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(54) Title: INHALABLE INFLUENZA VACCINE COMPOSITIONS AND METHODS

(57) Abstract: Dry powder inhalable compositions and methods for using and making the compositions are disclosed for vaccinating a subject against disease. In particular, the compositions are inhalable dry powders useful for preventing and/or treating diseases caused by microorganisms, for example, microorganisms such as viral or bacterial pathogens, including, the influenza virus. In particular, the method comprises the administration of an inhalable composition comprising virus-like particles and/or specific protein/s or antigenic peptides, peptide fragments and/or derivatives thereof using a dry powder drug delivery system.

INHALABLE INFLUENZA VACCINE COMPOSITIONS AND METHODS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit under 35 U. S. C. §119(e) from United States Provisional Patent Application Serial No. 61/719,308, filed October 26, 2012, the content of which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0002] Methods and compositions are disclosed for vaccinating a subject against disease. In particular, the compositions are inhalable dry powders suitable and useful for preventing and/or treating diseases caused by microorganisms, for example, microorganisms such as the influenza virus and other viral, parasitic or bacterial infections using an inhalation system. In particular, the method comprises the administration of a composition comprising single or multiple native or recombinant antigens, native particles or subunits or virus-like particles (VLPs) displaying homologous or heterologous antigens and/or specific peptide fragments and/or derivatives thereof into the lungs, pulmonary circulation and/or nasal cavities by oral or nasal inhalation using a dry powder drug delivery system.

BACKGROUND

[0003] Drug delivery systems for the treatment of disease or prophylactic vaccination for the prevention of disease which introduce active ingredients into the body are numerous and include oral, intranasal, transdermal, subcutaneous, intramuscular and intravenous administration. While these systems have been used for quite a long time and can deliver sufficient medication for the treatment or prevention of many diseases, there are numerous challenges associated with these drug delivery mechanisms, in particular, delivery of biological active agents or immunogenic compositions that should be able to elicit a protective immune response. Delivery of effective amounts of proteins, peptides and nucleic acids to prevent or treat a target disease has been problematic. Many factors are involved in introducing the right amount of the active agent in an appropriate drug composition or vaccine delivery system so that the formulation used contains the proper amount of active agent that can reach its target site(s) of action in order to attain a prophylactic or therapeutically effective response.

[0004] Vaccines are the most efficient and cost-effective means for disease prevention and treatment, however they comprise biological active agents which can require special manufacturing and storage conditions. Most vaccines are injectable and either provided in liquid form, or as a powder formulation which is reconstituted with a liquid prior to immunization. Injectable (intradermal, subcutaneous, intramuscular, intraperitoneal, etc.) vaccines can elicit systemic immune responses effectively. In certain cases, local immune responses to the injection are produced as side effects such as

secretory immunoglobulin A (IgA or S-IgA) production at the injection site that can reduce the magnitude of the expected/desired target systemic effects, which in turn, does not consistently translate to systemic immunity. Local immune reactions to the injection can also lead to uncomfortable irritation, itching, and swelling. In some situations, booster or repeated injections of the vaccine are required to attain effective immunization as well as co administered adjuvants.

[0005] Liquid vaccine formulations often require cold-chain storage and transport to maintain biological activity, potency and therapeutic effectiveness prior to administration, and can also require a trained healthcare professional for their administration, and proper disposal of needle and syringe. Further, the individual being vaccinated may be obliged to visit a clinic or the office of the professional provider. These limitations make immunization campaigns difficult, particularly during a pandemic situation, an intentional release of an infectious agent or in geographic locations where infrastructure for vaccine storage, distribution and delivery is deficient or non-existent.

[0006] Thus uninterrupted maintenance of low temperatures within a specified range during storage and transport of vaccines is required to ensure preservation of biological activity and potency. Disruption of the cold chain system may diminish or completely eliminate vaccine efficacy underlying the importance of a fully functional cold chain network while bringing vaccine from producers to immunization sites to preserving product efficacy. Operating and maintaining an effective cold chain network is expensive and susceptible to interruptions, particularly in under-developed regions. In addition, conflict areas and certain developing countries often lack the infrastructure required for a cold chain system. The need of a refrigerated system poses a serious burden for delivering vaccine in conflict zones, developing countries and in emergency situations.

[0007] To overcome the limitations of existing injected vaccines, alternative vaccination strategies, for example, mucosal vaccination systems have been evaluated. Mucosal-associated lymphoid tissues (MALT) form the largest mammalian lymphoid organ system, contributing almost 80% of all immunocytes. Mucosal immunization not only induces systemic immunity (IgG and long lasting cell mediated immune response), but also induces local (s-IgA) immunity at the site of mucosal entry (common mucosal immune response). This common mucosal immune system is associated with organ selectivity demonstrated as enhanced memory at the site of mucosal priming. In addition, mucosal delivery is needle-free and painless, so it is likely to improve patient acceptance and is suitable for mass vaccination campaigns. FLUMIST® is an example of a Food and Drug Administration (FDA)-approved intranasal mucosal vaccine for immunizing against the influenza virus which uses a cold-adapted and temperature sensitive live virus as vaccine.

[0008] As mentioned above, current methods of manufacturing, transporting/storing and delivering vaccines prior to immunization is burdened by two major factors; 1) the vaccine must be maintained refrigerated prior to immunization and 2) gathering people for mass immunization. In some situations, these factors are critical and create extraordinary situations, including during outbreaks of pandemic influenza such as the 1918 flu (Spanish flu), and influenza A virus subtype H5N1, also known as "bird flu" and the H1N1 also known as "Swine flu."

[0009] Pulmonary immunization against airborne pathogens in some cases has been shown useful, because the lungs contain a highly responsive immune system. The utility, effectiveness, and safety of pulmonary vaccination are well established for some agents such as the measles virus with the pioneering studies of Sabin in the early 1980s, and more recently with the mass vaccination campaigns in Mexico and South Africa. Published studies of inhaled vaccines for measles, influenza, and TB, however, have mostly involved nebulization of solutions, a method of administration that can be plagued by poor efficiency, lengthy administration time, and complex equipment. Accordingly, there is a need to develop new compositions and methods for immunizing against diseases, in particular, infectious and pandemic diseases which would provide a stable composition with a prolonged shelf-life in an easy to administer form and dispenser.

SUMMARY

[0010] Disclosed herein are stable, dry powder vaccine compositions, which are easy to use as an immunization system for oral or nasal inhalation. The dry powder vaccines can be for self-administration and can be manufactured to treat diseases of different origins. In an exemplary embodiment, the present vaccines are used to prevent and/or treat viral disease, wherein the target antigens are administered using an inhalation system to induce a local immune response and/or a systemic effect to the target antigen resulting in a subject being immunized. The vaccines can be monovalent or multivalent, stable dry powder formulations that can be stored at room temperature for a prolonged period of time. The dry powder formulations increase vaccine efficacy and overcome the limitations of nebulized formulations or injectable vaccines by improving storage conditions and distribution to a population.

[0011] Embodiments include an influenza vaccine comprising a dry powder composition comprising a diketopiperazine and an immunogenic components such as influenza virus-like particles, an antigenic protein, an antigenic peptide, fragments of said influenza virus-like particle, a subunit protein or multiple subunit proteins or fragments of said antigenic protein(s), fragments of said antigenic peptide, or combinations thereof. In embodiments the composition can comprise a diketopiperazine microparticle comprising the immunogenic component. In an embodiment the diketopiperazine can be a diketopiperazine salt.

[0012] In embodiments the immunogenic component can comprise a hemagglutinin and/or a neuraminidase protein and/or a matrix protein, or peptide or fragments thereof.

