (RSV), human metapneumovirus (hMPV), orthomyxoviruses, influenza virus A (including all strains varying in their HA and NA proteins, such as (non-limiting examples) H1N1, H1N2, H2N2, H3N2, H7N7, and H3N8); influenza B, influenza C, thogoto virus (including Dhori, Batken virus, SiAR 126 virus), Chicken anemia virus, isavirus (e.g., infectious salmon anemia virus) and the like. In another embodiment, the infection pathogen is a bacterial pathogen, such as *Burkholderia mallei*, *Borrelia burgdorferi*, *Chlamydia trachomatis*, *Elizabethkingia meningoseptica*, and the like..

[0038] An antigen, as used therein, designates any molecule which can cause a T-cell or B-cell immune response in a subject. An antigen specific for a pathogen is, typically, an element obtained or derived from said pathogen, which contains an epitope, and which can cause an immune response against the pathogen. Depending on the pathogenic agent, the antigen may be of various nature, such as a (poly)peptide, protein, nucleic acid, lipid, cell, etc. Live weakened forms of pathogens (e.g., bacteria, viruses), or killed or inactivated forms thereof may be used as well, or purified material therefrom such as proteins, peptides, lipids, etc. The antigen may be naturally-occurring or artificially created. It may be exogenous to the treated mammal, or endogenous (e.g., tumor antigens). The antigen may be produced by techniques known per se in the art, such as for instance synthetic or recombinant technologies, or enzymatic approaches.

[0039] In a particular embodiment, the antigen is a protein, polypeptide and/or peptide. The terms “polypeptide”, “peptide” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues. The terms also apply to amino acid polymers in which one or more amino acid residues may be modified or non-naturally occurring residues, such as an artificial chemical mimetic of a

corresponding naturally occurring amino acid. It should be understood that the term “protein” also includes fragments or variants of different antigens, such as epitope-containing fragments, or proteins obtained from a pathogen and subsequently enzymatically, chemically, mechanically or thermally modified.

[0040] It will be appreciated by one of skill in the art that any bacterial or viral antigen could be used in the system of the present invention to increase an immune response against desired antigens associated with the bacterial or viral antigen by being expressed as an AOS. Further contemplated within scope of this invention are antigens that usually are not presented to the immune system such as those in the cytosol which also when combined with the system of the present invention to present these antigens on the outer surface of a virion thus allowing the immune system to mount an immunogenic response to these antigens which previously were “hidden” from the immune system.

[0041] A “therapeutically effective amount” means the amount of a compound (e.g., a CVB-based composition as described herein) that, when administered to a subject for treating a state, disorder or condition, is sufficient to effect such treatment. The “therapeutically effective amount” will vary depending on the compound or bacteria administered as well as the disease and its severity and the age, weight, physical condition and responsiveness of the mammal to be treated.

[0042] The phrase “pharmaceutically acceptable”, as used in connection with compositions of the disclosure, refers to molecular entities and other ingredients of such compositions that are physiologically tolerable and do not typically produce untoward reactions when administered to a mammal (e.g., a human). Preferably, as used herein, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in mammals, and more particularly in humans.

[0043] The term “pharmaceutically acceptable composition” as used herein refers to a composition comprising at least one compound as disclosed herein formulated together with one or more pharmaceutically acceptable carriers.

[0044] The term “administration” refers to the introduction of an amount of a predetermined substance into a patient by a certain suitable method. The composition

disclosed herein may be administered via any of the common routes, as long as it is able to reach a desired tissue, for example, but is not limited to, inhaling, intraperitoneal, intravenous, intramuscular, subcutaneous, intradermal, oral, topical, intranasal, intrapulmonary, or intrarectal administration. However, since peptides are digested upon oral administration, active ingredients of a composition for oral administration should be coated or formulated for protection against degradation in the stomach.

[0045] The term “dose” means a single amount of a compound or an agent that is being administered thereto; and/or “regimen” which means a plurality of pre-determined doses that can be different in amounts or similar, given at various time intervals, which can be different or similar in terms of duration. In some embodiments, a regimen also encompasses a time of a delivery period (e.g., agent administration period, or treatment period). Alternatively, a regimen is a plurality of predetermined plurality pre-determined vaporized amounts given at pre-determined time intervals.

[0046] The term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water or aqueous solution saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Alternatively, the carrier can be a solid dosage form carrier, including but not limited to one or more of a binder (for compressed pills), a glidant, an encapsulating agent, a flavorant, and a colorant. Suitable pharmaceutical carriers are described in “Remington's Pharmaceutical Sciences” by E. W. Martin.

[0047] The terms “treat” or “treatment” of a state, disorder or condition include: (1) preventing or delaying the appearance of at least one clinical or sub-clinical symptom of the state, disorder or condition developing in a subject that may be afflicted with or predisposed to the state, disorder or condition but does not yet experience or display clinical or subclinical symptoms of the state, disorder or condition; or (2) inhibiting the state, disorder or condition, i.e., arresting, reducing or delaying the development of the disease or a relapse thereof (in case of maintenance treatment) or at least one clinical or sub-clinical symptom thereof; or (3) relieving the

disease, i.e., causing regression of the state, disorder or condition or at least one of its clinical or sub-clinical symptoms. The benefit to a subject to be treated is either statistically significant or at least perceptible to the patient or to the physician.

[0048] By “comprising” or “containing” or “including” is meant that at least the named compound, element, particle, or method step is present in the composition or article or method, but does not exclude the presence of other compounds, materials, particles, method steps, even if the other such compounds, material, particles, method steps have the same function as what is named.

[0049] As used herein, the term “parainfluenza virus 5” (PIV5) includes, for example and not limitation, strains KNU-11, CC-14, D277, 1168-1, and 08-1990. Non-limiting examples of PIV5 genomes are listed in GenBank Accession Nos. NC_006430.1, AF052755.1, KC852177.1, KP893891.1, KC237065.1, KC237064.1 and KC237063.1, which are hereby incorporated by reference.

[0050] As used herein, the term “expression” refers to the process by which polynucleic acids are transcribed into mRNA and translated into peptides, polypeptides, or proteins. If the polynucleic acid is derived from genomic DNA, expression may, if an appropriate eukaryotic host cell or organism is selected, include splicing of the mRNA. In the context of the present invention, the term also encompasses the yield of the F gene mRNA and F proteins achieved following expression thereof.

[0051] As used herein, the term “F protein” or “Fusion protein” or “F protein polypeptide” or “Fusion protein polypeptide” refers to a polypeptide or protein having all or part of an amino acid sequence of an RSV Fusion protein polypeptide. Numerous RSV Fusion and Attachment proteins have been described and are known to those of skill in the art. WO/2008/114149, which is herein incorporated by reference in its entirety, sets out exemplary F and G protein variants (for example, naturally occurring variants).

[0052] As used herein, the term “combination” of a CVB-based composition as described herein and at least a second pharmaceutically active ingredient means at least two, but any desired combination of compounds can be delivered simultaneously or sequentially (e.g., within a 24-hour period). It is contemplated that when used to treat various diseases, the compositions and methods of the present disclosure can be

utilized with other therapeutic methods/agents suitable for the same or similar diseases. Such other therapeutic methods/agents can be co-administered (simultaneously or sequentially, in any order) to generate additive or synergistic effects. Suitable therapeutically effective dosages for each agent may be lowered due to the additive action or synergy. Also, two or more embodiments of the disclosure may be also co-administered to generate additive or synergistic effects.

[0053] As used herein, “gram-negative bacterial ” is a term used herein for the purposes of the specification and claims may describe without limitation one or more (i.e., one or a combination) of the following bacterial species: *Acinetobacter baumannii*, *Acinetobacter haemolyticus*, *Actinobacillus actinomycetemcomitans*, *Aeromonas hydrophila*, *Bacteroides fragilis*, *Bacteroides theataiomacron*, *Bacteroides distasonis*, *Bacteroides ovatus*, *Bacteroides vulgatus*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Brucella melitensis*, *Burkholderia cepacia*, *Burkholderia pseudomallei*, *Burkholderia mallei*, *Prevotella corporis*, *Prevotella intermedia*, *Prevotella endodontalis*, *Porphyromonas asaccharolytica*, *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter fetus*, *Citrobacter freundii*, *Citrobacter koseri*, *Edwardsiella tarda*, *Eikenella corrodens*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Enterobacter agglomerans*, *Escherichia coli*, *Francisella tularensis*, *Haemophilus influenzae*, *Haemophilus ducreyi*, *Helicobacter pylori*, *Kingella kingae*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Klebsiella rhinoscleromatis*, *Klebsiella ozaenae*, *Legionella pneumophila*, *Moraxella catarrhalis*, *Morganella morganii*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Plesiomonas shigelloides*, *Proteus mirabilis*, *Proteus vulgaris*, *Proteus penneri*, *Proteus myxofaciens*, *Providencia stuartii*, *Providencia rettgeri*, *Providencia alcalifaciens*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Salmonella typhi*, *Salmonella paratyphi*, *Serratia marcescens*, *Shigella flexneri*, *Shigella boydii*, *Shigella sonnei*, *Shigella dysenteriae*, *Stenotrophomonas maltophilia*, *Streptobacillus moniliformis*, *Streptococcus pneumoniae*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Vibrio alginolyticus*, *Yersinia enterocolitica*, *Yersinia pestis*, *Yersinia pseudotuberculosis*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Rickettsia prowazekii*, *Coxiella burnetii*, *Ehrlichia chaffeensis*, or *Bartonella henselae*.

[0054] The mention of one or more method steps does not preclude the presence of additional method steps or intervening method steps between those steps

expressly identified. Similarly, it is also to be understood that the mention of one or more components in a composition does not preclude the presence of additional components than those expressly identified.

[0055] The materials described as making up the various elements of the disclosure are intended to be illustrative and not restrictive. Many suitable materials that would perform the same or a similar function as the materials described herein are intended to be embraced within the scope of the disclosure. Such other materials not described herein can include, but are not limited to, for example, materials that are developed after the time of the development of the disclosure.

II. Compositions

[0056] Presented herein are compositions using PIV5 (W3A, CPI, or CVB backbone) to express modified bacterial proteins to enhance expression of the bacterial Antigen On the Surface of cells (AOS), and therefore enhance the immunogenicity of the vaccine candidates.

A. Parainfluenza Virus 5 (PIV5)

[0057] Parainfluenza virus 5 (PIV5), a negative-stranded RNA virus, is a member of the Rubulavirus genus of the family Paramyxoviridae which includes many important human and animal pathogens such as mumps virus, human parainfluenza virus type 2 and type 4, Newcastle disease virus, Sendai virus, HPIV3, measles virus, canine distemper virus, rinderpest virus and respiratory syncytial virus. PIV5 was previously known as Simian Virus-5 (SV5). Although PIV5 is a virus that infects many animals and humans, no known symptoms or diseases in humans have been associated with PIV5. Unlike most paramyxoviruses, PIV5 infect normal cells with little cytopathic effect. As a negative stranded RNA virus, the genome of PIV5 is very stable. As PIV5 does not have a DNA phase in its life cycle and it replicates solely in cytoplasm, PIV5 is unable to integrate into the host genome. Therefore, using PIV5 as a vector avoids possible unintended consequences from genetic modifications of host cell DNAs. PIV5 can grow to high titers in cells, including Vero cells which have been approved for vaccine production by WHO and FDA. Thus, PIV5 presents many advantages as a vaccine vector.

[0058] A PIV5 vaccine vector of the present invention may be constructed using any of a variety of methods, including, but not limited to, the reverse genetics system described in more detail in He et al. (Virology; 237(2):249-60, 1997). PIV5

encodes eight viral proteins. Nucleocapsid protein (NP), phosphoprotein (P) and large RNA polymerase (L) protein are important for transcription and replication of the viral RNA genome. The V protein plays important roles in viral pathogenesis as well as viral RNA synthesis. The fusion (F) protein, a glycoprotein, mediates both cell-to-cell and virus-to-cell fusion in a pH-independent manner that is essential for virus entry into cells. The structures of the F protein have been determined and critical amino acid residues for efficient fusion have been identified. The hemagglutinin-neuraminidase (HN) glycoprotein is also involved in virus entry and release from the host cells. The matrix (M) protein plays an important role in virus assembly and budding. The hydrophobic (SH) protein is a 44-residue hydrophobic integral membrane protein and is oriented in membranes with its N terminus in the cytoplasm. For reviews of the molecular biology of paramyxoviruses see, for example, Whelan et al., 2004, *Curr Top Microbiol Immunol*; 283:61-119; and Lamb & Parks, (2006). *Paramyxoviridae: the viruses and their replication*. In *Fields Virology*, 5th edn, pp. 1449–1496. Edited by D. M. Knipe & P. M. Howley. Philadelphia, PA: Lippincott Williams & Wilkins.

