inexpensively manufactured and has a long shelf life. Importantly, it should generate robust protective immune responses using minimal antigen, and provide cross-protection against genetically distinct viruses, and ideally, those from different clades. In addition, the vaccine must also be safe.

SUMMARY

[0009a] In one aspect, the present invention provides the use of composition in the manufacture of a medicament for immunizing a subject in need thereof against an influenza virus, the composition comprising:

(a) a recombinant hemagglutinin (rHA) from an influenza virus, and

(b) an adjuvant, wherein the adjuvant comprises a disaccharide having a reducing and a non-reducing terminus each independently selected from glucosyl and amino substituted glucosyl, where a carbon at a 1 position of the non-reducing terminus is linked through either an ether (-O-) or amino (-NH-) group to a carbon at a 6' position of the reducing terminus, the disaccharide being bonded to a phosphate group through a 4' carbon of the non-reducing terminus and to a plurality of lipid groups through amide (-NH-C(O)-) and/or ester (-O-C(O)-) linkages, where the carbonyl (-C(O)-) group of the ester or amide linkage is directly linked to the lipid group, and each lipid group comprises at least 8 carbons,

wherein the composition is dosage sparing, wherein the composition is to be administered by way of a single injection and wherein the amount of rHA per dose is in the range of 15 to 0.1µg.

[0009b] In one aspect, the present invention provides the use of a composition in the manufacture of a medicament for immunizing a subject in need thereof against an influenza virus by way of a single injection of the medicament, the composition comprising:

(a) a recombinant hemagglutinin (rHA) from an influenza virus and

(b) an adjuvant, wherein the adjuvant comprises a disaccharide having a reducing and a non-reducing terminus each independently selected from glucosyl and amino substituted glucosyl, where a carbon at a 1 position of the non-reducing terminus is linked through either an ether (-O-) or amino (-NH-) group to a carbon at a 6' position of the reducing terminus, the disaccharide being bonded to a

phosphate group through a 4' carbon of the non-reducing terminus and to a plurality of lipid groups through amide (-NH-C(O)-) and/or ester (-O-C(O)-) linkages, where the carbonyl (-C(O)-) group of the ester or amide linkage is directly linked to the lipid group, and each lipid group comprises at least 8 carbons and wherein the amount of rHA per dose is in the range of 15 to 0.1μg.

[0010] The present ~~invention~~disclosure is directed to compositions for use as vaccines and methods of immunizing subjects with the vaccines, in which the vaccines comprise a recombinant hemagglutinin from a pre-pandemic or pandemic influenza virus and an adjuvant. In one ~~embodiment~~example, the adjuvant is a compound (a DSLP compound) described as a disaccharide having a reducing and a non-reducing terminus each independently selected from glucosyl and amino substituted glucosyl, where a carbon at a 1 position of the non-reducing terminus is linked through either an ether (-O-) or amino (-NH-) group to a carbon at a 6' position of the reducing terminus, the disaccharide being bonded to a phosphate group through a 4' carbon of the non-reducing terminus and to a plurality of lipid groups through amide (-NH-C(O)-) and/or ester (-O-C(O)-) linkages, where the carbonyl (-C(O)-) group of the ester or amide linkage is directly linked to the lipid group, and each lipid group comprises at least 8 carbons. In particular compositions and methods, the adjuvant is GLA (see, e.g., U.S. patent application publication 2008/0131466), which may, in various ~~embodiments~~examples, be oil-free, formulated as an oil-in-water emulsion or formulated with other adjuvants such as alum, an aluminum salt. Hemagglutinins of particular interest include H5 from highly pathogenic H5N1 viruses and H1 from H1N1 ("swine flu") pandemic influenzas. Compositions may be dosage-sparing and/or the recombinant hemagglutinin may be present in an amount that is dose-sparing. Methods may comprise immunizing subjects with a single injection, i.e., not multiple injections, of the compositions. For example, the composition may be defined by one or more (i.e., any combination of) the following: the rHA is present at an amount that is dose-sparing; the rHA is present at a concentration that does not provide protective immunity in the absence of the adjuvant; the rHA is from a pathogenic strain of avian influenza; the rHA is from a pathogenic