[0013] In embodiments the immunogenic component can comprise an antigenic protein or peptide such as a neuraminidase derived from influenza virus type A, B or C, or any subtype of influenza A, or fragments thereof or combinations thereof.

[0014] In embodiments the immunogenic component can comprise an antigenic protein or peptide such as hemagglutinin derived from influenza A, B or C or any subtype of influenza A, or fragments thereof, or combinations thereof.

[0015] In embodiments the immunogenic component can comprise an antigenic protein or peptide such as hemagglutinin A, or another protein of the influenza virus such as NP, M1, M2, NS1, NS2, polymerase subunits PB1, PB2 or PA or fragments thereof or combinations thereof.

[0016] In an embodiment the diketopiperazine is *bis*[3,6-(*N*-X-4-aminobutyl)]-2,5-diketopiperazine, wherein X is fumaryl, glutaryl, succinyl, maleyl, malonyl, or oxalyl. In an embodiment the diketopiperazine is *bis*[3,6-(*N*-fumaryl-4-aminobutyl)]-2,5-diketopiperazine.

[0017] An embodiment includes an influenza vaccination system, comprising an inhalation system comprising a dry powder inhaler comprising a dry powder formulation comprising microparticles of *bis*[3,6-(*N*-fumaryl-4-aminobutyl)]-2,5-diketopiperazine and an antigen selected from influenza virus protein consisting of hemagglutinin A or neuraminidase A, or other influenza protein and/ or combinations thereof. In an embodiment the hemagglutinin A is a recombinant HA3 antigen. In an embodiment the dry powder inhaler is configured as a single use disposible inhaler for self-administration.

[0018] Embodiments include a method for immunizing a subject against a virus infection, for example an influenza virus infection, comprising administering to the subject a therapeutically effective amount of a dry powder vaccine comprising a diketopiperazine and an immunogenic component comprising at least one of a VLP, an antigenic protein, an antigenic peptide, fragments of said virus-like particle, or a subunit protein or multiple distinct subunits proteins or fragments of said antigenic protein/s, fragments of said antigenic peptide, or combinations thereof, wherein the vaccine is self-administered by inhalation. In an embodiment the diketopiperazine is *bis*[3,6-(*N*-X-4-aminobutyl)]-2,5-diketopiperazine, wherein X is fumaryl, glutaryl, succinyl, maleyl, malonyl, or oxalyl. In an embodiment the diketopiperazine is *bis*[3,6-(*N*-fumaryl-4-aminobutyl)]-2,5-diketopiperazine. In an embodiment the antigenic peptide is a recombinant HA3 antigen or fragment thereof. Embodiments can further include providing a booster vaccine comprising the influenza vaccine.

[0019] In one embodiment, the vaccine comprises a dry powder inhaler and a dry powder composition comprising an antigen, and/or fragments thereof, or combinations thereof for delivery of the antigen to the respiratory tract including the lungs to induce local as well as systemic immune responses. In some embodiments, the dry powder inhaler is disposable and configured for single use. In embodiments herewith, the dry powder vaccine can be a crystalline powder, an amorphous powder and/or combinations thereof. In another embodiment any format of the powder vaccine is delivered via the intradermal route utilizing micro-needles, dissolvable micro-needles or a vaccine patch.

[0020] In a particular embodiment, the vaccine composition against viral infections comprises a dry powder comprising diketopiperazine microparticles and an antigen, antibody, fragment thereof, and/or combinations thereof. In one embodiment, the antigens in the vaccine composition comprise Virus-Like Particles (VLP), peptides, proteins, including glycoproteins, and/or fragments of a VLP, antigenic proteins and/or antigenic peptides, or combinations thereof. The dry powder composition comprises highly immunogenic, non-infectious monovalent and/or polyvalent vaccines using an antigen formed from a cell-based manufacturing system, and delivered using a dry powder inhaler.

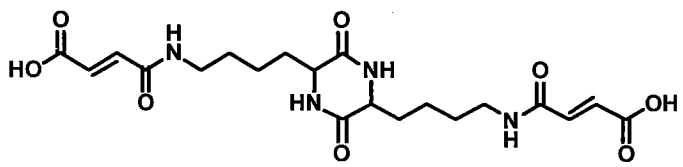
[0021] Embodiments include a dry powder formulation comprising a diketopiperazine and an antigen or antibody for treating influenza. In this embodiment, the vaccine composition comprises an immunogenic VLP, and/or antigenic proteins or peptides thereof wherein the antigenic protein(s) or peptides are viral in nature , including, viral proteins and peptides corresponding to those of influenza virus, including influenza virus A, influenza virus B and influenza virus C. In this embodiment, the vaccine comprises a breath powered, dry powder, single use inhaler comprising a dry powder composition comprising peptide(s), protein(s), glycoprotein(s) and/or combinations therein including, Hemagglutinin (HA) or a subtype thereof selected from subtypes H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H4, H15, H16, and/or H17, or other mutants thereof, and/or a neuraminidase (NA) or a subtype thereof selected from subtypes N1, N2, N3, N4, N5, N6, N7, N8, N9, and/or N10, or mutants thereof, fragments thereof, or combinations thereof. In one embodiment, an influenza vaccine comprises a dry powder composition comprising a recombinant peptide and/or protein antigen, for example, recombinant HA3 (rHA3) and a diketopiperazine in a single use dry powder inhaler.

[0022] In alternate embodiments, the dry powder vaccines can comprise one or more neuraminidase specific antigen(s). In another embodiment, the vaccine composition comprises combinations of HA and NA antigens, or antibodies or fragments thereof against HA or NA and/or their subtypes. In embodiments for treating influenza, the vaccines can be administered as prophylactic in a healthy subject alone, or in combination with a neuraminidase inhibitor, including Oseltamivir (TAMIFLU®) and Zanamivir (RELENZA®) for treatment of influenza A and B viral infections, or M2

inhibitors, including Amantidine (SYMMETREL®) and Rimantadine (FLUMADINE®) or analogs thereof for treatment of influenza A viral infections. In these embodiments, the neuraminidase inhibitor can be administered separately by inhalation or other route of administration.

[0023] In one embodiment, an inhalable dry powder vaccine comprising microparticles of a diketopiperazine and a VLP, an antigenic protein, an antigenic peptide, fragments of the VLP, fragments of the antigenic protein, fragments of the antigenic peptide, or combinations thereof, wherein the microparticles of the diketopiperazine are made from, for example, *bis*[3,6-(*N*-X-4-aminobutyl)]-2,5-diketopiperazine, wherein X is fumaryl, glutaryl, succinyl, maleyl, malonyl, oxalyl, or a salt thereof; and a VLP, protein, peptide, glycoprotein and/or fragments thereof.

[0024] In one embodiment, an inhalable dry powder vaccine comprises microparticles of a diketopiperazine having the formula *bis*[3,6-(*N*-X-4-aminobutyl)]-2,5-diketopiperazine, wherein X is fumaryl, glutayl, succinyl, maleyl, or malonyl; and a VLP, glycoprotein, protein, peptide, fragments thereof, and/or combinations thereof. In a particular embodiment, the diketopiperazine is fumaryl diketopiperazine (FDKP) having the formula:



or a salt thereof, including disodium, dipotassium, magnesium, lithium, and the like.