[0059] Previously, recombinant PIV5 viruses expressing foreign genes from numerous pathogens, including Influenza, Rabies, Respiratory Syncytial Virus, *Tuberculosis*, *Burkholderia*, and MERS-CoV have been generated and tested as vaccine candidates (Li, Z., et al., *J Virol*, 87(1):354 (2013); Chen, Z., et al., *J Virol*, 87(6): 2986 (2013); Wang, D., et al., *J Virol*, 91(11) (2017); Chen, Z., et al., *Vaccine*, 33(51):7217 (2015); Lafontaine, E.R., et al., *Vaccine X*, 1:100002 (2018); Li, K., et al., *mBio*, 11(2) (2020)). Because it actively replicates in the respiratory tract following intranasal immunization, PIV5-vectored vaccines can generate mucosal immunity that includes antigen-specific IgA antibodies and long-lived IgA plasma cells (Wang, D., et al., *J Virol*, 91(11) (2017). Xiao, P., et al., *Front Immunol*, 12:623996 (2021)). Recently a PIV5-vectored vaccine expressing the spike protein from SARS-CoV-2 Wuhan (WA1; CVXGA1) has been shown to be efficacious in mice and ferrets. A single, intranasal dose of CVXGA1 induced WA1-neutralizing antibodies and protected K18-hACE2 mice against lethal infection with SARS-CoV-2 WA1. Furthermore, a single, intranasal dose of CVXGA1 protected ferrets from infection with SARS-CoV-2 WA1 and blocked transmission to cohoused naïve ferrets (An, D., et al., *Sci Adv*, 7(27) (2021)). While these studies determined its

efficacy against SARS-CoV-2 WA1, further studies were necessary to establish its efficacy against SARS-CoV-2 variants.

1. PIV5 - W3A

[0060] In one embodiment, the PIV5-based vaccine vector of the present invention is based on any of a variety of wild type, mutant, or recombinant (rPIV5) strains. Wild type strains include, but are not limited to, the PIV5 strains W3A, WR (ATCC® Number VR-288TM), canine parainfluenza virus strain 78-238 (ATCC number VR-1573) (Evermann et al., 1980, J Am Vet Med Assoc; 177:1132-1134; and Evermann et al., 1981, Arch Virol; 68:165-172), canine parainfluenza virus strain D008 (ATCC number VR-399) (Binn et al., 1967, Proc Soc Exp Biol Med; 126:140-145), MIL, DEN, LN, MEL, cryptovirus, CPI+, CPI-, H221, 78524, T1 and SER. See, for example, Chatziandreou et al., 2004, J Gen Virol; 85(Pt 10):3007-16; Choppin, 1964, Virology: 23:224-233; and Baumgartner et al., 1987, Intervirology; 27:218-223. Additionally, PIV5 strains used in commercial kennel cough vaccines, such as, for example, BI, FD, Merck, and Merial vaccines, may be used.

2. PIV5 – CPI

[0061] In another embodiment, the PIV5-based vaccine vector of the present invention is based on the PIV5 CPI strain vector backbone. The most notable difference between the PIV5 CPI and PIV5 W3A strain backbones is in the PIV5 F protein of the CPI strain that consists of an additional 22 amino acid extension as part of its cytoplasmic tail. The extension of the F protein is thought to result in inhibition of the fusogenic properties of the virus. CPI based viruses are more lytic and produce more progeny virus in infected cells compared to the W3A based viruses that does not have the extended PIV5 F protein tail and possess additional amino acid difference compared with CPI.

3. PIV5-CVB

[0062] In yet another embodiment, the PIV5 viral vaccine of the present invention will have a mutation, alteration, or deletion in one or more of the eight proteins of the PIV5 genome. For example, a PIV5 viral expression vector may include one or more mutations, including, but not limited to any of those described herein. In some aspects, a combination of two or more (two, three, four, five, six, seven, or more) mutations may be advantageous and may demonstrated enhanced activity.

[0063] In one embodiment, the PIV5 vector was further modified by introducing the mutations in the PIV5 V/P gene and by deletion of the PIV5 SH gene, further enhancing vaccine efficiency. S157F and S308A in the PIV5 V/P genes have been shown previously to increase viral polymerase activities and improve viral titer or yield (Timani KA, Sun D, Sun M, et al. *J Virol.*, 82(18):9123-9133 (2008); Sun D, Luthra P, Li Z, He B., *PLoS Pathog.*, 5(7):e1000525 (2009)). PIV5 W3A strain-based RSV vaccine with a single S157F mutation was shown to induce higher levels of neutralizing antibodies than PIV5 CPI-vectored RSV vaccine in cotton rats (See table 19). PIV5 W3A strain lacking the SH gene and expressing influenza virus H5 HA protein induced higher levels of antibodies and provided better protection against influenza virus challenge (Li Z, Gabbard JD, Mooney A, et al., *J Virol.*, 87(17): 9604–9609 (2013)). In one embodiment, a newly generated modified PIV5 viral vector backbone is presented herein and named as CVB through introducing S157F into the V/P gene, and deleting the SH gene from the PIV5 W3A viral genome. In another embodiment, CVB -vectored SARS-CoV-2 vaccines for intranasal immunization were generated.

[0064] A mutation includes, but is not limited to, a mutation of the V/P gene, a mutation of the shared N-terminus of the V and P proteins, a mutation of residues 26, 32, 33, 50, 102, 156, and/or 157 of the shared N-terminus of the V and P proteins, a mutation lacking the C-terminus of the V protein, a mutation lacking the small hydrophobic (SH) protein, a mutation of the fusion (F) protein, a mutation of the phosphoprotein (P), a mutation of the large RNA polymerase (L) protein, a mutation incorporating residues from canine parainfluenza virus, and/or a mutation that enhances syncytial formation.

[0065] A mutation may include, but is not limited to, rPIV5-V/P-CPI-, rPIV5-CPI-, rPIV5-CPI+, rPIV5V Δ C, rPIV-Rev, rPIV5-RL, rPIV5-P-S156N, rPIV5-P-S157A, rPIV5-P-S308A, rPIV5-L-A1981D and rPIV5-F-S443P, rPIV5-MDA7, rPIV5 Δ SH-CPI-, rPIV5 Δ SH-Rev, and combinations thereof.

[0066] PIV5 can infect cells productively with little cytopathic effect (CPE) in many cell types. In some cell types, PIV5 infection causes formation of syncytia, i.e., fusion of many cells together, leading to cell death. A mutation may include one or more mutations that promote syncytia formation (see, for example Paterson et al., 2000, *Virology*; 270:17-30).

[0067] The V protein of PIV5 plays a critical role in blocking apoptosis induced by virus. Recombinant PIV5 lacking the conserved cysteine-rich C-terminus (rPIV5V Δ C) of the V protein induces apoptosis in a variety of cells through an intrinsic apoptotic pathway, likely initiated through endoplasmic reticulum (ER)-stress (Sun et al., 2004, J Virol; 78:5068-5078). Mutant recombinant PIV5 with mutations in the N-terminus of the V/P gene products, such as rPIV5-CPI-, also induce apoptosis (Wansley and Parks, 2002, J Virol; 76:10109-10121). A mutation includes, but is not limited to, rPIV5 Δ SH, rPIV5-CPI-, rPIV5V Δ C, and combinations thereof.

4. PIV5-AVLP

[0068] The disclosure provides PIV5-based AVLP compositions and methods for the use as bacterial vaccines. The disclosure relates to PIV5- based AVLP compositions, and constructs for their manufacture, which can be utilized to introduce expressible polynucleotide sequences of interest into host cells. In some embodiments, the PIV5-based AVLP composition is an isolated polynucleotide sequence that transcribes a single stranded RNA encoding a portion of a negative stranded PIV5 genome, wherein said polynucleotide sequence transcribes a single stranded RNA encoding at least a portion of the negative stranded NP, V/P and L genes, and wherein said polynucleotide sequence lacks, or is otherwise incapable of transcribing, one or more of the M, F, SH and HN genes, and wherein said polynucleotide sequence contains an heterologous non-PIV5 nucleotide sequence inserted between the V/P and L genes (e.g., Fig 1A). In some embodiments, one or all of the M, F, SH and HN genes are completely removed and replaced with the expressible heterologous nucleotide sequence of interest. In some embodiments, the isolated polynucleotide comprises all of each of the NP, V/P and L genes. In some embodiments, the isolated polynucleotide comprises all of at least one of the NP, V/P and L genes.

[0069] In some embodiments, the PIV5-based AVLP compositions comprise at least one AVLP particle comprising an isolated polynucleotide as described herein. In other embodiments, the PIV5-based AVLP compositions comprise a plurality of AVLP particles comprising an isolated polynucleotide as described herein.

[0070] The compositions of the present disclosure may be formulated in pharmaceutical preparations in a variety of forms adapted to the chosen route of

administration. One of skill will understand that the composition will vary depending on mode of administration and dosage unit.

[0071] The agents of this invention can be administered in a variety of ways, including, but not limited to, intravenous, topical, oral, intranasal, subcutaneous, intraperitoneal, intramuscular, and intratumor deliver. In some aspects, the agents of the present invention may be formulated for controlled or sustained release. One advantage of intranasal immunization is the potential to induce a mucosal immune response.

B. Bacterial antigens expressed by PIV5 vector

[0072] Presented herein are compositions wherein the PIV5, AVLP, CPI or CVB backbone was used as a viral vector to express a bacterial antigen on a virus infected cell surface through the introducing of the PIV5 HN N-terminal cytoplasmic tail or cytoplasmic tail with the transmembrane domain. The invention overcomes the difficulty of display bacterial antigen on cell surface to improve antigen-specific immune responses. Here we present three examples of bacterial antigens that we have successfully expressed by PIV5 vector: *Burkholderia mallei* Pal protein, Lyme Disease (*Borrelia burgdorferi*) OspA protein and *Chlamydia trachomatis* MOMP protein.

1. *Burkholderia mallei* Pal protein

[0073] Lipopolysaccharide (LPS, endotoxin) is one of the bacterial components released from Gram-negative bacteria and has been shown to play a major role in the induction of sepsis. An early seminal study showed that, in humans, polyclonal antisera raised against heat-killed *Escherichia coli* (*E. coli*) J5 (featuring an exposed LPS core) reduced death by GNS in half. Subsequent studies showed that antibodies to the LPS core alone were not protective. Later, IgG in J5 antisera was shown to bind three *E. coli* outer membrane proteins: Lpp, OmpA, and peptidoglycan-associated lipoprotein (Pal). Since those studies, results from in vitro and in vivo experiments have further implicated Pal in the pathology of GNS.

[0074] *Burkholderia mallei* is a host-adapted Gram-negative bacterium and causes the incapacitating and highly fatal zoonotic disease glanders, which affects primarily horses, donkeys, and mules. The organism is extremely contagious through

contact with diseased equids and humans are susceptible to infection (Khan I, et al., *Transbound Emerg Dis.* 60(3):204–221 (2013)).

[0075] Comparative analyses indicate that *B. mallei* evolved from the bacterium *Burkholderia pseudomallei* through genomic reduction. The latter is commonly found in water and wet soils of countries bordering the equator and causes the global emerging tropical disease melioidosis (Wiersinga WJ, et al., *Nat Rev Dis Primers* 4:17107 (2018); Limmathurotsakul D, G et al., *Nat Microbiol.* 1:1 (2016)). The genes retained by *B. mallei* have a high level of sequence identity with their *B. pseudomallei* orthologs and the two species share many virulence determinants. The clinical and pathological manifestations of disease caused by the organisms are also very similar. In humans, infection typically occurs through punctured skin and the respiratory route, and the most common manifestations are life-threatening pneumonia and bacteremia (Carr-Gregory B, Waag DM. Glanders. In: Dembek ZF, editor. *Medical aspects of biological warfare*. Borden Institute, Office of the Surgeon General, AMEDD Center and School, Texas, US; 2007. p. 121–146.; Wiersinga WJ, et al., *Nat Rev Dis Primers* 4:17107 (2018)). Pathogenesis is complex and entails the synchronized expression of genes supporting intracellular and extracellular replication of bacteria, colonization of deep tissues and organ systems, and development of hallmark chronic lesions that are difficult to treat and eliminate.

[0076] Peptidoglycan-associated lipoprotein (Pal) is highly conserved among Enterobacteriaceae, but can be found in most Gram-negative bacteria. Pal is a component of the Tol-Pal system. These proteins create a complex that stretches the inner and outer membrane of gram-negative bacteria and play a role in maintaining cell wall integrity (Dyke, J. S. et al., *Virulence* 11, 1024-1040 (2020)). Pal can be exploited for the entry of macromolecules such as bacteriocins and bacteriophages (Godlewska R, et al., *FEMS Microbiol Lett.* 298(1):1–11 (2009); Duche D, *EcoSal Plus.* 8:2 (2019)).