[0025] In certain embodiments, the vaccine composition comprises a diketopiperazine inhalable dry powder to which is incorporated one or more VLPs, glycoproteins, proteins, peptides and/or peptide fragments; for example those related to influenza viruses. In an embodiment, the influenza VLP, virus glycoprotein, protein, peptide and/or fragments thereof can include one or more Hemagglutinin (HA) proteins or subtype thereof, for example, subtypes H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H4, H15, H16, and/or H17, or other mutants thereof, and/or a neuraminidase (NA) or subtype thereof, for example, subtypes N1, N2, N3, N4, N5, N6, N7, N8, N9, and/or N10, or mutants thereof, fragments thereof, or combinations thereof. In an embodiment, the antigenic peptides or proteins are H1, H2, H3, H5, N1, N2, N5, N7 and/or N8, fragments thereof or combinations thereof, for example, H3 and N2, H2 and N2, H1 and N1, H5 and N1 and the like. In other embodiments, the VLP, glycoprotein, protein, peptide and/or fragment thereof comprises other influenza proteins such as NP, M1, M2, NS1, NS2 or the polymerase subunits PB1, PB2, and PA or combination thereof.

[0026] The amount of an antigen for use in the compositions can be determined by the immunogenicity of the antigen. In some embodiments, the amount of peptide or protein in the compositions can range from about 0.001 μg to more than 1 mg, or .001 μg to 1 mg, or .01 μg to 1 mg, or .1 μg to .1 mg, or .1 μg to .05 mg, or the like,. For example, an influenza vaccine can comprise a recombinant HA3 peptide (for example, rHA3; derived from influenza A/Perth/2009 (H3N1)) in amounts ranging from about 0.05 mg to 50mg of bulk powder with a rHA3 content of approximately 5% (e.g. 0.5 mg powder/0.025 mg rHA3; 500 μg powder/25 μg rHA3). In some embodiments, the amount of dry powder to be administered to a subject can be, for example, greater than 0.5 mg, greater than 1 mg, greater than 2 mg, greater than 5 mg, greater than 10 mg, greater than 15 mg, greater than 20 mg, greater than 30 mg, or the like.

[0027] Further embodiments include vaccine delivery systems comprising an inhaler, a unit dose dry powder medicament container, and a powder comprising the microparticles disclosed herein and an immunogen including, for example, one or more antigens. In some embodiments the container can be a cartridge that is loaded into the inhaler; in other embodiments the container is integral with the inhaler. In one embodiment, the delivery system for use with the dry powders includes an inhalation system comprising a high resistance inhaler having air conduits which impart a high resistance to airflow through the conduits for deagglomerating and dispensing the powder. In one embodiment, the inhalation system has a resistance value of, for example, approximately 0.065 to about 0.200 (√kPa)/liters per minute, or 0.1 to about 0.200 (√kPa)/liters per minute, or 0.1 to about 0.150 (√kPa)/liters per minute, or the like. In certain embodiments, the dry powders can be delivered effectively by inhalation with an inhalation system wherein the peak inhalation pressure differential can range from about 2 to about 20 kPa, from about 4 to about 16 kPa, or from about 10 to about 12 kPa, which can produce resultant peak flow rates of about between 7 and 70 liters per minute. In certain embodiments, the inhalation system are configured to provide a single dose by discharging powder from the inhaler as a continuous flow, or as one or more pulses of powder delivered to a patient in, for example, less than 5 seconds, or less than 4 seconds, or less than 3 seconds, or less than 2 seconds or less than 1 second.

[0028] In some embodiments disclosed herewith, the dry powder inhaler system comprises a predetermined mass flow balance within the inhaler. For example, a flow balance of approximately 10% to 70% of the total flow exiting the inhaler and into the patient is delivered by one or more dispensing ports, which airflow passes through the area containing the powder formulation, and wherein approximately 30% to 90% air flow is generated from other conduits of the inhaler. Moreover, bypass flow, or flow not entering and exiting the area of powder containment such as through a cartridge, can recombine with the flow exiting the powder dispensing port within the inhaler to dilute, accelerate and

ultimately deagglomerate the fluidized powder prior to exiting the mouthpiece. In one embodiment, flow rates ranging from about 7 to 70 liters per minute result in greater than 75% of the container or the cartridge contents dispensed in fill masses between 1 and 30 mg. In certain embodiments, an inhalation system as described above can emit a respirable fraction/fill of a powder dose at percentages greater than 40% in a single inhalation, greater than 50%, greater than 60%, greater than 70%, greater than 80%, or greater than 90%, or a range between any of these values.

[0029] In particular embodiments, an inhalation system is provided comprising a dry powder inhaler, a dry powder formulation comprising microparticles of fumaryl diketopiperazine having an FDKP trans isomer content between 45% and 65% and one or more immunogens, antigens or active agents. In some aspects of this embodiment of the inhalation system, the dry powder formulation is provided in a unit dose cartridge. Alternatively, the dry powder formulation can be preloaded in the inhaler. In this embodiment, the structural configuration of the inhalation system allows the deagglomeration mechanism of the inhaler to produce respirable fractions greater than 50%; that is, more than half of the powder contained in the inhaler (cartridge) is emitted as particles of less than 5.8 μm. The inhalers can discharge greater than 85% of a powder medicament (*e.g.,* vaccine) contained within a container during dosing. In certain embodiments, the inhalers can discharge greater than 85% of a powder medicament contained in a single inhalation. In one embodiment, the inhalers can discharge greater that 90% of the cartridge contents or container contents in less than 3 seconds at pressure differentials between 2 and 5 kPa with fill masses ranging up to 30 mg, 40 mg, or 50 mg.

[0030] In an alternate embodiment, dry powder vaccines as described herein can comprise pharmaceutically acceptable carriers and/or excipients, including amino acids for example, leucine, alanine, glycine, isoleucine, norleucine; sugars, for example, mannitol, xylitol, and the like at amounts ranging from about 0.5 % to about 30 % by weight, from about 0.9 % to about 25 % by weight, from about 5 % to about 20 % by weight, from about 10 % to about 15 % by weight,. Optionally, vaccine compositions can comprise surfactants, including polysorbates, for example, PS 80, TWEEN®s, and the like. Additionally, vaccine compositions can include an adjuvant, an immunodulator or substances that enhance the effectiveness of the vaccine product.

[0031] In yet an alternate embodiment, a method for immunizing a subject is provided, comprising administering to the subject a therapeutically or prophylactically effective amount of a dry powder vaccine composition comprising a diketopiperazine and a VLP, for example an influenza virus glycoprotein, protein, peptide selected from HA or NA or a subtype thereof, fragments thereof or combinations thereof; wherein the subject inhales the vaccine composition in a single inhalation. In this

embodiment, booster inhalations can be given after, for example, one week, two weeks, three weeks or a month of administration of a first dose.

[0032] In an alternate embodiment, the dry powder formulation can be reconstituted with a suitable diluent, for example, saline solution or a buffer, including phosphate buffer for administering the antigen by injection or intradermal application via micro-needles or vaccine patch. In this embodiment, the dry powder formulation is stable at room temperature for an extended period of time compared to current vaccination formulations requiring refrigeration.

DEFINITION OF TERMS

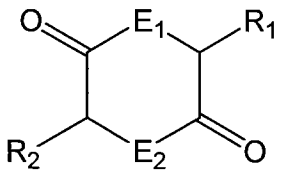
[0033] Prior to setting forth the invention, it may be helpful to provide an understanding of certain terms that will be used hereinafter:

[0034] Analog: As used herein, an "analog" includes compounds having structural or functional similarity to another compound. For example, the anti-viral compound acyclovir is a nucleoside analogue and is structurally similar to the nucleoside guanosine which is derived from the base guanine. Thus, acyclovir mimics guanosine (is biologically analogous with) and interferes with DNA synthesis by replacing (or competing with) guanosine residues in the viral nucleic acid and prevents translation/transcription. Thus, compounds having structural similarity to another (a parent compound) that mimic the biological or chemical activity of the parent compound are analogs. There are no minimum or maximum numbers of elemental or functional group substitutions required to qualify a compound as an analog provided the analog is capable of mimicking, in some relevant fashion, either identically, complementarily or competitively, with the biological or chemical properties of the parent compound. Analogs can be, and often are, derivatives of the parent compound (see "derivative" infra). Analogs of the compounds disclosed herein may have equal, lesser or greater activity than their parent compounds.

[0035] Derivative: As used herein, a "derivative" is a compound made from (or derived from), either naturally or synthetically, a parent compound. A derivative may be an analog (see "analog" supra) and thus may possess similar chemical or biological activity. However, unlike an analog, a derivative does not necessarily have to mimic the biological or chemical activity of the parent compound. There are no minimum or maximum numbers of elemental or functional group substitutions required to qualify a compound as a derivative. For example, while the antiviral compound ganciclovir is a derivative of acyclovir, ganciclovir has a different spectrum of anti-viral activity and different toxicological properties

than acyclovir. Derivatives of the compounds disclosed herein may have equal, less, greater or even distinct activities when compared to their parent compounds.

[0036] Diketopiperazine: As used herein, "diketopiperazine" or "DKP" includes diketopiperazines and salts, derivatives, analogs and modifications thereof falling within the scope of the general Formula 1, wherein the ring atoms E_1 and E_2 at positions 1 and 4 are either O or N and at least one of the side-chains R_1 and R_2 located at positions 3 and 6 respectively contains a carboxylic acid (carboxylate) group. Compounds according to Formula 1 include, without limitation, diketopiperazines, diketomorpholines and diketodioxanes and their substitution analogs.



Formula 1

[0037] Diketopiperazines, in addition to making aerodynamically suitable microparticles, also facilitate the delivery of drugs by enabling absorption into the circulation. Diketopiperazines can be formed into particles that incorporate a drug or particles onto which a drug can be adsorbed. The combination of a drug and a diketopiperazine can impart improved drug stability. These particles can be administered by various routes of administration. As dry powders these particles can be delivered by inhalation to specific areas of the respiratory system, depending on particle size. Additionally, the particles can be made small enough for incorporation into an intravenous suspension dosage form. Oral delivery is also possible with the particles incorporated into a suspension, tablets or capsules. Diketopiperazines may also facilitate absorption of an associated drug.

[0038] In one embodiment, the diketopiperazine is 3,6-di(fumaryl-4-aminobutyl)-2,5-diketopiperazine (fumaryl diketopiperazine, FDKP). The FDKP can comprise microparticles in its acid form or salt forms which can be aerosolized or administered in a suspension.

[0039] In another embodiment, the DKP is a derivative of 3,6-di(4-aminobutyl)-2,5-diketopiperazine, which can be formed by (thermal) condensation of the amino acid lysine. Exemplary derivatives include bis—[3,6-(N-X-4-aminobutyl)]-2,5-diketopiperazine; wherein X is fumaryl, glutaryl, succinyl, maleyl, malonyl, or oxalyl and the compounds are 3,6-di(succinyl-4-aminobutyl)-, 3,6-di(maleyl-4-aminobutyl)-, 3,6-di(glutaryl-4-aminobutyl), 3,6-di(malonyl-4-aminobutyl)-, 3,6-di(oxalyl-4-aminobutyl)-, and 3,6-di(fumaryl-4-aminobutyl)-2,5-diketopiperazine. The use of DKPs for drug delivery

is known in the art (see for example U.S. Patent Nos. 5,352,461; 5,503,852; 6,071,497; 6,331,318; 8,227,409; 8,227,409; each of which is incorporated herein by reference for all that they teach regarding diketopiperazines and diketopiperazine-mediated drug delivery). The use of DKP salts is described in U.S. Patent Nos. 7,820,676 and 8,278,308, which are hereby incorporated by reference for all they teach regarding diketopiperazine salts. Pulmonary drug delivery using DKP microparticles is disclosed in U.S. Patent No. 6,428,771, which is hereby incorporated by reference in its entirety. Nasal drug delivery using DKP microparticles is disclosed in U.S. Patent No. 7,833,550 which is hereby incorporated by reference in its entirety. Further details related to adsorption of active agents onto crystalline DKP particles can be found in co-pending U.S. Patent Nos. 7,799,344 and 7,803,404, which are hereby incorporated by reference in their entirety.

[0040] Drug delivery system: As used herein, "drug delivery system" refers to a system for delivering one or more active agents.

[0041] Dry powder: As used herein, "dry powder" refers to a fine particulate composition that is not suspended or dissolved in a propellant, liquid, or other carrier. It is not meant to necessarily imply a complete absence of all water molecules.

[0042] Immunogen: As used herein, "immunogen" refers to a molecule that induces an immune response when administered to subject (*e.g.,* human or non-human animal), including inducing the production of antibodies or expansion of antigen-specific T cells in the subject against the molecule after administration. It can be naturally derived, produced by a recombinant process, or by a synthetic process.

[0043] Microparticles: As used herein, the term "microparticles" includes particles of generally 0.5 to 100 microns in diameter and particularly those less than 10 microns in diameter. Various embodiments will entail more specific size ranges. The microparticles can be assemblages of crystalline plates with irregular surfaces and internal voids as is typical of those made by pH controlled precipitation of the DKP acids. In such embodiments the active agents can be entrapped by the precipitation process or coated onto the crystalline surfaces of the microparticle. The microparticles can also be spherical shells or collapsed spherical shells comprised of DKP salts with the active agent dispersed throughout. Typically such particles can be obtained by spray drying a co-solution of the DKP and the active agent. The DKP salt in such particles can be amorphous. The forgoing descriptions should be understood as exemplary. Other forms of microparticles are contemplated and encompassed by the term.

[0044] Pulmonary inhalation: As used herein, "pulmonary inhalation" is used to refer to administration of pharmaceutical preparations by inhalation so that they reach the lungs and in particular

embodiments the alveolar regions of the lung. Typically inhalation is through the mouth, but in alternative embodiments in can entail inhalation through the nose.

[0045] Therapeutically effective amount: As used herein, the term "therapeutically effective amount" of a composition, when administered to a human or non-human patient, to provide a therapeutic or prophylactic benefit such as amelioration of symptoms or prevention of a transmissible disease, *e.g.*, an amount effective to stimulate the production and secretion of antibodies that neutralize an infective agent as well as a T lymphocyte response against the agent. Thus a therapeutically effective amount of a composition is also an amount sufficient to prevent the onset of symptoms of a disease or reduce their severity.

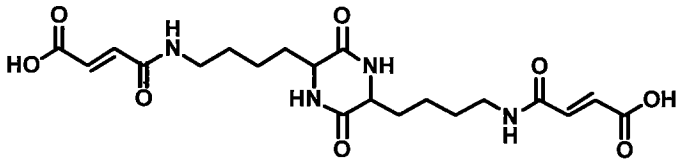
DETAILED DESCRIPTION

[0046] In embodiments described herein, there is provided a dry powder immunization system and methods that allow for improved delivery of vaccines to a subject for self-administration and which vaccines have an increased shelf-life and can be stored at room temperature for various periods of times while maintaining efficacy. The present vaccine compositions are for inhalation using a dry powder inhaler which can be self administered without the need to visit a clinic, nurse, or a physician. In particular, the vaccine compositions are intended for the treatment and prevention of transmissible diseases including viral infections such as influenza.

[0047] In one embodiment, dry powder vaccine compositions comprise a diketopiperazine and one or more antigenic components, including virus-like particles (VLPs), immunogenic proteins, peptides or fragments of the viral proteins. In certain embodiments, antigenic molecules which can also be delivered by the methods herein include, antigens derived from pathogenic agents such as parasites, or bacteria, tumor antigens, allergens or self-antigens; or antibodies thereof, can be used to vaccinate or treat against corresponding diseases.