2. Lyme Disease (*Borrelia burgdorferi*) OspA protein

[0077] Lyme disease is a tick-borne disease caused by *Borrelia burgdorferi sensu lato* (*s.l.*). The disease is typically characterized by the development of an expanding red rash at the site of the tick bite that may be followed by systemic complications including meningitis, carditis or arthritis. Almost all cases of Lyme disease are caused by one of three genospecies, *Borrelia afzelii*, *Borrelia garinii* and

Borrelia burgdorferi sensu stricto (s.s.). In Europe, all three species which infect humans are found. However, in North America only a single species, *Borrelia burgdorferi sensu stricto*, is found. *Borrelia burgdorferi* is a species of Gram negative bacteria of the spirochete class of the genus *Borrelia*. Antibiotic treatment of Lyme disease is usually effective but some patients develop a chronic disabling form of the disease involving joints or nervous system, which does not substantially improve even after parenteral antibiotic therapy, thus highlighting the need for a vaccine for high-risk populations.

[0078] Outer surface protein A (OspA) is a 31 kDa antigen, expressed by *Borrelia burgdorferi* s./ species present in the midgut of Ixodes ticks. OspA has proven to be efficacious in preventing Lyme disease in North America (Steere et al., N. Engl. J. Med. 339: 209-15, 1998; Sigal et al., N. Engl. J. Med. 339:216-22, 1998; erratum in: N. Engl. J. Med. 339:571, 1998). The amino terminus of fully processed OspA is a cysteine residue that is post-translationally modified with three fatty-acyl chains that anchor the protein to the outer surface of the bacterial membrane (Bouchon et al., Anal. Biochem. 246: 52-61, 1997). Lipidation of OspA is reported to stabilize the molecule (US8623375B2) and is essential for protection in the absence of a strong adjuvant (Erdile et al., Infect. Immun. 61: 81-90, 1993). A soluble, recombinant form of the protein lacking the amino-terminal lipid membrane anchor was co-crystallized with the Fab fragment of an agglutinating mouse monoclonal antibody to determine the structure of OspA, which was shown to comprise 21 anti-parallel β -strands followed by a single α -helix (Li et al., *Proc. Natl. Acad. Sci. U.S.A.* 94:3584-9, 1997).

[0079] A monovalent OspA-based vaccine (LYMERix®) was marketed in the USA for the prevention of Lyme disease. However, in Europe heterogeneity in OspA sequences across the three genospecies precludes broad protection with a vaccine based on OspA from a single strain (Gern et al., *Vaccine* 15:1551-7, 1997). Seven principal OspA serotypes have been recognized among European isolates (designated serotypes 1 to 7, Wilske et al., *J. Clin. Microbiol.* 31:340-50, 1993). OspA serotypes correlate with species; serotype 1 corresponds to *B. burgdorferi* s.s., serotype 2 corresponds to *B. afzelii* and serotypes 3 to 7 correspond to *B. garinii*.

[0080] Protective immunity acquired through immunization with OspA is unusual since the interaction between the host's immune response and the pathogen does not take place in the host, but in the mid-gut of the tick vector. In the case of Lyme disease, a tick acts as a vector or carrier for the transmission of Lyme disease from animals to humans. OspA specific antibody acquired during feeding by an infected tick prevents transmission of *B. burgdorferi* s.l. to the immunized mammalian host (de Silva et al., *J. Exp. Med.* 183: 271-5, 1996). Protection is antibody-mediated and is mainly affected through bactericidal antibody although an antibody that blocks attachment of the spirochete to a receptor on the lining of the tick gut epithelium may also be efficacious (Pal et al., *J. Immunol.* 166: 7398-403, 2001).

[0081] Rational development of effective OspA vaccines requires identification of the protective epitopes such as that defined by the protective monoclonal antibody LA-2 (Golde et al., *Infect. Immun.* 65: 882-9, 1997). X-ray crystallography and NMR analysis have been used to identify immunologically important hypervariable domains in OspA and have mapped the LA-2 epitope to amino acids 203-257 (Ding et al., *J. Mol. Biol.* 302: 1153-64, 2000; Luft et al. *J Infect Dis.* 185 (Suppl. 1): S46-51, 2002).

3. *Chlamydia trachomatis* MOMP protein

[0082] *Chlamydia* is a prevalent sexually transmitted infection that affects over 100 million people worldwide. Although most individuals infected with *Chlamydia trachomatis* are initially asymptomatic, symptoms can arise if left undiagnosed. Long-term infection can result in debilitating side effects such as pelvic inflammatory disease, infertility, and blindness. *Chlamydia* infection, therefore, constitutes a significant public health threat and underscores the need for a vaccine.

[0083] *Chlamydia* strains express a major outer membrane protein (MOMP) that is shown to be an effective vaccine antigen. However, in view of poor solubility, low yield and protein misfolding of the *Chlamydia* MOMP protein production of a functional recombinant MOMP protein for vaccine development has been challenging.

[0084] *Chlamydiale* bacteria are obligate intracellular pathogens of eukaryotic cells. Four chlamydial species are currently known— *C. trachomatis*, *C.*

pneumoniae, *C. pecorum* and *C. psittaci*— and genomic sequences for each of these are publicly available ((1999) Nature Genetics 21 :385-389; (2000) Nucleic Acids Res 28: 1397-1406; (2000) Nucleic Acids Res 28:2311-2314; (1998) Science 282:754-759).

[0085] *C. trachomatis* organisms are dimorphic, and alternate between 1) infectious "elementary bodies" (EBs) which are endocytosed by mucosal cells into vesicular inclusions; and 2) metabolically active, intracellular "reticulate bodies" (RBs). RBs replicate and redifferentiate into EBs before being released to infect neighbouring cells.

[0086] Treatment of EBs with n-lauroyl sarcosine produces "chlamydial OM complexes" (COMCs) containing three relatively detergent-resistant, cysteine-rich proteins: the Major Outer Membrane Protein (MOMP), encoded by *ompA*, and *OmcB* and *OmcA*, encoded by *omp2* and *omp3*, respectively (BMC Microbiology 2005, 5 :5, and references therein). There are a number of other Chlamydial outer membrane proteins such as for example, *PorB*, *PmpB*, *PmpC*, *PmpD*, *PmpG*, and *PmpH*.

[0087] MOMP is expressed in both EBs and RBs and is situated on the outer membrane where it functions as a porin. It constitutes about 60% of the membrane protein of the infectious EB. Structural and functional analysis has shown that native MOMP exists as an oligomer; the native conformation of *C. trachomatis* MOMP is a trimer with monomers that have a β -barrel, β -sheet secondary structure. In EB, MOMP forms trimers with disulfide bridges within and between its individual monomers (~ 40 kDa) and also between trimers (J. Bacteriol. 189:6222-6235, 2007).

[0088] There are at least 19 different *C. trachomatis* serovars capable of infecting humans (i.e., A to K, Ba, Da, Ia, Ja, LI to L3 and L2a) and these serovars have been typed based on serological differentiation of the antigenic epitopes on MOMP. The MOMPs encoded by each of these 19 different serovars share five well-conserved regions and four variable sequence segments or domains (termed VS or VD 1 to VD 4). The subspecies and serovar specific antigenic epitopes or determinants are located on the variable domains. Based on amino acid homology, the serovars have been subdivided into the following serogroups or classes: B class (B, Ba, D, Da, E, LI, L2 and L2a), C class (A, C, H, I, Ia, J, K and L3), and

intermediate class (F and G). Infection with any *C. trachomatis* serovar may result in disease: serovars A, B, Ba and C cause trachoma; serovars L1-L3 are the agents of lymphogranuloma venereum; serovars D-K cause sexually transmitted infections; and serovars G, I and D have been associated with cervical cancer.

C. Viral antigens expressed by PIV5 vector

[0089] Vaccines often contain a plurality of antigen components, e.g., derived from different proteins, and/or from different epitopic regions of the same protein. For example, a vaccine against a viral disease can comprise one or more polypeptide sequences obtained from the virus which, when administered to a host, elicit an immunogenic or protective response to viral challenge.

[0090] As mentioned, the disclosure can also be utilized to prepare polypeptide multimers, e.g., where an antigenic preparation is produced which is comprised of more than one polypeptide. For instance, virus capsids can be made up of more than one polypeptide subunit. By transducing a host cell with vectors carrying different viral envelope sequences, the proteins, when expressed in the cell, can self-assemble into three-dimensional structures containing more than one protein subunit (e.g., in their native configuration).

[0091] In further embodiments, the expressible heterologous nucleotide sequence is derived from another virus, other than PIV5. For example, the heterologous nucleotide sequence may encode (from any strain) influenza HA, RSV F, HIV Gag and/or Env, etc. Such embodiments can be useful for developing vaccines and/or methods of vaccination. The examples given here are non-limiting, as it will be understood by those in the art that nucleotide sequences from a variety of pathogenic agents (including also bacteria, parasites, etc.) may be desirable to use for an AVLP vaccine composition and/or method of vaccination.

[0092] Examples of viruses to which vaccines can be produced in accordance with the disclosure include, e.g., orthomyxoviruses, influenza virus A (including all strains varying in their HA and NA proteins, such as (non-limiting examples) H1N1, H1N2, H2N2, H3N2, H7N7, and H3N8); influenza B, influenza C, thogoto virus (including Dhori, Batken virus, SiAR 126 virus), respiratory syncytial virus (RSV), and isavirus (e.g., infectious salmon anemia virus), coronaviruses and the like. These include influenza isolated or transmitted from all species types,

including isolates from invertebrates, vertebrates, mammals, humans, non-human primates, monkeys, pigs, cows, and other livestock, birds, domestic poultry such as turkeys, chickens, quail, and ducks, wild birds (including aquatic and terrestrial birds), reptiles, etc. These also include existing strains which have changed, e.g., through mutation, antigenic drift, antigenic shift, recombination, etc., especially strains which have increased virulence and/or interspecies transmission (e.g., human-to-human).

D. Composition sequences

1. PIV5 vaccine sequences

a. pMHD11- PIV5-Pal

[0093] The pMHD11- PIV5-Pal nucleic acid sequence is provided herein from 5' to 3':

ACCAAGGGGAAAATGAAGTGGTGACTCAAATCATCGAAGACCCTCGAGATTACATAGGT
CCGGAACCTATGGCCTTCGTGACCGACCTCGAGTCAGAGTAGTTCAATAAGGACCTATCA
AGTTTGGGCAATTTTCGTCCCCGACACAAAATGTCATCCGTGCTTAAAGCATATGAGC
GATTCACGCTCACTCAAGAACTGCAAGATCAGAGTGAGGAAGGTACAATCCACCTACA
ACACTAAAACCGGTAATCAGGGTATTTATACTAACCTCTAATAACCCAGAGCTAAGATCC
CGGCTTCTTCTATTCTGCCTACGGATTGTTCTCAGTAATGGTGCAAGGGATTCCCATCGCT
TTGGAGCATTACTCACAATGTTTTCGCTACCATCAGCCACAATGCTCAATCATGTCAAATT
AGCTGACCAGTCACCAGAAGCTGATATCGAAAGGGTAGAGATCGATGGCTTTGAGGAGG
GATCATTCCGCTTAATCCCCAATGCTCGTTCAGGTATGAGCCGTGGAGAGATCAATGCCT
ATGCTGCACTTGCAAGAAGATCTACCTGACACACTAAACCATGCAACACCTTTCGTTGATT
CCGAAGTCGAGGGAACCTGCATGGGATGAGATTGAGACTTTCTTAGATATGTGTTACAGTG
TCCTAATGCAGGCATGGATAGTGACTTGCAAGTGCATGACTGCGCCAGACCAACCTGCTG
CTTCTATTGAGAAACGCCTGCAAAAATATCGTCAGCAAGGCAGGATCAACCCGAGATAT
CTCCTGCAACCGGAGGCTCGACGAATAATCCAGAATGTAATCCGGAAGGGAATGGTGGT
CAGACATTTCTCACCTTTGAACTGCAGCTTGCCCGAGCACAAAGCCTTGATCAAATAG
GTATTATGCTATGGTAGGGGATGTTGGAAAATATATAGAGAATTGTGGAATGGGAGGCT
TCTTTTGACACTAAAATATGCATTAGGAACTAGATGGCCACACTTGCTTTAGCTGCATT
TTCAGGAGAGCTAACAAAGCTAAAGTCCCTCATGGCATTATACCAGACCCTTGGTGAGCA
GGCCCGATATTTGGCCCTATTGGAGTCACCACATTTGATGGATTTTGCTGCAGCAAACTA
CCCCTGCTATATAGCTATGCTATGGGAATAGGCTATGTGTTAGATGTCAACATGAGGAA
CTACGCTTCTCCAGATCATACATGAACAAGACATATTTCCAATTGGGAATGGAACTGC
AAGAAAACAACAGGGTGACGTTGACATGAGGATGGCAGAAAGATCTCGGTCTAACTCAAG
CCGAACGCACCGAGATGGCAAATACACTTGCCAAATTGACCACAGCAAATCGAGGGGCA
GACACCAGGGGAGGAGTCAACCCGTTCTCATCTGTCACTGGGACAACCTCAGGTGCCCGCT
GCAGCAAACAGGTGACACACTCGAGAGTTACATGGCAGCGGATCGACTGAGGCAGAGATA