[0048] In an exemplary embodiment, the vaccine composition comprises a dry powder comprising a VLP and a diketopiperazine selected from 3,6-di(fumaryl-4-aminobutyl)-2,5-diketopiperazine (fumaryl diketopiperazine, FDKP); 3,6-di(succinyl-4-aminobutyl)-2,5-diketopiperazine, 3,6-di(maleyl-4-aminobutyl)-2,5-diketopiperazine, 3,6-di(glutaryl-4-aminobutyl)-2,5-diketopiperazine, 3,6-di(malonyl-4-aminobutyl)-2,5-diketopiperazine, 3,6-di(oxalyl-4-aminobutyl)-2,5-diketopiperazine or derivatives of analogs thereof.

[0049] In a particular embodiment, fumaryl diketopiperazine (*bis*—[3,6-(N-fumaryl-4-aminobutyl)]-2,5-diketo-diketopiperazine (FDKP) is used as the diketopiperazine for pulmonary inhalation applications:



[0050] In this embodiment, the diketopiperazine can be in crystalline or amorphous dry powder form or combinations thereof. In one embodiment, the dry powder composition can comprise the disodium salt of the diketopiperazine or other salts including, dipotassium, magnesium, lithium and the like.

[0051] In embodiments utilizing FDKP in the compositions, FDKP provides a beneficial microparticle matrix because it has low solubility in acid, but is readily soluble near neutral or at basic pH. These properties allow FDKP to crystallize and the crystals to self-assemble into form microparticles under acidic conditions. The particles dissolve readily under physiological conditions where the pH is neutral. As noted, microparticles having a diameter of between about 0.5 and about 10 microns can reach the lungs, successfully passing most of the natural barriers. Particles in this size range can be readily prepared from FDKP as a crystalline powder, an amorphous powder, or combinations thereof by several methods.

[0052] In a particular embodiment, the vaccine composition against viral infections is provided comprising a dry powder composition comprising diketopiperazine microparticles and an antigen, antibody, fragment thereof, and/or combinations thereof. In one embodiment, the antigens in the vaccine composition comprise VLPs comprising peptides, proteins, including glycoproteins, and/or individual antigenic components or fragments of a VLP, fragments of the antigenic proteins and/or antigenic peptides, or combinations thereof. In this embodiment, the dry powder vaccine composition comprises highly immunogenic, non-infectious monovalent and/or polyvalent vaccines using an antigen produced from a cell-based manufacturing system, and delivered using a dry powder inhaler for single use.

[0053] In an embodiment inhaled FDKP particles carry the immunogens to the lungs or nasal cavities, wherein the particles dissolve immediately upon contact with wet tissue surfaces at the prevailing physiological pH due to FDKP's characteristics consisting of high solubility at pH $\geq$ 6. Once the particles are dissolved, the immunogenic material is available for local and systemic absorption. Active factors, including, nucleic acids, proteins and peptides ranging in molecular weight from 300 to

100,000 Da can be administered in the vaccine compositions and are absorbed readily. FDKP delivered to the lungs is absorbed, but is not metabolized and is excreted intact, primarily in urine. FDKP does not directly facilitate drug absorption, but functions solely as the particle matrix.

[0054] Exemplary FDKP powders can be delivered using patient friendly, breath-powered inhalers for self-administration and can be configured for single use. In use, a patient's inhalation effort generates the air flow necessary to fluidize, de-agglomerate, and disperse the dry powders from the inhaler with efficiency greater than 90% powder emission from the inhaler. Timing and/or coordination of the inhalation effort with the powder dispersion is not required. In an embodiment, this delivery system comprises high air flow resistance rigid conduits that change the direction of flow at angles that promote deagglomeration. This feature keeps the powder moving slowly during delivery to reduce unwanted throat impaction and promote deep lung deposition. Additionally, the dry powder inhalation system can be designed and configured to prevent powder dispersion during reverse air flow, for example, if a patient accidentally exhales into the device when in use so as to prevent loss of, for example, the vaccine dose being administered. The inhalation system provides consistent delivery. Powder dispersion from these devices begins with the onset of the inhalation effort and ends quickly within approximately 300-500 ms, ensuring proper delivery even with a short or interrupted patient inhalation.

[0055] In embodiments described herewith, an inhalation system for pulmonary delivery by nasal or oral inhalation is provided, comprising a dry powder inhaler having rigid air conduits and high resistance to airflow characteristics which delivers a vaccine formulation at pressure differentials greater than 2 kPa; wherein the vaccine formulation comprises a dry powder comprising a diketopiperazine and an antigen or antibody for immunizing a subject against the influenza virus and the vaccine formulation is self-administered by a subject in a single inhalation. In this embodiment, the vaccine composition comprises immunogenic material(s), for example, VLPs, and/or antigenic proteins or peptides thereof, wherein the antigenic protein or peptides are selected for one or more viruses, including, native or recombinant viral glycoproteins, proteins and peptides isolated or derived from an influenza virus, including influenza virus A, influenza virus B and influenza virus C. Non-limiting examples of VLPs that can be incorporated into the formulations and systems as described herein include those disclosed in U.S. Patent Publication Nos. 20080031895; 20090022762 and 20110212128. In this embodiment, the vaccine comprises a breath powered, dry powder, single use inhaler comprising a dry powder formulation comprising a peptide, protein and/or glycoprotein, including, Hemagglutinin (HA) or subtype thereof selected from subtypes H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H4, H15, H16, and/or H17, or other mutants thereof, and/or a neuraminidase (NA) or subtype thereof selected from subtypes N1, N2, N3, N4, N5, N6, N7, N8, N9, and/or N10, or mutants thereof, fragments thereof, or combinations

thereof. In another embodiment, the formulation (*e.g.,* VLP) comprises additional influenza proteins such as NP, M1, M2, NS1, NS2 or the polymerase subunits PB1, PB2, and PA or combination thereof. In an example embodiment, an influenza vaccine comprises a dry powder composition comprising a recombinant peptide and/or protein antigen, for example, recombinant HA3 (rHA3) and a diketopiperazine in a single use dry powder inhaler. In one embodiment, the diketopiperazine comprises microparticles of, for example, fumaryl diketopiperazine or disodium salt of fumaryl diketopiperazine.

[0056] In alternate embodiments, the dry powder vaccines can comprise one or more neuraminidase related antigen(s). In another embodiment, the vaccine composition comprises combinations of HA and NA antigens, or antibodies or fragments thereof against HA or NA and/or their subtypes. In embodiments for treating influenza, the vaccines can be administered as prophylactic in healthy subject alone, or in combination with a neuraminidase inhibitor, including Oseltamivir (TAMIFLU®) and Zanamivir (RELENZA®) for treatment of influenza A and B viral infections, or M2 inhibitors, including Amantidine (SYMMETREL®) and Rimantadine (FLUMADINE®) or analogs thereof for treatment of influenza A viral infections. In these embodiments, the neuraminidase inhibitor can be administered separately by inhalation or another route of administration.

[0057] In further embodiments, there is provided a drug delivery systems comprising an inhaler, a unit dose dry powder medicament container, for example, a reservoir configured to hold a dry powder, and a dry powder comprising microparticles disclosed herein and an immunogen or antigen including, an influenza virus particle antigen, hemagglutinin A and neuraminidase and/or fragments thereof. In one embodiment, the delivery system for use with the dry powders includes an inhalation system comprising a high resistance inhaler having air conduits which impart a high resistance to airflow through the conduits for deagglomerating and dispensing the powder. In one embodiment, the inhalation system has a resistance value of, for example, approximately 0.065 to about 0.200 ($\sqrt{kPa}$)/liter per minute. In certain embodiments, the dry powders can be delivered effectively by inhalation with an inhalation system wherein the peak inhalation pressure differential can range from about 2 to about 20 kPa, which can produce resultant peak flow rates of about between 7 and 70 liters per minute. In certain embodiments, the inhalation system are configured to provide a single dose by discharging powder from the inhaler as a continuous flow, or as one or more pulses of powder delivered to a patient.

[0058] In one embodiment, there is provided a vaccine composition comprising an anti-infective composition against bacteria that can produce disease, including bacteria use as biological warfare agents such as anthrax bacteria. In an example embodiment, the vaccine comprises a dry powder composition composition for inhalation comprising diketopiperazine particles and an immunogenic peptide either from the bacteria, or prepared separately from the bacteria. In one embodiment, for example, the dry powder

vaccine composition comprises fumaryl diketopiperazine microparticles and an antigen; wherein the antigen is an immunogenic peptide, for example, a protective antigen or PA peptides. In another embodiment, an inhalable dry powder vaccine composition for immunizing against bacteria including, anthrax or any infectious bacteria comprises particles of a diketopiperazine and antibodies and/or antibody fragments thereof against PA peptides from the infectious bacteria. In this and other embodiments, a method is provided which comprises providing a subject in need of immunization against an infectious bacteria or bacterial spores, a vaccine composition comprising particles of a diketopiperazine and an antigenic peptide of the infectious bacterium, and having the subject inhale the vaccine composition by oral inhalation; wherein the vaccine composition is provided in a single use disposable dry powder inhaler. In the methods, subjects can be immunized with a single administration of the vaccine composition; or a single administration of the vaccine composition and booster vaccine administrations can be administered at 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks, or longer after initial administration. In one embodiment, multiple booster vaccines can be administered up to 4 to 6 doses. Vaccine doses can vary depending on the subject, but can be greater than 0.10 mg of powder to about 10 mg of powder, such as 0.20 mg, 0.40 mg, 0.80 mg, 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, or 10 mg. In some embodiments, the amount of peptide antigen in the composition can be at least 0.001 μg, at least 0.050 μg, at least 0.075 μg, or at least 0.10 μg of peptide in the vaccine composition.

[0059] In some embodiments disclosed herewith, the dry powder inhaler system comprises a predetermined mass flow balance within the inhaler. For example, a flow balance of approximately 10% to 70% of the total flow exiting the inhaler and into the patient is delivered by one or more dispensing ports, which airflow passes through the area containing the powder formulation, and wherein approximately 30% to 90% air flow is generated from other conduits of the inhaler. Moreover, bypass flow, or flow not entering and exiting the area of powder containment such as through a cartridge, can recombine with the flow exiting the powder dispensing port within the inhaler to dilute, accelerate and ultimately deagglomerate the fluidized powder prior to exiting the mouthpiece. In one embodiment, flow rates ranging from about 7 to 70 liters per minute result in greater than 75% of the container or the cartridge contents dispensed in fill masses between 1 and 30 mg. In certain embodiments, an inhalation system as described above can emit a respirable fraction/fill of a powder dose at percentages greater than 40%, greater than 50%, greater than 60%, or greater than 70% in a single inhalation,.

[0060] In particular embodiments, an inhalation system is provided comprising a dry powder inhaler, a dry powder formulation comprising microparticles of fumaryl diketopiperazine having an FDKP trans isomer content between 45% and 65% and one or more than one active agents. In some aspects of this embodiment of the inhalation system, the dry powder formulation is provided in a unit

dose cartridge. Alternatively, the dry powder formulation can be preloaded in the inhaler. In this embodiment, the structural configuration of the inhalation system allows the deagglomeration mechanism of the inhaler to produce respirable fractions greater than 50%; that is, more than half of the powder contained in the inhaler (cartridge) is emitted as particles of less than 5.8 μm. The inhalers can discharge greater than 85% of a powder medicament or vaccine contained within a container during dosing. In certain embodiments, the inhalers can discharge greater than 85% of a powder medicament or vaccine contained in a single inhalation. In one embodiment, the inhalers can discharge greater that 90% of the cartridge contents or container contents in less than 3 seconds at pressure differentials between 2 and 5 kPa with fill masses ranging up to 50 mg.

[0061] A method of immunizing a subject is also provided herewith comprising administering to a subject in need of immunization a therapeutically effective amount of a dry powder vaccine as described above and comprising an antigen, antibody, fragments thereof, or combinations thereof, and a diketopiperazine, including FDKP. In one embodiment, the antigen, antibody, or combinations thereof are for the treatment and prevention of a viral infection, including, influenza, and the dry powder vaccine is administered by inhalation and the inhalation system is configured to deliver a dose of the vaccine in a single inhalation in less than 1 seconds, or less than 2 seconds.

[0062] In an alternate embodiment, the dry powder formulation can be reconstituted with a solution or diluent, including phosphate buffered saline as an injectable formulation. In this embodiment, stable dry powder can be stored at room temperature up to about three months or longer, and reconstituted into a solution at the time of administration.

EXAMPLES

[0063] The following examples are included to demonstrate certain embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples elucidate representative techniques that function well in the practice of the present invention. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention. The injectable vaccine can be administered to a subject, for example, by an intramuscular injection.

EXAMPLE 1

[0064] **Preparation of vaccine compositions as an Inhalable Dry Material**

[0065] In these experiments, a crystalline dry powder vaccine formulation was made using a hemagglutinin A3 antigen adsorbed onto preformed fumaryl diketopiperazine microparticles. FDKP

powder was dissolved in a solution containing ammonium hydroxide and PS80 then precipitated with acetic acid solution and washed in water to form a suspension. A separately prepared solution (7.5 ml) containing HA3 in Tris-HCl buffer, sodium chloride and 1 % glycerol was added to 3.3 g of the suspension with stirring at room temperature to yield a 5 % HA3 content in the mixture. The resulting pH of the suspension was pH 4.1 and the suspension was pelletized in liquid nitrogen and lyophilized overnight under vacuum. Lyophilized FDKP-H3A powder was stored in a dessicator at ambient temperature. In an additional study, dry powder vaccine formulation was made as described above except that the suspension containing the FDKP-HA3 particles were instead sprayed dried to remove the solvents instead of pelletizing and lyophilizing. Lyophilized and spray-dried powders were tested for the presence of the HA3 in the formulation by various assays and methods including HPLC analysis (Waters Alliance 2695 HPLC equipped with a 2996 photodiode array detector and a Phenomenex, Aeris widepore 3.6 XB-C8, 15 x 2.10 mm column) and Column mobile phase used. Albumin at 1 mg/ml was used as standard using Mobile Phase A (0.1% TFA in water, pH 3.0 ± 0.1; pH adjusted with 7% aqueous NH_4OH) and Mobile Phase B (0.1% TFA in CAN). Particle size distribution of the powder formulation was assessed using a dry powder inhaler (Mannkind Corp.) and Cascade Impaction apparatus using a cascade impactor (Andersen) at 4 kPa pressure differential.

[0066] The results from the HPLC analysis of the powder indicated that FDKP-HA3 formulation had a total protein content of 5.05 % (w/w). Particle size distribution resulting from cascade impaction studies are presented in Table 1. The Data show that the dry powder made by spray-drying and lyophilization is effectively delivered out of the inhaler and more than 45% of the particles produced are within the respirable range.

Table 1

Vaccine Formulation	**% Respirable Fraction on Contents Delivered**	**% Respirable Fraction of Fill Contents**	**Total Amt. All Stages/ Total Emptied**	*% Cartridge Emptying*
Lyophilized FDKP-HA3	45.5	45.2	72.2	99.2
Spray-Dried FDKP-HA3	53.8	53.1	65.4	98.7

EXAMPLE 2

[0067] *Immunization of mice with FDKP-HA3 Inhalation Powder.*

[0068] Dry powder vaccine for inhalation containing HA3 prepared as described in Example 1 was used in these experiments.