TGCTGATGCAGGCACCCATGATGATGAGATGCCACCATTGGAAGAGGAGGAAGAGGACG
ACACATCTGCAGGTCCACGCACTGGACCAACTCTTGAACAAGTGGCCTTGGACATCCAGA
ACGCAGCAGTTGGAGCTCCCATCCATACAGATGACCTGAATGCCGCACTGGGTGATCTTG
ACATCTAGACAATTCAGATCCCAATCTAAAATTGACATACCTAATTGATTAGTTAGATGG
AACTACAGTGGATTCCATAAGGTTCTGCCTACCATCGGCTTTAAAGAAAAAAATAGGCC
CGGACGGGTAGCAACAAGCGACTGCCGGTGCCAACAGCGCAATCCACAATCTACAATG
GATCCCACTGATCTGAGCTTCTCCCCAGATGAGATCAATAAGCTCATAGAGACAGGCCCTG
AATACTGTAGAGTATTTTACTTCCCAACAAGTCACAGGAACATCCTCTCTTGGAAAQAAT
ACAATACCACCAGGGGTCACAGGACTACTAACCAATGCTGCAGAGGCCAAAGATCCAAGA
GTCAACTAACCATCAGAAGGGCTCAGTTGGTGGGGGTGCAAAACCAAAGAAACCGCGAC
CAAAAATTGCCATTGTGCCAGCAGATGACAAAACAGTGCCCGGAAAGCCGATCCCAAAC
CCTCTATTAGGTCTGGACTCCACCCCGAGCACCCAAACTGTGCTTGATCTAAGTGGGAAA
ACATTACCATCAGGATCCTATAAGGGGGTTAAGCTTGCGAAAATTTGGAAAAGAAAATCT
GATGACACGGTTCATCGAGGAACCCAGAGAGAATCCTATCGCAACCAGTTCCCCCATCG
ATTTTAAGAGGGGCAGGGATACCGGCGGGTTCATAGAAGGGAGTACTCAATCGGATGG
GTGGGAGATGAAGTCAAGGTCAGTGAAGTGGTGAATCCATCCTGTTCTCCAATCACCGCT
GCAGCAAGGCGATTGAATGCACTTGTCACCAGTGTCCAGTCACTTGCTCTGAATGTGAA
CGAGATACTTAATACAGTGAGAAAATTTGGACTCTCGGATGAATCAACTGGAGACAAAAG
TAGATCGCATTCTCTCATCTCAGTCTCTAATCCAGACCATCAAGAATGACATAGTTGGAC
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CCGACTGGCCATTGAACTGTTGCTCATGACTATGATGTGATAGTTATTGACAGCGCGCC
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GCCTGCTGAGTTGTTTGACTACACCTCCGCACTGCAGTTTTTCGATATGCTTCGTGATCTG
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GCTGCATCCGATGCAAGTGTGTCGCTGTCGAGTTTAAACGGATCTGACGTCTAAGAAACC
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CGGGTGTGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTAAGTGTGAGAGTGACCC
ATATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCTAA
TACGACTCACTATAGGG (SEQ ID NO:1).

[0094] The underlined sequence is the PIV5 genomic nucleic acid sequence.
The italicized and underlined sequence is the whole Pal nucleic acid sequence.

b. pMHD39-PIV5-NTTM-Pal

[0095] The pMHD39-PIV5-NTTM-Pal nucleic acid sequence is provided herein from 5' to 3':

ACCAAGGGGAAAATGAAGTGGTGACTCAAATCATCGAAGACCCTCGAGATTACATAGGT
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GATTCACGCTCACTCAAGAACTGCAAGATCAGAGTGAGGAAGGTACAATCCCACCTACA
ACACTAAAACCGGTAATCAGGGTATTTATACTAACCTCTAATAACCCAGAGCTAAGATCC
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TTGGAGCATTACTACAATGTTTCGCTACCATCAGCCACAATGCTCAATCATGTCAAATT
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 ACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCTAATACGACTCACTATAGGG
 (SEQ ID NO:2).

[0096] The underlined sequence is the PIV5 genomic nucleic acid sequence.
 The italicized and underlined sequence is the whole Pal nucleic acid sequence. The
 double underlined sequence is the AOS modified NTM-Pal nucleic acid sequence.

c. pMHD35- PIV5-NTM-OspA_{BPBK}

[0097] The pMHD35- PIV5-NTM-OspA_{BPBK} nucleic acid sequence is
 provided herein from 5' to 3':

ACCAAGGGGAAAATGAAGTGGTGACTCAAATCATCGAAGACCCTCGAGATTACATAGGT
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 GCTGCTGTGCTCTGCTTATCCACAACATTTTGCACACGGTTATGTGGACAAAATACCTGG
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 AGTTTAAACGGATCTGACGTCTAAGAAAACATTATTATCATGACATTAACCTATAAAAAAT
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 CACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACA
 AGCCCGTCAGGGCGCGTCAGCGGGTGTGGCGGGTGTGCGGGCTGGCTTAACTATGCGG
 CATCAGAGCAGATTGTACTGAGAGTGCACCATATATGCGGTGTGAAATACCGCACAGAT
 GCGTAAGGAGAAAATACCGCATCAGGCTAATACGACTCACTATAGGG (SEQ ID NO:3).

[0098] The underlined sequence is the PIV5 genomic nucleic acid sequence.
 The italicized and underlined sequence is the whole OspA nucleic acid sequence.
 The double underlined sequence is the AOS modified NTM-OspA_{BPBk} nucleic acid sequence.

d. pMHD36- PIV5-NTM-OspA_{B31}

[0099] The pMHD36- PIV5-NTM-OspA_{B31} nucleic acid sequence is provided herein from 5' to 3':

ACCAAGGGGAAAATGAAGTGGTGACTCAAATCATCGAAGACCTCGAGATTACATAGGT
CCGGAACCTATGCCCTTCGTGACCGACCTCGAGTCAGAGTAGTTCAATAAGGACCTATCA
AGTTTGGGCAATTTTCGTCCCCGACACAAAAATGTCATCCGTGCTTAAAGCATATGAGC
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ACACTAAAACCGTAATCAGGGTATTATACTAACCTCTAATAACCCAGAGCTAAGATCC
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TTGGAGCATTACTCACAATGTTTTTCGCTACCATCAGCCACAATGCTCAATCATGTCAAATT
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 AGCAGATTGTACTGAGAGTGCACCATATATGCGGTGTGAAATACCGCACAGATGCGTAA
 GGAGAAAATACCGCATCAGGCTAATACGACTCACTATAGGG (SEQ ID NO:4).

[00100] The underlined sequence is the PIV5 genomic nucleic acid sequence.
 The italicized and underlined sequence is the whole OspA nucleic acid sequence.
 The double underlined sequence is the AOS modified NTM-OspA_{B31} nucleic acid sequence.

2. AVL P vaccine sequences

a. pMHD30- AVL P-Pal

[00101] The pMHD30- AVL P-Pal nucleic acid sequence is provided herein from 5' to 3':

ACCAAGGGGAAAATGAAGTGGTGACTCAAATCATCGAAGACCCTCGAGATTACATAGGT
CCGGAACCTATGGCCTTCGTGACCGACCTCGAGTCAGAGTAGTTCAATAAGGACCTATCA
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GATTCACGCTCACTCAAGAACTGCAAGATCAGAGTGAGGAAGGTACAATCCACCTACA
ACACTAAAACCGTAATCAGGGTATTTATACTAACCTCTAATAACCCAGAGCTAAGATCC
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 CTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCCGGCGTC
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 GACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAAGCGGATGCCGGGAGCAGA
 CAAGCCCGTCAGGGCGCGTCAGCGGGTGTGGCGGGTGTGCGGGCTGGCTTAACCTATGC
 GGCATCAGAGCAGATTGTACTGAGAGTGCACCATATATGCGGTGTGAAATACCGCACAG
 ATGCGTAAGGAGAAAATACCGCATCAGGCTAATACGACTCACTATAGGG (SEQ ID
 NO:5).

[00102] The underlined sequence is the PIV5-AVLP genomic nucleic acid sequence. The italicized and underlined sequence is the whole Pal nucleic acid sequence.

b. pGOB10- AVLP-NTTM-Pal

[00103] The pGOB10- AVLP-NTTM-Pal nucleic acid sequence is provided herein from 5' to 3':

ACCAAGGGGAAAATGAAGTGGTGACTCAAATCATCGAAGACCTCGAGATTACATAGGT
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ACACTAAAACCGGTAATCAGGGTATTTATACTAACCTCTAATAACCCAGAGCTAAGATCC
CGGCTTCTTCTATTCTGCCTACGGATTGTTCTCAGTAATGGTGCAAGGGATTCCCATCGCT
TTGGAGCATTACTACAATGTTTTTCGCTACCATCAGCCACAATGCTCAATCATGTCAAATT
AGCTGACCAGTCACCAGAAGCTGATATCGAAAGGGTAGAGATCGATGGCTTTGAGGAGG
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 ACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATATGCGGTGTGAAATAC
 CGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCTAATACGACTCACTATAGGG
 (SEQ ID NO:6).

[00104] The underlined sequence is the PIV5-AVLP genomic nucleic acid sequence. The italicized and underlined sequence is the whole Pal nucleic acid sequence. The double underlined sequence is the AOS modified NTTM-Pal nucleic acid sequence.

3. CPI vaccine sequences

a. pMHD106- CPI-NTTM-OspA_{BPBPK}

[00105] The pMHD106- CPI-NTTM-OspA_{BPBPK} nucleic acid sequence is provided herein from 5' to 3':

ACCAGGGGGAAAAACGAAGTGGTGACTCAAATCATCGAAGACCTCGAGATTACATAGGT
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CGGCTTCTTCTATTCTGCCTACGGATTGTCTCAGTAATGGTGCAAGGGATTCCCATCGCT
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GCTATGGACTGACAGGTGCTGGGAACCTCTTTACACGATCACGTGAACCTCTCTGGTGACC
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GATCATAATGATCCTGATTTAATCACGTATATCGAAAATAGAGAAGGCATAATCATCATA
ACCCCTGAACCTGGTAGCATTATTTAACACTGAGAATCATACTAACATACATGACCTTT
GAAATTGTACTGATGGTTTCAGATATGTACGAAGGTCGTCACAACATTTTATCACTATGC
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GATAACTTAGCTTTTCAGATAGGTGATGCTGTATATAACATAATTGCTTTGCTAGAAATCCT
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 AGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAATGTTGAATACTC
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 ACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCGCGCACATTTCCCCGAA
 AAGTGCCACCTGACGTCAATTCTAATACGACTCACTATAGGG (SEQ ID NO:7).

[00106] The underlined sequence is the PIV5-CPI genomic nucleic acid sequence. The italicized and underlined sequence is the whole OspA nucleic acid sequence. The double underlined sequence is the AOS modified NTM-OspA_{BPBPK} nucleic acid sequence.

b. pMHD175- CPI-MOMP_{Δsp}-CD5sp

[00107] The pMHD175- CPI-MOMP_{Δsp}-CD5sp nucleic acid sequence is provided herein from 5' to 3':

ACCAGGGGGAAAAACGAAGTGGTGACTCAAATCATCGAAGACCTCGAGATTACATAGGT
CCGGAACCTATGGCCTTCGTGACCGACCTCGAGTCAGAGTAGTTCAATAAGGACCTATCA
AGTTTGGGCAATTTTTCGTCCCCGACACAAAAATGTCATCCGTGCTTAAAGCATATGAGC
GATTCACACTCACTCAAGAACTGCAAGATCAGAGTGAGGAAGGTACAATCCACCTACA
ACACTAAAACCGTAATCAGGGTATTTATACTAACCTCTAATAACCCAGAGCTAAGATCC
CGGCTTCTTCTATTCTGCCTACGGATTGTTCTCAGTAATGGTGCAAGGGATTCCCATCGCT
TTGGAGCATTACTTACAATGTTTTCGCTACCATCAGCCACAATGCTCAATCATGTCAAATT
AGCTGACCAAGTCACCAGAAGCTGATATCGAAAGGGTAGAGATCGATGGCTTTGAGGAGG
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 CTGACGTCGAATTCTAATACGACTCACTATAGGG (SEQ ID NO:8).

[00108] The underlined sequence is the PIV5-CPI genomic nucleic acid sequence. The italicized and underlined sequence is the whole MOMP nucleic acid sequence. The double underlined sequence is the AOS modified MOMP Δ sp nucleic acid sequence.

c. pMHD177- CPI-NT-MOMP Δ sp

[00109] The pMHD177- CPI-NT-MOMP Δ sp nucleic acid sequence is provided herein from 5' to 3':

ACCAGGGGGAAAACGAAGTGGTGACTCAAATCATCGAAGACCTCGAGATTACATAGGT
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 TAATACGACTCACTATAGGG (SEQ ID NO:9).

[00110] The underlined sequence is the PIV5-CPI genomic nucleic acid sequence. The italicized and underlined sequence is the whole MOMP nucleic acid sequence. The double underlined sequence is the AOS modified NT-MOMP Δ sp nucleic acid sequence.

3. CVB vaccine sequences

a. pMHD181- CVB-MOMP Δ sp-CD5sp

[00111] The pMHD181- CPI-MOMP Δ sp-CD5sp nucleic acid sequence is provided herein from 5' to 3':

GTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGA
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 ACTGTGATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAA (SEQ ID
 NO:10).

[00112] The underlined sequence is the PIV5-CVB genomic nucleic acid sequence. The italicized and underlined sequence is the whole MOMP nucleic acid sequence. The double underlined sequence is the AOS modified MOMP Δ sp-CD5sp nucleic acid sequence.

b. pMHD182- CVB-NT-MOMP Δ sp

[00113] The pMHD182- CPI-NT-MOMP Δ sp nucleic acid sequence is provided herein from 5' to 3':

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ACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACG
CTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAA

GTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGT
 AAGTAGTTCGCCAGTTAATAGTTTGCACAACGTTGTTGCCATTGCTACAGGCATCGTGGT
 GTCACGCTCGTCGTTTGGTATGGCTTCATTACAGCTCCGGTCCCAACGATCAAGGCGAGT
 TACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTC
 AGAAGTAAGTTGGCCGCAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTT
 ACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCT
 GAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACC
 GCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAA
 ACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAA
 CTGATCTTCAGCATCTTTTACTTTACACGCGTTTCTGGGTGAGCAAAAAACAGGAAGGCA
 AAATGCCGCAAAAAAGGGAATAAGGGGCGACACGGAAATGTTGAATACTCATACTCTTCC
 TTTTTCATATTATTGAAGCATTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGA
 ATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCAC
 CTGACGTCTAAGAAACCATATTATCATGACATTAACCTATAAAAAATAGGCGTATCACGA
 GGCCCTTTCGTCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCC
 CGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGC
 GCGTCAGCGGGTGTGTCGGGGTGTGCGGGCTGGCTTAACCTATGCGGCATCAGAGCAGAT
 TGTACTGAGAGTGCACCATATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAA
 ATACCGCATCAGGCTAATACGACTCACTATAGGG (SEQ ID NO:11).

[00114] The underlined sequence is the PIV5-CVB genomic nucleic acid sequence. The italicized and underlined sequence is the whole MOMP nucleic acid sequence. The double underlined sequence is the AOS modified NT-MOMP Δ sp nucleic acid sequence.

c. pMHD116- CVB-NTTM-OspA_{BPBPk}

[00115] The pMHD116- CVB-NTTM-OspA_{BPBPk} nucleic acid sequence is provided herein from 5' to 3':

ACCAAGGGGAAAATGAAGTGGTGACTCAAATCATCGAAGACCCTCGAGATTACATAGGT
CCGGAACCTATGGCCTTCGTGACCGACCTCGAGTCAGAGTAGTTCAATAAGGACCTATCA
AGTTTGGGCAATTTTCGTCCCCGACACAAAATGTCATCCGTGCTTAAAGCATATGAGC
GATTCACGCTCACTCAAGAACTGCAAGATCAGAGTGAGGAAGGTACAATCCACCTACA
ACACTAAAACCGGTAATCAGGGTATTTATACTAACCTCTAATAACCCAGAGCTAAGATCC
CGGCTTCTTCTATTCTGCCTACGGATTGTTCTCAGTAATGGTGCAAGGGATTCCCATCGCT
TTGGAGCATTACTACAATGTTTTTCGTACCATCAGCCACAATGCTCAATCATGTCAAATT
AGCTGACCAGTCACCAGAAGCTGATATCGAAAGGGTAGAGATCGATGGCTTTGAGGAGG
GATCATTCGGCTTAATCCCCAATGCTCGTTCAGGTATGAGCCGTGGAGAGATCAATGCCT
ATGCTGCACTTGCAAGATCTACCTGACACACTAAACCATGCAACACCTTTCGTTGATT
CCGAAGTCGAGGGAAGTGCATGGGATGAGATTGAGACTTTCTTAGATATGTGTTACAGTG
TCCTAATGCAGGCATGGATAGTGACTTGCAAGTGCATGACTGCGCCAGACCAACCTGCTG

CTTCTATTGAGAAACGCCTGCAAAAATATCGTCAGCAAGGCAGGATCAACCCGAGATAT
CTCCTGCAACCGGAGGCTCGACGAATAATCCAGAATGTAATCCGGAAGGGAATGGTGGT
CAGACATTTTCTCACCTTTGAACTGCAGCTTGCCCCGAGCACAAAGCCTTGTATCAAATAG
GTATTATGCTATGGTAGGGGATGTTGGAAAGTATATAGAGAATTGTGGAATGGGAGGCT
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GCAGCAACAGGTGACACACTCGAGAGTTACATGGCAGCGGATCGACTGAGGCAGAGATA
TGCTGATGCAGGCACCCATGATGATGAGATGCCACCATTGGAAGAGGAGGAAGAGGACG
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GATCCCACTGATCTGAGCTTCTCCCCAGATGAGATCAATAAGCTCATAGAGACAGGCCCTG
AATACTGTAGAGTATTTTACTTCCCAACAAGTCACAGGAACATCCTCTCTTGGAAGAAT
ACAATACCACAGGGGTACAGGACTACTAACC AATGCTGCAGAGGCAAAAGATCCAAGA
GTCAACTAACCATCAGAAGGGCTCAGTTGGTGGGGGTGCAAAACCAAGAAACCGCGAC
CAAAAATTGCCATTGTGCCAGCAGATGACAAAACAGTGCCCGGAAAGCCGATCCCAAC
CCTCTATTAGGTCTGGACTCCACCCCGAGCACCCAACTGTGCTTGATCTAAGTGGGAAA
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CCAACAACCTTTGCATTCCAGACTGTCTCGCAGGCAACAGGAATGGCGATGACTTTAACTG
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AGCAAGAACGTATCGTAGCAGAAGCAATTGATAAGGTGGTTCTAGGAGAAAATGCTCTA
TTTAATTCGAGTGATAATGAATTAATTCTCAAATGTGGAGGGACACCAAATGCACGGAAT
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TTGACAACCCACTTGAAGGAAAATAATTGATATAACAAGGTCTGGAACCCAGGATTATGA
AAGTTTATTACTCCTTACAATTTAGGTCTTCTTGGTAAAATCAGTACGATAGTGAGA
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GCAGGCTACATTAGGAAATTGATTGAGGGGGATTGCAATATCGATCTAACCAGACCTATC
CAAAAGCAAATCTGGAAAGCATTAGGTTGTGTAGTCTATTGTCACGATCCAATGGATCAA
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CCCTTCGGGAAGCGTGCGCTTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTA
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 GAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCG
 GCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGG
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 CCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGA
 GCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTGGCGGGTGTGGGGCTGGCTTAAC
 TATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATATGCGGTGTGAAATACCG
 CACAGATGCGTAAGGAGAAAATACCGCATCAGGCTAATACGACTCACTATAGGG (SEQ
 ID NO:12).

[00116] The underlined sequence is the PIV5-CVB genomic nucleic acid sequence. The italicized and underlined sequence is the whole OspA nucleic acid sequence. The double underlined sequence is the AOS modified NTM-OspA_{BBPCK} nucleic acid.

III. Methods of Making

[00117] Also included in the present invention are methods of making and using PIV5 (W3A, CPI, or CVB backbone) viral expression vectors, including, but not limited to any of those described herein, to express modified bacterial proteins.

[00118] For example, the present invention includes methods of expressing modified bacterial proteins in a cell by contacting or infecting the cell with a PIV5 viral expression vector, viral particle, or composition as described herein.

[00119] The present invention includes methods of inducing an immune response in a subject to a modified bacterial protein, by administering a viral expression vector, viral particle, or composition as described herein to the subject. The immune response may include a humoral immune response and/or a cellular immune response. The immune response may enhance an innate and/or adaptive immune response.

[00120] The present invention includes methods expressing a heterologous modified bacterial protein in a subject by administering a viral expression vector, viral particle, or composition as described herein to the subject.

[00121] The present invention includes methods expressing a modified bacterial protein in a subject by administering a viral expression vector, viral particle, or composition as described herein to the subject.

IV. Methods of Treatment

[00122] The disclosure can be used in gene therapy and/or therapeutic approaches for the treatment of disease which involve the increase or decrease of a nucleotide sequence of interest in a host-cell. In these embodiments, the expressible heterologous nucleotide sequence may be derived from a mammalian genome. It may be particularly useful in some embodiments to have the expressible heterologous nucleotide sequence derived from a human genome, wherein expression of the wild-type RNA and/or protein can produce therapeutic effects in a patient. For example, the expressible heterologous nucleotide sequence can encode CFTR, NeuroD1, Cas9 and Guide RNAs, or any other such sequence. In other embodiments, the heterologous nucleotide sequence encodes a secreted protein.

[00123] In other embodiments, the expressible heterologous nucleotide sequence responds to positive selection stimuli. In other embodiments, the expressible heterologous nucleotide sequence also responds to negative selection stimuli. In further embodiments, it may be useful for the polynucleotide sequences to further comprise a reporter gene. For example, the report gene can be a luciferase or green fluorescent protein.

[00124] In some embodiments, PIV5 expresses one or more nucleotide sequences (e.g., siRNAs) that modify the translation and/or transcription of a host-cell nucleotide sequence of interest within a host cell. In some embodiments, transcription and/or translation of the expressible heterologous nucleotide sequence is modified so that its nucleotide sequence is codon degenerated with respect to the endogenous gene in a cell. Additionally, the expressible heterologous nucleotide sequence can be modified so that it co-expresses inhibitory or silencing sequences capable of inhibiting or silencing a host-cell nucleotide sequence of interest within a host cell.

[00125] In other embodiments, the expressible heterologous nucleotide of interest generates a product that stabilizes host-cell RNA nucleotide sequences. Such a product can be inducible or continually expressed. For example, the 3' RhoB untranslated region (UTR) can stabilize target RNAs that express either toxic proteins or other proteins of interest in response to serum. Another example is linking the eotaxin 3' untranslated region to the target gene of interest, which normally has a low half-life, but is stabilized with the addition of TNF-alpha and IL-4 to the cells. Alternatively, sequences contained in 16 mer sequence in the 5' coding region of CYP2E 1 and CYP2B 1 mRNA destabilizes target RNAs in the presence of insulin. Upon the removal of insulin the target RNAs are stabilized and the proteins can be expressed (Trong et al., Biochem J., Dec. 23, 2004).

[00126] Further non-limiting examples of expressible heterologous sequences that can be used in the invented compositions and methods include sequences can produce proteins, including, for example, e.g., interferons (alpha, beta, gamma, epsilon), erythropoietin, Factor VIII, clotting factors, antibodies and fragments thereof (e.g., including single chain, Fab, and humanized), insulin, chemokines, cytokines, growth factors, angiogenesis modulatory factors, apoptosis modulatory factors, e.g., Growth Factors, including, e.g., Amphiregulin, B-lymphocyte stimulator, Interleukin 16 (IL16), Thymopoietin, TRAIL, Apo-2, Pre B cell colony enhancing factor, Endothelial differentiation-related factor 1 (EDF1), Endothelial monocyte activating polypeptide II, Macrophage migration inhibitory factor MIF, Natural killer cell enhancing factor (NKEFA), Bone morphogenetic protein 8 (osteogenic protein 2), Bone morphogenetic protein 6, Connective tissue growth factor (CTGF), CGI-149 protein (neuroendocrine differentiation factor), Cytokine A3 (macrophage inflammatory protein 1-alpha), Glioblastoma cell differentiation-related protein (GBDR1), Hepatoma-derived growth factor, Neuromedin U-25 precursor, any tumor gene, oncogene, proto-oncogene or cell modulating gene (which can be found at condor.bcm.tmc.edu/oncogene), Vascular endothelial growth factor (VEGF), Vascular endothelial growth factor B (VEGF-B), T-cell specific RANTES precursor, Thymic dendritic cell-derived factor 1; Receptors, such as Activin A receptor, type II (ACVR2), β -signal sequence receptor (SSR2), CD14 monocyte LPS receptor, CD36 (collagen type 1/thrombospondin receptor)-like 2, CD44R (Hermes antigen gp90 homing receptor), G protein coupled receptor 9,

Chemokine C×C receptor 4, Colony stimulating factor 2 receptor β (CSF2RB), FLT-3 receptor tyrosine kinase, Similar to transient receptor potential C precursor, Killer cell lectin-like receptor subfamily B, Low density lipoprotein receptor gene, low-affinity Fc-gamma receptor IIC, MCP-1 receptor, Monocyte chemoattractant protein 1 receptor (CCR2), Nuclear receptor subfamily 4, group A, member 1, Orphan G protein-coupled receptor GPRC5D, Peroxisome proliferative activated receptor gamma, Pheromone related-receptor (rat), Vasopressin-activated calcium mobilizing putative receptor, Retinoic×receptor, Toll-like receptor 6, Transmembrane activator and CAML interactor (TACI), B cell maturation peptide (BCMA), CSF-1 receptor, Interferon (α , β and gamma) receptor 1 (IFNAR1). Pathways that can be modulated to increase antibody production include, e.g., ubiquitin/proteasome; telomerase; FGFR3; and Mcd-1, etc.