[0069] *Experimental Vaccine:* Powder composition of a rHA3 protein (derived from influenza A/Perth/2009 (H3N1). BALB/c mice were used and the mice were divided into groups. The first group, a group of female mice, 6-8 weeks old received powdered rHA3 vaccine (Vac-Powder) via the intra-pulmonary route (IPu) using an insufflation technique (Penn Century, Inc. Model DP-4M. A second group of mice received the equivalent dose of vaccine reconstituted into a liquid form (Vac-liquid), prior to inoculation via the intramuscular route (IM). The second group is used as a control to the intra-pulmonary route in the experiments. In addition, the third group of mice did not receive these materials and served as the untreated controls.

[0070] *Dosage:* A Vac-Powder vaccine dose contains 0.5mg of bulk powder with a rHA3 content of approximately 5% (*e.g.* 0.5 mg powder/0.025 mg rHA; 500 μg powder/25 μg rHA3). Pre- and post-insufflator device weights are measured to determine actual vaccine powder deliveries.

[0071] The Vac-liquid vaccine for intramuscular immunization was prepared by dissolving an equivalent amount of powder (0.5 mg powder/0.025 mg rHA3) with 40 μl of 100mM Sodium Phosphate buffer, pH 7.24, and filtering the solution through a 0.22 μm filter prior to injection.

[0072] *Immunization regimen:* The mice received two doses of the corresponding treatment administered by the indicated route, two weeks apart. Animal handling and care were consistent with the current NIH "Guide for the Care and Use of Laboratory Animals" Federal and State Law and current operating procedures maintained by the Institution's Animal Facility. Mice were anesthetized prior to retro-orbital bleeding and for the insufflation procedure.

[0073] *Experimental Groups:* Thirty six mice are randomized into three experimental units: 1) Control; 2) Vac-Powder-Intra-pulmonary powder vaccine, and 3) Vac-Liquid Intramuscular liquid form.

[0074] *Treatments:* Three days prior to the initiation of the experiment, blood samples were collected from 5 mice per group (randomly selected) to assess pre-immunization immunological status. At day zero, all animals in each group received a first dose of vaccine or placebo, and two weeks later, all animal received a booster dose of vaccine or a second placebo treatment via the same route as the primary treatment. Two weeks after the booster dose, blood samples were collected from all animals (vaccine and controls) for appraising the immune response elicited by vaccine and control treatment. If deemed adequate, protective efficacy was be assessed by challenging all mice with $1x10^6$ PFU of a homologous influenza virus [A/Perth/2009 (H3N1)].

[0075] Table 2 and 3 below summarize schedule of treatment and experimental details of the study, respectively.

Table 2. Schedule of Treatments

Table 3.

[0076] Also, a dry powder vaccine as described in Table 3, Vac-Powder was reconstituted in PBS and administered intramuscularly to 12 mice to test the efficacy of the powder composition by another route of administration.

[0077] ***Assessment of immunogenicity and protective efficacy:***

[0078] *Immunogenicity:* The level of serum antibody elicited by the rHA powder vaccine and control immunizations was evaluated by ELISA, micro-neutralization assay and hemagglutination inhibition (HAI). Serum samples from three mice of each group were pooled to assure sufficient material for the serological determinations.

[0079] If the elicited immune response is deemed adequate, animal in all groups are challenged with a homologous influenza virus [A/Perth/2009 (H3N1)].

[0080] *Protective Efficacy:* The protective efficacy afforded by the rHA powdered vaccine or control immunizations was evaluated by challenging all animals with $1x10^6$PFU of influenza A/Perth/2009 (H3N1). Protection was assessed by measuring virus load in the upper and lower respiratory tract at days 3, 6, and 8 post-challenge in 4 mice per time point. Body weight measurements and clinical signs of disease were monitored daily until the last time point. Animals that experience greater than 20% body weight decrease or show severe symptoms of influenza such as labored breathing or paralysis were euthanized. In a separate group, mice were given an equivalent dose of a dry powder

vaccine reconstituted into a liquid using 100 mM sodium phosphate buffer, pH 7.24 which was administered intramuscularly.

[0081] ***Immunization Results:***

[0082] The results from immunization are shown in Table 4 below. The data are from mice immunized as described above receiving two doses of dry powder vaccine formulations as described herein and containing the Influenza virus HA3 antigen and delivered using an insufflation device (Penn Century). Mice were given a dose of the formulation at day 1 and a second dose 20 days after the initial vaccination. The data in Table 4 illustrate the amounts of HA3 and FDKP powder each mouse received at initial dose by insufflation followed by a booster dose 20 days after initial vaccination.

[0083] The data indicate that each mouse received an amount of the dry powder vaccine containing the HA3 antigen in amounts ranging from 2.5 μg to 30.35 μg by insufflation in both doses administered. The results from the animal studies indicate that the dry powder vaccine composition comprising fumaryl diketopiperazine particles and a viral rHA3 antigen stimulated the production of antibodies against the HA3 antigen *in vivo* as shown by a significant antibody response obtained from positive hemagluttination inhibition studies of sera obtained from dry powder vaccine intramuscularly immunized mice when compared to controls. Overall mice immunized with powderized rHA3 delivered either by insufflation (Vac-powder) or intramuscularly as a suspension (IM-Vac-powder) had reduced lung virus titers compared to intramuscular injections of saline solution [IM-PBS], standard VLPs administered intramuscularly (positive control), and lactose powder vaccine administered by insufflation.

Table 4.

Penn Century discharge gravimetric data

		1st insufflation 7-Sep			2nd insufflation / Booster dose 27-Sep		
	mouse ID	powder lot	powder delivered (mg)	HA3 delivered (µg)	powder lot	powder delivered (mg)	HA3 delivered (µg)
Vaccine delivery	814	Spray Dry	0.261	13.05	Lyo'd*	0.493	24.65
	815	Spray Dry	0.103	5.15	Lyo'd	0.198	9.9
	816	Spray Dry	0.607	30.35	Lyo'd	0.369	18.45
	818	Spray Dry	0.193	9.65	Lyo'd	0.565	28.25
	819	Lyo'd	0.445	22.25	Lyo'd	0.373	18.65
	820	Lyo'd	0.464	23.2	Lyo'd	0.385	19.25
	821	Spray Dry	0.21	10.5	Lyo'd	0.126	6.3
	822	Lyo'd	0.155	7.75	Lyo'd	0.523	26.15
	823	Lyo'd	0.193	9.65	Lyo'd	0.299	14.95
	824	Lyo'd	0.412	20.6	Lyo'd	0.239	11.95
	825	Lyo'd	0.46	23	Lyo'd	0.338	16.9
	839	Lyo'd	0.412	20.6	Lyo'd	0.258	12.9
	840	Lyo'd	0.43	21.5	Lyo'd	0.176	8.8
	842	Lyo'd	0.402	20.1	Lyo'd	0.433	21.65
	843	Spray Dry	0.187	9.35	Lyo'd	0.447	22.35
	845	Lyo'd	0.19	9.5	Lyo'd	0.05	2.5

* Lyo'd denotes lyophilized powder

EXAMPLE 3

[0084] ***Influenza vaccine administration:*** A 35 year old, healthy female who is needlephobic reports to her physician to receive her annual flu vaccine. Following examination, her physician provides her with a dry powder vaccine comprising 5 mg of powder containing fumaryl diketopiperazine microparticles and 50 µg of H3N1 antigen in a high resistance dry powder inhaler. The female patient places the mouthpiece of the inhaler in her mouth and deeply inhales the dry powder. After inhalation of the dry powder, the inhaler is safely discarded and the patient is discharged being immunized.