[00127] In certain embodiments of the disclosure, PIV5 compositions can be utilized to prepare antigenic preparations that be used as vaccines.. Any suitable antigen(s) can be prepared in accordance with the disclosure, including antigens obtained from prions, viruses, *mycobacterium*, protozoa (e.g., *Plasmodium falciparum* (malaria)), trypanosomes, bacteria (e.g., *Streptococcus*, *Neisseria*, etc.), etc.

[00128] Host cells can be transfected with single PIV5 particles containing one or more heterologous polynucleotide sequences, or with a plurality of PIV5 particles, where each comprises the same or different heterologous polynucleotide sequence(s). For example, a multi-subunit antigen (including intracellular and cell-surface multi-subunit components) can be prepared by expressing the individual subunits on separate vectors, but infecting the same host cell with all the vectors, such that assembly occurs within the host cell.

[00129] Vaccines often contain a plurality of antigen components, e.g., derived from different proteins, and/or from different epitopic regions of the same protein. For example, a vaccine against a viral disease can comprise one or more polypeptide sequences obtained from the virus which, when administered to a host, elicit an immunogenic or protective response to viral challenge.

[00130] As mentioned, the disclosure can also be utilized to prepare polypeptide multimers, e.g., where an antigenic preparation is produced which is comprised of

more than one polypeptide. For instance, virus capsids can be made up of more than one polypeptide subunit. By transducing a host cell with vectors carrying different viral envelope sequences, the proteins, when expressed in the cell, can self-assemble into three-dimensional structures containing more than one protein subunit (e.g., in their native configuration).

[00131] In further embodiments, the expressible heterologous nucleotide sequence is derived from another virus, other than PIV5. For example, the heterologous nucleotide sequence may encode (from any strain) influenza HA, RSV F, HIV Gag and/or Env, etc. Such embodiments can be useful for developing vaccines and/or methods of vaccination. The examples given here are non-limiting, as it will be understood by those in the art that nucleotide sequences from a variety of pathogenic agents (including also bacteria, parasites, etc.) may be desirable to use for an CVB vaccine composition and/or method of vaccination.

[00132] Examples of viruses to which vaccines can be produced in accordance with the disclosure include, e.g., coronaviruses, orthomyxoviruses, influenza virus A (including all strains varying in their HA and NA proteins, such as (non-limiting examples) H1N1, H1N2, H2N2, H3N2, H7N7, and H3N8); influenza B, influenza C, thogoto virus (including Dhori, Batken virus, SiAR 126 virus), isavirus (e.g., infectious salmon anemia virus), gram negative bacteria (including *Burkholderia mallei*, *Borrelia burgdorferi*, *Chlamydiae* bacteria and the like). These include gram negative bacteria isolated or transmitted from all species types, including isolates from invertebrates, vertebrates, mammals, humans, non-human primates, monkeys, pigs, cows, and other livestock, birds, domestic poultry such as turkeys, chickens, quail, and ducks, wild birds (including aquatic and terrestrial birds), reptiles, etc. These also include existing strains which have changed, e.g., through mutation, antigenic drift, antigenic shift, recombination, etc., especially strains which have increased virulence and/or interspecies transmission (e.g., human-to-human).

V. Methods of Administration

A. Administration by Vaccination

[00133] The present invention includes methods of vaccinating a subject by administering a viral expression vector, viral particle, or composition as described herein to the subject.

[00134] The disclosure provides vaccines against gram negative bacteria, including existing serotypes, derivatives thereof, and recombinants thereof, such as subtypes and recombinants which have the ability to spread from human-to-human.

[00135] The disclosure also provides methods for producing PIV5 compositions. Examples of host cells which can be utilized to produce PIV5 compositions, include, any mammalian or human cell line or primary cell. Non-limiting examples include, e.g., 293, HT1080, Jurkat, and SupT1 cells. Other examples are CHO, 293, Hela, Vero, L929, BHK, NIH 3T3, MRC-5, BAE-1, HEP-G2, NSO, U937, Namalwa, HL60, WEHI 231, YAC 1, U 266B1, SH-SY5Y, CHO, e.g., CHO-K1 (CCL-61), 293 (e.g., CRL-1573). Cells are cultured under conditions effective to produce transfection and expression. Such conditions include, e.g., the particular milieu needed to achieve protein production. Such a milieu, includes, e.g., appropriate buffers, oxidizing agents, reducing agents, pH, co-factors, temperature, ion concentrations, suitable age and/or stage of cell (such as, in particular part of the cell cycle, or at a particular stage where particular genes are being expressed) where cells are being used, culture conditions (including cell media, substrates, oxygen, carbon dioxide, glucose and other sugar substrates, serum, growth factors, etc.).

[00136] The disclosure also provides various treatment methods involving delivering PIV5 to host cells in vivo. In some embodiments, PIV5 is delivered into a subject for treating or preventing gram negative bacterial infections. In other embodiments, PIV5 is delivered into a subject for treating or preventing *Burkholderia mallei*, *Borrelia burgdorferi*, *Chlamydiae* bacterial infections or eliciting an immune response to *Burkholderia mallei*, *Borrelia burgdorferi*, *Chlamydiae* bacterial infections in a subject.

[00137] It is contemplated that when used to treat various diseases, the compositions and methods of the disclosure can be combined with other therapeutic agents suitable for the same or similar diseases. Also, two or more embodiments of the disclosure may be also co-administered to generate additive or synergistic effects. When co-administered with a second therapeutic agent, the embodiment of the disclosure and the second therapeutic agent may be simultaneously or sequentially (in any order). Suitable therapeutically effective dosages for each agent may be lowered due to the additive action or synergy.

[00138] As a non-limiting example, the disclosure can be combined with other therapies that block inflammation through (e.g., via inhibition, reduction and/or blockage of IL1, INF α/β , IL6, TNF, L13, IL23, etc.). In some embodiments, PIV5 compositions and methods disclosed herein are useful to enhance the efficacy of vaccines directed to gram negative bacterial infections. The compositions and methods of the disclosure can be administered to a subject either simultaneously with or before (e.g., 1-30 days before) a reagent (including but not limited to small molecules, antibodies, or cellular reagents) that acts to elicit an immune response (e.g., to an infection). The compositions and methods of the disclosure can be also administered in combination with an antibody directed at a pathogenic antigen or allergen.

[00139] The pharmaceutical compositions of the invention can be readily employed in a variety of therapeutic or prophylactic applications, e.g., for treating gram negative bacterial infection or eliciting an immune response to gram negative bacteria infecting a subject. In various embodiments, the vaccine compositions can be used for treating or preventing infections caused by a pathogen from which the displayed immunogen polypeptide in the PIV5-based vaccine is derived. Thus, the vaccine compositions of the invention can be used in diverse clinical settings for treating or preventing infections caused by various gram negative bacteria. As exemplification, a *Burkholderia mallei* and *pseudomallei* Pal-based vaccine composition can be administered to a subject to induce an immune response to sepsis, e.g., to induce production of broadly neutralizing antibodies to the *Burkholderia mallei* and *pseudomallei* bacteria. In another example, a *Borrelia burgdorferi* OspA-based vaccine composition can be administered to a subject to induce an immune response to Lyme disease, e.g., to induce production of broadly neutralizing antibodies to the *Borrelia burgdorferi* bacteria. In yet another example, a *Chlamydia* MOMP -based vaccine composition can be administered to a subject to induce an immune response to *Chlamydia*, e.g., to induce production of broadly neutralizing antibodies to the *Chlamydia* bacteria. For subjects at risk of developing these bacterial infections, a vaccine composition of the invention can be administered to provide prophylactic protection against viral infection. Therapeutic and prophylactic applications of vaccines derived from the other immunogens described herein can be similarly performed. Depending on the specific subject and conditions,

pharmaceutical compositions of the invention can be administered to subjects by a variety of administration modes known to the person of ordinary skill in the art, for example, topical, oral, intranasal, intramuscular, subcutaneous, intravenous, intra-arterial, intra-articular, intraperitoneal, or parenteral routes. In some aspects, administration is to a mucosal surface. A vaccine may be administered by mass administration techniques such as by placing the vaccine in drinking water or by spraying the animals' environment. When administered by injection, the immunogenic composition or vaccine may be administered parenterally. Parenteral administration includes, for example, administration by intravenous, subcutaneous, intramuscular, or intraperitoneal injection.

1. Administration by Inhalation

[00140] In one embodiment, the disclosed PIV5 vaccine compositions are formulated to allow intranasal administration. Intranasal compositions may comprise an inhalable dry powder pharmaceutical formulation comprising a therapeutic agent, wherein the therapeutic agent is present as a freebase or as a mixture of a salt and a freebase. Pharmaceutical formulations disclosed herein can be formulated as suitable for airway administration, for example, nasal, intranasal, sinusoidal, peroral, and/or pulmonary administration. Typically, formulations are produced such that they have an appropriate particle size for the route, or target, of airway administration. As such, the formulations disclosed herein can be produced so as to be of defined particle size distribution.

[00141] For example, the particle size distribution for a salt form of a therapeutic agent for intranasal administration can be between about 5 μm and about 350 μm . More particularly, the salt form of the therapeutic agent can have a particle size distribution for intranasal administration between about 5 μm to about 250 μm , about 10 μm to about 200 μm , about 15 μm to about 150 μm , about 20 μm to about 100 μm , about 38 μm to about 100 μm , about 53 μm to about 100, about 53 μm to about 150 μm , or about 20 μm to about 53 μm . The salt form of the therapeutic agent in the pharmaceutical compositions of the invention can a particle size distribution range for intranasal administration that is less than about 200 μm . In other embodiments, the salt form of the therapeutic agent in the pharmaceutical compositions has a particle size distribution that is less than about 150 μm , less than about 100 μm , less than about 53 μm , less than about 38 μm , less than about 20 μm , less than about 10

µm, or less than about 5 µm. The salt form of the therapeutic agent in the pharmaceutical compositions of the invention can have a particle size distribution range for intranasal administration that is greater than about 5 µm, greater than about 10 µm, greater than about 15 µm, greater than about 20 µm, greater than about 38 µm, less than about 53 µm, less than about 70 µm, greater than about 100 µm, or greater than about 150 µm.

[00142] Additionally, the salt form of the therapeutic agent in the pharmaceutical compositions of the invention can have a particle size distribution range for pulmonary administration between about 1 µm and about 10 µm. In other embodiments for pulmonary administration, particle size distribution range is between about 1 µm and about 5 µm, or about 2 µm and about 5 µm. In other embodiments, the salt form of the therapeutic agent has a mean particle size of at least 1 µm, at least 2 µm, at least 3 µm, at least 4 µm, at least 5 µm, at least 10 µm, at least 20 µm, at least 25 µm, at least 30 µm, at least 40 µm, at least 50 µm, at least 60 µm, at least 70 µm, at least 80 µm, at least 90 µm, or at least 100 µm.

[00143] In some embodiments the disclosed cannabinoid compositions include one or more cannabinoids or pharmaceutically acceptable derivatives or salts thereof, a propellant, an alcohol, and a glycol and/or glycol ether. The alcohol may be a monohydric alcohol or a polyhydric alcohol, and is preferably a monohydric alcohol. Monohydric alcohol has a lower viscosity than a glycol or glycol ether. Accordingly, the composition is able to form droplets of a smaller diameter in comparison to compositions in which the monohydric alcohol is not present. The present inventors have surprisingly found that a specific ratio of monohydric alcohol to glycol or glycol ether results in a composition with a desired combination of both long term stability (for example the composition remains as a single phase for at least a week at a temperature of 2-40° C.) and small droplet size.

B. Booster Vaccines

[00144] The present disclosure provides for the administration of a booster PIV5 vaccine for use in such a method for inducing in a human subject an immune response, wherein said subject has previously received a primary vaccination against a bacterial infection.

[00145] The method of booster vaccination according to the disclosure comprises the step of administering the vaccine composition to the subject.

[00146] The immune response induced by the vaccine composition of the disclosure or by the method of the disclosure is preferably a humoral response, especially a response comprising the production of neutralizing antibodies against the gram negative bacteria, i.e. a neutralizing antibody response.

EXAMPLES

Example 1: PIV5- and AVLP-based *Burkholderia* (Pal) Constructs

[00147] To generate the PIV5-Pal (MHD11) construct, a gene fragment encoding amino acids 22–170 of the *B. mallei* (ATCC 23344) Pal protein was synthesized with codon usage optimized for human cells (GenScript custom gene synthesis services). The Pal protein was inserted between the PIV5 SH and HN junction to generate MHD11 (PIV5-Pal). For the PIV5-NTTM-Pal (MHD39) construct, the N-terminus and transmembrane domains of PIV5 HN glycoprotein were incorporated directly to the N-terminus of the Pal protein (Figure 1). The modified Pal cDNA was cloned between the SH and HN genes of PIV5 in the plasmid ZL185, which contains the entire genome of the PIV5 virus. The MHD11 and MHD39 viruses were rescued by co-transfecting the MHD11 and MHD39 plasmids along with the pPIV5-NP, pPIV5-P, pPIV5-L, and pT7-polymerase plasmids, encoding the NP, P, and L proteins and T7 RNA polymerase into BHK21 cells.

[00148] In order to test expression of the Pal protein in MHD11- and MHD39-infected cells, the cell lysate and supernatant were resolved on a 4-20% acrylamide gel by SDS-PAGE and transferred to Amersham Hybond LFP PVDF membranes. The membranes were incubated with mouse anti-Pal mAb mixture and mouse anti-PIV5-NP antibodies followed by incubation with Cy3-conjugated goat anti-mouse IgG (Figure 2). The addition of the PIV5 HN NT+TM domain increased expression of the Pal protein in the cell lysate compared to Pal alone, as well as incorporation of the Pal protein in the virion as seen in the culture supernatant.

[00149] To generate the AVLP-Pal (MHD30) construct, the same gene fragment as MHD11 was used to replace the EGFP gene in the pAE31 plasmid containing the AVLP genome. For the AVLP-NTTM-Pal (GOB10) construct, the PIV5 HN N-terminus and transmembrane domains were inserted into the N-terminus of the Pal protein. Stable BHK21 cells lines containing the MHD30 and GOB10

constructs were made by co-transfecting the MHD30 and GOB10 plasmids along with the pPIV5-NP, pPIV5-P, pPIV5-L, and pT7-polymerase plasmids, encoding the NP, P, and L proteins and T7 RNA polymerase under the selection of xxx. Once the cell lines were made, AVLPS were created by co-transfecting the PIV5-F, PIV5-HN, and PIV5-M plasmids and collecting the media. Descriptions of these constructs can be seen in Figure 3A.

[00150] Immunogenicity of the MHD30 and GOB10 constructs was measured in a mouse model (FIG. 3B). Briefly, 6- to 8-week-old BALB/c mice were immunized intranasally with either PBS, MHD30, or GOB10 at a dose of 3.8×10^5 AP. Mice were bled at 14 days post-immunization. At 21 days post-immunization, mice were boosted intranasally with the same dose as the prime. Seven days post-boost, mice were humanely euthanized, and blood was collected for serological assays.

[00151] An ELISA was performed to determine the levels of anti-Pal IgG antibodies in the serum at 14 days post-prime and 7 days post-boost (day 28). Briefly, Immulon® 2HB 96-well microtiter plates were coated with 1 µg/ml of purified Pal protein in PBS. Two-fold serial dilutions of the serum samples were made in blocking buffer and added to the plates. Secondary antibody was (horseradish peroxidase-labeled goat anti-mouse IgG) was added to each well after washing the serum. The endpoint titer was defined as the highest serum dilution at which the OD was greater than two standard deviations above the mean OD of the naïve serum. Figure 3B demonstrates that the addition of the PIV5 HN NT+TM significantly enhances the immunogenicity induced by the Pal protein at both 14 days post-prime and 7 days post-boost. MHD30 (AVLP-Pal) was not immunogenic in the mouse model.

[00152] A second mouse immunogenicity study was performed to compare the MHD30 and GOB10 constructs as well as the MHD11 and MHD39 vaccines. Briefly, 6- to 8-week-old BALB/c mice were immunized intranasally with either PBS, MHD30, GOB10, MHD11, or MHD39 at a dose of 3.8×10^5 AP or PFU. Mice were bled at 14 days post-immunization. At 21 days post-immunization, mice were boosted intranasally with the same dose as the prime. Seven days post-boost, mice were humanely euthanized, and blood was collected for serological assays.

[00153] An ELISA was performed to determine the levels of anti-Pal IgG antibodies in the serum at 14 days post-prime and 7 days post-boost (day 28). Briefly, Immulon® 2HB 96-well microtiter plates were coated with 1 µg/ml of purified Pal protein in PBS. Two-fold serial dilutions of the serum samples were made in blocking

buffer and added to the plates. Secondary antibody was (horseradish peroxidase-labeled goat anti-mouse IgG) was added to each well after washing the serum. The endpoint titer was defined as the highest serum dilution at which the OD was greater than two standard deviations above the mean OD of the naïve serum. Figure 3C demonstrates that the addition of the PIV5 HN NT+TM in both the replication-competent PIV5 backbone, as well as the replication-incompetent AVL P platform significantly enhances the immunogenicity induced by the Pal protein at both 14 days post-prime and 7 days post-boost. MHD30 (AVLP-Pal) was not immunogenic in the mouse model..

Example 2. PIV5-Based Lyme Disease (OspA) Constructs

[00154] The outer surface protein A (OspA) is a lipoprotein found in the periplasm and fluid outer membrane and it is present in *Borrelia burgdorferi* when the bacterium is in the tick midgut (Li, H., *et al.*, *Proceedings of the National Academy of Sciences* **94**, 3584-3589 (1997)).

[00155] To generate the MHD22 plasmid construct, the OspA BPBPk gene codon optimized for human was incorporated into the pCAGGS expression plasmid. For the MHD24 plasmid, the PIV5 HN protein N-terminus and transmembrane domain (TM) sequences were added directly upstream of the OspA antigen sequence. The MHD35 and MHD36 constructs were generated by introducing the NTTM-OspA_{BPBPk} or NTTM-OspA_{B31} sequences, respectively into the PIV5 genome in between the SH and HN genes. The OspA BPBPk sequence was created by replacing the *B. burgdorferi* B31 165-189 and 219-273 amino acid sequences with the respective sequences from *B. afzelii* strains to avoid any cross-reactivity with autoimmune epitopes (del Rio, B. *et al.*, *Clinical and vaccine immunology : CVI* **15**, 1429-1435 (2008)). The CVL106 constructs was generated by introducing the NTTM-OspA_{BPBPk} gene sequences into the CPI PIV5 genome between the SH and HN genes, and the CVL116 construct was generated by introducing the same antigen into the CVB PIV5 genome in replace of the SH gene. All viruses were rescued by co-transfecting the MHD35, MHD36, CVL106, and CVL116 plasmids along with the pPIV5-NP, pPIV5-P, pPIV5-L, and pT7-polymerase plasmids, encoding the NP, P, and L proteins and T7 RNA polymerase into cells. Description of these constructs are included in Figures 4A-4B.

[00156] The MHD22 and MHD24 pCAGGS constructs, along with an MHD control were transfected into BHK cells to do Western blot analysis. Briefly, cell

lysate was resolved on a 4-20% acrylamide gel by SDS-PAGE and transferred to Amersham Hybond LFP PVDF membranes. The membrane was incubated with mouse anti-OspA antibody and mouse anti-actin antibody followed by incubation with Cy3-conjugated goat anti-mouse IgG (Figures 5A-5B). These results show that the addition of the PIV5 HN NT+TM increases expression of the OspA_{ABPBK} protein in the cell lysate compared to OspA_{ABPBK} alone.

[00157] The pCAGGS constructs were used to determine expression of the OspA_{ABPBK} protein on the surface of cells through IFA. Cells were transfected with the MHD22 and MHD24 constructs, and protein expression was detected using the mouse anti-OspA antibody followed by anti-mouse Alexa Fluor 488 secondary antibody (Figures 6A-6C). Results show an increase in OspA_{ABPBK} protein expression in cells transfected with the MHD24 construct compared to MHD22, indicating that the addition of the PIV5 HN NT+TM enhances OspA_{ABPBK} expression on the cell surface.

[00158] The MHD35 (PIV5-ABPBK) and MHD36 (PIV5-AB_{B31}) vaccine constructs were analyzed for OspA protein expression through Western blot assay. Briefly, cell lysates were resolved on a 4-20% acrylamide gel by SDS-PAGE and transferred to Amersham Hybond LFP PVDF membranes. The membrane was incubated with mouse anti-OspA antibody and mouse anti-PIV5 NP antibody followed by incubation with Cy3-conjugated goat anti-mouse IgG (Figure 7). These results demonstrate that both the MHD35 and MHD 36 viruses express the OspA protein.

[00159] To assess humoral IgG immune responses induced by the PIV5-ABPBK, PIV5-AB_{B31} and SC OspA_{B31} vaccines, all blood collected before tick challenge was used for determination of anti-OspA antibody (1:100) by ELISA. In contrast to the controls, serum from mice vaccinated with OspA (IN and SC) collected before (D17) and after the boost (>D86) generally had anti-OspA IgG antibody OD₄₅₀ > 3.5. To quantify anti-OspA IgG endpoint titers we used serum from Study 3 (15-month challenge) collected at day 17 (pre-boost), and at months 3, 6 and 12 post-prime (Figure 8) diluted at 10²-10⁶ on ELISA. Mice from the SC rOspA_{B31}+Alum, PIV5-ABPBK, and PIV5-AB_{B31} vaccine groups had peak serum IgG end point titers (EPT) at 3 months post-prime, with geometric means of 5.5, 5.5 and 5.4 log₁₀, respectively. These values were significantly higher than serum IgG EPTs from day 17 post-prime, showcasing the boosting effect by the second dose of the vaccine. These levels of anti-OspA_{B31} serum IgG antibodies decreased over the next 9 months of the

experiment, with mice from the SC rOspA_{B31}+Alum group having the biggest decrease of 1.7 log₁₀ EPT compared to 0.7 log₁₀ EPT seen in mice from the PIV5-ABPBPK and PIV5-AB₃₁ vaccine groups. Mice from the SC PBS+Alum and IN PIV5 control groups had very low levels of cross-reactive IgG antibodies at some point throughout the study, but the peak titers for these control groups are 3 log₁₀ lower than the peak titers for all vaccine groups. These results suggest that immunization with an intranasal, PIV5-based vaccine may lead to longer-lasting protection than a protein-based vaccine given subcutaneously.

[00160] Similar to study 3, mice from study 2 (9-month, no-challenge) were immunized with either two-doses of alum alone (SC PBS+Alum) or alum plus 20 µg of rOspA protein (SC rOspA_{B31}+Alum) subcutaneously, or with two-doses of 10⁶ PFU of wild-type PIV5 (IN PIV5), PIV5-ABPBPK, or PIV5-AB₃₁ intranasally. Study 1 contained the same groups, except for the SC PBS+Alum group. For this study blood was collected on D117 for neutralization assays of *B. burgdorferi* in culture (Figure 9A) before the mice were challenged (4-month, pre-challenge). Because insufficient blood was collected from each mouse, we pooled the serum from each group for the neutralization assay (Figure 9A). For the longevity studies (Studies 2 and 3) we vaccinated additional groups of mice to do neutralization assays, thus sufficient serum was collected at euthanasia from each mouse on D270 (Study 2, 9-month, no challenge, Figure 9B) and D533 (Study 3, 18-month, no-challenge, Figure 9C). Total motile bacteria were measured at days 0, 2, 5, and 7 post neutralization for study 1, and days 0, 3, and 6 for studies 2 and 3. At 4-months after prime-boost vaccination (Study 1), the levels of motile bacteria in cultures incubated with serum from the SC rOspA_{B31}+Alum, PIV5-ABPBPK, and PIV5-AB₃₁ vaccine groups decreased by 1.6, 0.6, and 1.0 log₁₀, respectively at day 2 compared to day 0 (Figure 9A). These values further decreased on day 5 until they reach 0 for all vaccine groups at day 7 post-neutralization (Figure 9A). In contrast, the Bb BSK and IN PIV5 control groups had increasing number of motile bacteria at days 2 and 5 post-neutralization, with peak numbers at day 5 post-inoculation with geometric means of 7.6 and 7.8 log₁₀ of motile *B. burgdorferi* per milliliter of culture, respectively. These values decreased slightly on day 7 post-neutralization to 7.1 and 6.2 log₁₀ of motile *B. burgdorferi* per milliliter of culture, respectively. At 9-months after prime-boost immunization (Study 2), vaccine groups SC rOspA_{B31}+Alum, PIV5-ABPBPK, and PIV5-AB₃₁ show a significance decrease in numbers of motile bacteria in cultures at day 3 or 6 post-inoculation

compared to day 0, with almost all samples from each group reaching 0 at day 3 post-neutralization (Figure 9B). On the other hand, samples from the Bb BSK, SC PBS+Alum, and IN PIV5 control groups had an increase in the levels of motile bacteria in cultures on day 6 post-neutralization compared to day 0. On average, levels of motile *B. burgdorferi* per milliliter of culture in control groups increased by 0.2 log₁₀ on day 6 post-neutralization compared to day 0. At 18-months post prime-boost vaccination (Study 3), the data continues to show a significant reduction in levels of motile *B. burgdorferi* per milliliter of culture in samples from the SC rOspA_{B31}+Alum, PIV5-A_{B31}BPBPK, and PIV5-A_{B31} vaccine groups at days 3 and 6 post-neutralization compared to day 0, with the largest difference seen on day 6 with an average decrease of 0.8, 1.5 and 2.0 log₁₀, respectively (Figure 9C). In contrast, the Bb BSK and IN PIV5 control groups had either an increase or no change in numbers of motile *B. burgdorferi* per milliliter of culture at day 6 post-neutralization compared to day 0, with a 0.2 log₁₀ growth seen in the Bb BSK samples. These results demonstrate that an intranasal PIV5-based vaccine carrying OspA sequences from different *B. burgdorferi* sensu lato genospecies can generate robust neutralizing IgG immune responses that can last for up to 18 months, and these responses are higher than those seen with a recombinant protein-based vaccine.