[0085] **Vaccination against *B. anthracis:*** A 19 year old healthy male who has enlisted into the military service reports for duty. Having found the enlisted serviceman healthy, the doctor provides a dry powder vaccine to the serviceman. The vaccination takes place in front of the doctor in which the serviceman orally inhales the vaccine from a dry powder inhaler in a single inhalation. The vaccine administered comprises a dry powder containing fumaryl diketopiperazine microparticles and 50 μg of PA peptide and is inhaled from a high resistance dry powder inhaler. A second dry powder inhaler is given to the serviceman to take a booster immunization 4 weeks later. The anthrax vaccine is administered annually.

[0086] ***Influenza vaccine administration:*** A 55 year old, healthy female reports to her physician to receive her annual flu vaccine. Following examination, her physician provides her with a dry powder vaccine comprising 5 mg of powder containing fumaryl diketopiperazine microparticles and 50 μg of a combination of HA antigen and a neuraminidase inhibitor in a high resistance dry powder inhaler. The female patient places the mouthpiece of the inhaler in her mouth and deeply inhales the dry powder. After inhalation of the dry powder, the inhaler is safely discarded and the patient is discharged being immunized.

[0087] While the invention has been particularly shown and described with reference to particular embodiments, it will be appreciated that variations of the above-disclosed and other features and functions, or alternatives thereof, may be desirably combined into many other different systems or applications. Also that various presently unforeseen or unanticipated alternatives, modifications, variations or improvements therein may be subsequently made by those skilled in the art which are also intended to be encompassed by the following claims.

[0088] Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value,

however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0089] The terms "a," "an," "the" and similar referents used in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

[0090] Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member may be referred to and claimed individually or in any combination with other members of the group or other elements found herein. It is anticipated that one or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[0091] Certain embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Of course, variations on these described embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventor expects skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0092] Furthermore, numerous references have been made to patents and printed publications throughout this specification. Each of the above-cited references and printed publications are individually incorporated herein by reference in their entirety.

[0093] In closing, it is to be understood that the embodiments of the invention disclosed herein are illustrative of the principles of the present invention. Other modifications that may be employed are within the scope of the invention. Thus, by way of example, but not of limitation, alternative configurations of the present invention may be utilized in accordance with the teachings herein. Accordingly, the present invention is not limited to that precisely as shown and described.

[0094] Specific embodiments disclosed herein may be further limited in the claims using consisting of or consisting essentially of language. When used in the claims, whether as filed or added per amendment, the transition term "consisting of" excludes any element, step, or ingredient not specified in the claims. The transition term "consisting essentially of" limits the scope of a claim to the specified materials or steps and those that do not materially affect the basic and novel characteristic(s). Embodiments of the invention so claimed are inherently or expressly described and enabled herein.

[0095] Specific embodiments disclosed herein may be further limited in the claims using consisting of or consisting essentially of language. When used in the claims, whether as filed or added per amendment, the transition term "consisting of" excludes any element, step, or ingredient not specified in the claims. The transition term "consisting essentially of" limits the scope of a claim to the specified materials or steps and those that do not materially affect the basic and novel characteristic(s). Embodiments of the invention so claimed are inherently or expressly described and enabled herein.

We claim:

1. An influenza vaccine comprising a dry powder composition comprising a diketopiperazine and at least one of influenza virus-like particles, an antigenic protein, an antigenic peptide, fragments of said influenza virus-like particle, a subunit protein or multiple subunit proteins or fragments of said antigenic protein(s), fragments of said antigenic peptide, or combinations thereof.

2. The influenza vaccine of claim 1, wherein the virus-like particle comprises a hemagglutinin and/or a neuraminidase protein and/or a matrix protein, or peptide or fragments thereof.

3. The influenza vaccine of claim 1, wherein the antigenic protein or peptide is neuraminidase derived for influenza virus type A, B or C, or any subtype of influenza A, or fragments thereof or combinations thereof.

4. The influenza vaccine of claim 1, wherein the antigenic protein or peptide is hemagglutinin derived from influenza A, B or C or any subtype of influenza A, or fragments thereof, or combinations thereof.

5. The influenza vaccine of claim 4, wherein the antigenic protein or antigenic peptide is hemagglutinin A, or wherein the antigenic protein or subunit is another protein of the influenza virus such as NP, M1, M2, NS1, NS2, polymerase subunits PB1, PB2 or PA or fragments thereof or combinations thereof.

6. The influenza vaccine of claim 1, wherein the diketopiperazine is *bis*[3,6-(*N*-X-4-aminobutyl)]-2,5-diketopiperazine.

7. The influenza vaccine of claim 6, wherein X is fumaryl, glutaryl, succinyl, maleyl, malonyl, or oxalyl.

8. The influenza vaccine of claim 1, wherein the diketopiperazine is *bis*[3,6-(*N*-fumaryl-4-aminobutyl)]-2,5-diketopiperazine.

9. An influenza vaccine, comprising an inhalation system comprising a dry powder inhaler comprising a dry powder formulation comprising microparticles of *bis*[3,6-(*N*-fumaryl-4-aminobutyl)]-2,5-diketopiperazine and an antigen selected from influenza virus protein consisting of hemagglutinin A or neuraminidase A, or other influenza protein and/ or combinations thereof.

10. The influenza vaccine of claim 9, wherein the hemagglutinin A is a recombinant HA3 antigen.

11. The influenza vaccine of claim 9, wherein the dry powder inhaler is configured as a single use disposable inhaler for self-administration.

12. A method for immunizing a subject against an influenza virus infection, comprising administering to the subject a therapeutically effective amount of a dry powder vaccine comprising a diketopiperazine and a virus-like particle, an antigenic protein, an antigenic peptide, fragments of said virus-like particle, or a

subunit protein or multiple distinct subunits proteins or fragments of said antigenic protein/s, fragments of said antigenic peptide, or combinations thereof, wherein the vaccine is self-administered by inhalation.

13. The method of claim 12, wherein the diketopiperazine is *bis*[3,6-(*N*-X-4-aminobutyl)]-2,5-diketopiperazine.

14. The method of claim 13, wherein X is fumaryl, glutaryl, succinyl, maleyl, malonyl, or oxalyl.

15. The method of claim 14, wherein the diketopiperazine is *bis*[3,6-(*N*-fumaryl-4-aminobutyl)]-2,5-diketopiperazine.

16. The method of claim 12, wherein the antigenic peptide is a recombinant HA3 antigen or fragment thereof.

17. A method of immunizing a subject against an influenza viral infection comprising, administering to said subject in need of immunization the influenza vaccine of claim 9.

18. The method of claim 17, further comprising providing a booster vaccine dose to said subject, said booster vaccine comprising the influenza vaccine of claim 9.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/066969

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/12 A61K39/145 A61K9/00 A61P31/16
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C12N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2010/144789 A2 (MANNKIND CORP [US]; GRANT MARSHALL L [US]; STOWELL GRAYSON W [US]; MEN) 16 December 2010 (2010-12-16) paragraphs [0004], [0007], [0019], [0034], [0040] - [0042], [0045], [0046], [0067]; claims 5,15	1-18

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 January 2014	27/01/2014

Name and mailing address of the ISA/	Authorized officer
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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	SMITH D J ET AL: "Evaluation of novel aerosol formulations designed for mucosal vaccination against influenza virus", VACCINE, ELSEVIER LTD, GB, vol. 21, no. 21-22, 20 June 2003 (2003-06-20), pages 2805-2812, XP004429678, ISSN: 0264-410X, DOI: 10.1016/S0264-410X(03)00224-X abstract	1-18
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2013/066969

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