[00161] Mice from the 3 studies were challenged with ticks infected with multiple strains of *B. burgdorferi* at 4-months (Study 1), 9-months (Study 2), or 15-months (Study 3) post-prime. To assess *B. burgdorferi* dissemination to target tissues, bladder, joint, and heart samples were tested for *B. burgdorferi* FlaB load by qPCR (Figure 10A-10C). Furthermore, heart and bladder tissues were cultured in BSK-H medium to evaluate mobility/viability of *B. burgdorferi* under a dark field microscope that was confirmed by FlaB PCR from the cultures (Figure 10C-10F). In Study 1, the challenge done 4 months after prime-boost vaccination with SC rOspA_{B31}, IN PIV5-A_{B31}BPBPK and IN PIV5-A_{B31} resulted in absence of *B. burgdorferi* FlaB DNA in heart, bladder and joint, as well as absence of viable *B. burgdorferi* in cultures from heart, in contrast to the controls that received intranasal (IN) PIV5 or subcutaneous (SC) PBS+alum. In Study 2, the challenge done 9 months after prime-boost vaccination with IN PIV5-A_{B31}BPBPK and IN PIV5-A_{B31} resulted in absence of *B. burgdorferi* FlaB DNA in heart, bladder and joint, as well as absence of viable *B. burgdorferi* in cultures from bladder, in contrast to 1/4 mice that received SC rOspA_{B31} and all controls that received intranasal (IN) PIV5 or subcutaneous (SC) PBS+alum. In Study

3, the challenge done 15 months after prime-boost vaccination with IN PIV5-AB₃₁ resulted in absence of *B. burgdorferi* FlaB DNA in heart, bladder and joint, as well as absence of viable *B. burgdorferi* in cultures from bladder, in contrast to 1/3 IN PIV5-ABPBK, 3/3 mice that received SC rOspAB₃₁, and all controls that received intranasal (IN) PIV5 or subcutaneous (SC) PBS+alum.

Example 3. PIV5-Based *Chlamydia* (MOMP) Constructs

[00162] The major outer membrane protein (MOMP) from *Chlamydia trachomatis* is a member of the general porin class of proteins and it comprises ~60% of the outer membrane protein mass, making it an excellent antigen for vaccine development (Feher, V. A. *et al.*, *PLoS One* **8**, e68934 (2013)). To generate the CVL168 plasmid construct, the MOMP gene codon optimized for human was incorporated into the pCAGGS expression plasmid (Figure 11). The CVL169-172 plasmids were generated by removing the MOMP signal peptide and replacing it with the CD5 signal peptide (CVL169), adding the PIV5 HN N-terminus cytoplasmic tail plus transmembrane domain to the N-terminus of the MOMP gene (CVL170), removing the MOMP signal peptide and replacing it with the PIV5 HN N-terminus plus transmembrane domain to the N-terminus of the MOMP gene (CVL171), or removing the MOMP signal peptide and replacing it with the PIV5 HN cytoplasmic tail to the N-terminus of the MOMP gene (CVL172) (Figure 11).

[00163] The CVL175 and CVL181 constructs for generating recombinant viruses were generated by introducing the MOMP Δ sp-CD5sp gene sequence into the CPI genome between the SH and HN genes, and the CVB genome in replace of the SH gene. The CVL177 and CVL182 constructs were generated by introducing the NT-MOMP Δ sp gene sequence into the CPI genome between the SH and HN genes, and the CVB genome in replace of the SH gene. All viruses were rescued by co-transfecting the CVL175, CVL177, CVL181, and CVL182 plasmids along with the pPIV5-NP, pPIV5-P, pPIV5-L, and pT7-polymerase plasmids, encoding the NP, P, and L proteins and T7 RNA polymerase into cells. Description of these constructs are included in Figure 12.

[00164] The CVL168-CVL172 pCAGGS constructs were transfected into Vero cells to determine expression of the MOMP protein on cells through IFA. Protein expression was detected using the mouse anti-MOMP antibody followed by anti-

mouse Cy3 secondary antibody (Figures 13A-13F). Results show expression of the MOMP protein in all cells transfected with the different pCAGGS constructs.

[00165] The CVL168-CVL172 pCAGGS constructs were transfected into Vero cells for western blot analysis. Briefly, the transfected cell lysate was resolved on a 4-12% acrylamide gel by SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was incubated with mouse anti-OspA antibody followed by incubation with Cy3-conjugated goat anti-mouse IgG (Figure 14). Replacing the MOMP protein signal peptide with either the CD5 signal peptide (CVL169) or the PIV5 HN N-terminus (CVL172) increases expression of the MOMP protein in the transfected cell lysate compared to native MOMP protein (pCVL168).

Example 4: PIV5- and AVL P-based viral construct

PIV5 and AVL P vectored vaccine constructs directed to viral antigens such as coronaviruses, RSV, and influenza are disclosed in PCT/US22/76732, PCT/US23/78661, PCT/US24/14505, US10752916B2, and are hereby incorporated by reference. It is contemplated that PIV5-vectored vaccines directed to viral antigens may express modified viral proteins to enhance expression of the viral Antigen On the Surface of cells (AOS). The PIV5-vectored vaccines directed to viral antigens contemplated herein comprises a PIV5-based viral expression vector and a gene expressing an antigen in mammalian cells, wherein the gene expressing the antigen is modified to enhance expression of the antigen on the surface of cells (AOS); wherein the gene expressing the modified antigen is placed in the PIV5 genome; and wherein the antigen is a viral antigen. The modification may be attained by AOS modification of the NTTM-Pal nucleic acid sequence, as shown in the examples presented herein and results in an increase in the immunogenicity to the antigen.

[00166] The complete disclosure of all patents, patent applications, and publications, and electronically available material (including, for instance, nucleotide sequence submissions in, e.g., GenBank and RefSeq, and amino acid sequence submissions in, e.g., SwissProt, PIR, PRF, PDB, and translations from annotated coding regions in GenBank and RefSeq) cited herein are incorporated by reference. In the event that any inconsistency exists between the disclosure of the present application and the disclosure(s) of any document incorporated herein by reference, the disclosure of the present application shall govern. The foregoing detailed description and examples have been given for clarity of understanding only. No

unnecessary limitations are to be understood therefrom. The invention is not limited to the exact details shown and described, for variations obvious to one skilled in the art will be included within the invention defined by the claims.

What is claimed is:

1. A recombinant vaccine comprising a PIV5-based antigen expression vector, the vector comprising:
 - a PIV5-based viral expression vector; and
 - a gene expressing an antigen in mammalian cells,wherein the gene expressing the antigen is modified to enhance expression of the antigen on the surface of cells (AOS);
 - wherein the gene expressing the modified antigen is placed in the PIV5 genome; and
 - wherein the antigen is a viral antigen or a bacterial antigen.
2. The vaccine of claim 1, wherein the PIV5-based antigen expression vector has a heterologous nucleic acid sequence with at least 98% sequence identity to SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12.
3. The vaccine of claim 1, wherein the PIV5-based viral expression vector comprises a PIV5-AVLP, PIV5-W3A, PIV5-CPI, or PIV5-CVB backbone.
4. The vaccine of claim 1, wherein the modified antigen gene is modified by replacing the signal peptide of the gene with a PIV5 HN protein N-terminal domain (NT) or a PIV5 HN protein N-terminal domain plus transmembrane domain (NTTM).
5. The vaccine of claim 1, wherein the modified antigen gene is modified by adding the PIV5 HN protein N-terminal domain (NT) only or the PIV5 HN protein N-terminal domain plus transmembrane domain (NTTM) sequences directly upstream of the antigen without removing the signal peptide.
6. The vaccine of claim 1, wherein the modified antigen gene is placed between SH and HN genes or HN and L genes of the PIV5 genome.
7. The vaccine of claim 1, wherein the modified antigen gene replaces the SH gene of the PIV5 genome.

8. The vaccine of claim 1, wherein the modified antigen gene is expressed on the surface of the mammalian cell.
9. The vaccine of claim 1, wherein the bacterial antigen is that of a gram negative bacteria selected from a group consisting of *Burkholderia mallei*, *Borrelia burgdorferi*, and *Chlamydia trachomatis* and wherein the viral antigen is that of a virus selected from a group consisting of coronaviruses, respiratory syncytial virus (RSV), influenza virus A, influenza B, influenza C, orthomyxoviruses, thogoto virus, and isavirus.
10. The vaccine of claim 9, wherein the gene expressing a bacterial antigen is modified by replacing the N-terminus of a *Burkholderia mallei* Pal protein with the N-terminus and transmembrane domains of PIV5 HN glycoprotein.
11. The vaccine of claim 9, wherein the gene expressing a bacterial antigen is modified by placing the N-terminus and transmembrane domains of PIV5 HN glycoprotein directly upstream of the *Borrelia burgdorferi* OspA BPBPk protein.
12. The vaccine of claim 9, wherein the gene expressing a bacterial antigen is modified by replacing the signal peptide of *Chlamydia trachomatis* major outer membrane protein (MOMP) with the N-terminus alone or the N-terminus and transmembrane domains of PIV5 HN glycoprotein, or by placing the N-terminus and transmembrane domains of PIV5 HN glycoprotein directly upstream of the antigen without removing the signal peptide.
13. A PIV5-based antigen vaccine composition comprising
a PIV5-based viral expression vector; and
a gene expressing a bacterial antigen in mammalian cells,
wherein the antigen is a viral antigen or a bacterial antigen;
wherein the PIV5-based viral expression vector comprises a PIV5-AVLP,
PIV5-W3A, PIV5-CPI, or PIV5-CVB backbone;
wherein the gene expressing the antigen is modified by replacing the signal peptide of the gene with a PIV5 HN protein N-terminal domain (NT) or a PIV5 HN protein N-terminal domain plus transmembrane domain (NTTM), or by adding the

PIV5 HN protein N-terminal domain (NT) only or the PIV5 HN protein N-terminal domain plus transmembrane domain (NTTM) sequences directly upstream of the antigen without removing the signal peptide; and

wherein the gene expressing the modified antigen is placed between the SH and HN or HN and L genes of the PIV5 genome, or by replacing the SH gene.

14. The composition of claim 13, wherein the PIV5-based antigen vector has a nucleic acid sequence with at least 98% sequence identity to SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12.

15. The composition of claim 13, wherein the composition is a vaccine against a bacterial or viral infection.

16. The composition of claim 13, wherein the bacterial infection is caused by a gram negative bacteria selected from a group consisting of *Burkholderia mallei*, *Borrelia burgdorferi*, and *Chlamydia trachomatis* and the viral infection is caused by a virus selected from a group consisting of coronaviruses, respiratory syncytial virus (RSV), influenza virus A, influenza B, influenza C, orthomyxoviruses, thogoto virus, and isavirus.

17. A method of inducing an immune response in a subject in need thereof comprising administering a PIV5-based antigen vaccine of claim 1 to the subject, wherein the immune response comprises a humoral immune response and/or a cellular immune response, and wherein the subject is susceptible to a bacterial or viral infection.

18. The method of claim 17, wherein the subject was previously vaccinated against the bacteria or viral infection, the method comprising administering the composition to the subject.

19. The method of claim 17, wherein the bacterial infection is caused by a gram negative bacteria is selected from a group consisting of *Burkholderia mallei*, *Borrelia burgdorferi*, and *Chlamydia trachomatis*, and the viral infection is caused by a virus selected from a group consisting of coronaviruses, respiratory syncytial virus (RSV),

influenza virus A, influenza B, influenza C, orthomyxoviruses, thogoto virus, and isavirus.

20. The method of claim 17, wherein the composition is administered intranasally, intramuscularly, topically, parenterally or orally.