strain of H5N1 influenza; the rHA is from clade 1 or clade 2; the rHA is from a pandemic swine flu virus strain; the rHA is from a pandemic H1N1 strain; the composition comprises a single, i.e., not more than one distinct recombinant protein; the amount of rHA per dose is in the range of about 15 to about 0.1 μg; the rHA is expressed from either insect or mammalian cells in order to achieve a preferred level of glycosylation; the rHA is expressed as a fusion protein; the adjuvant is only or includes GLA; the adjuvant is only or includes 3D-MPL, the composition is oil-free; the composition comprises less than about 1% v/v oil or less than about 0.1% v/v oil; the adjuvant is formulated as an aqueous solution prior to being combined with antigen; the adjuvant is formulated in a liposome-containing composition prior to be being combined with antigen; the composition further comprises an aluminum salt or saponin.

[0011] In one ~~embodiment~~example, the ~~invention~~disclosure provides a method of immunizing a subject in need thereof against a pre-pandemic or pandemic influenza virus, comprising administering a single injection of a pharmaceutical composition comprising (a) a recombinant hemagglutinin (rHA) from a pre-pandemic or pandemic influenza virus and (b) an adjuvant, wherein the adjuvant comprises a disaccharide having a reducing and a non-reducing terminus each independently selected from glucosyl and amino substituted glucosyl, where a carbon at a 1 position of the non-reducing terminus is linked through either an ether (-O-) or amino (-NH-) group to a carbon at a 6' position of the reducing terminus, the disaccharide being bonded to a phosphate group through a 4' carbon of the non-reducing terminus and to a plurality of lipid groups through amide (-NH-C(O)-) and/or ester (-O-C(O)-) linkages, where the carbonyl (-C(O)-) group of the ester or amide linkage is directly linked to the lipid group, and each lipid group comprises at least 8 carbons, where the administration achieves seroconversion after the single injection. In various ~~embodiments~~examples, which may individually or in any combination further define the ~~invention~~disclosure: the composition does not include an emulsion; the adjuvant is GLA, the adjuvant is 3D-MPL.

[0012] For example, the ~~invention~~disclosure provides, in one ~~embodiment~~example, a pharmaceutical composition comprising: (a) a recombinant hemagglutinin (rHA) from a

pre-pandemic or pandemic influenza virus and (b) an adjuvant, wherein the adjuvant comprises a disaccharide having a reducing and a non-reducing terminus each independently selected from glucosyl and amino substituted glucosyl, where a carbon at a 1 position of the non-reducing terminus is linked through either an ether (-O-) or amino (-NH-) group to a carbon at a 6' position of the reducing terminus, the disaccharide being bonded to a phosphate group through a 4' carbon of the non-reducing terminus and to a plurality of lipid groups through amide (-NH-C(O)-) and/or ester (-O-C(O)-) linkages, where the carbonyl (-C(O)-) group of the ester or amide linkage is directly linked to the lipid group, and each lipid group comprises at least 8 carbons, *for use in a method of immunizing a subject in need thereof against a pre-*pandemic or pandemic influenza virus by way of a single injection of the pharmaceutical composition. The pharmaceutical composition may be used in a method of immunizing a population against a pre-pandemic or pandemic influenza virus wherein administration of the composition achieves seroconversion in at least 50%, or at least 60% or more of the population after the single injection. In one ~~aspect~~example, the pharmaceutical composition does not include any oil, i.e., is oil-free, or contains a minimal amount of oil that does not impact the seroconversion efficacy of the compositions, and does not include any oil-containing emulsion. In combination with any of these ~~embodiments~~examples, one ~~aspect~~example of the ~~invention~~disclosure is to use GLA as the adjuvant. In another ~~aspect~~example, 3D-MPL may be used as the adjuvant.

[0013] As another example, the ~~invention~~disclosure provides a pharmaceutical composition for use in a method of immunizing a subject in need thereof against a pre-pandemic or pandemic influenza virus, where the composition comprisess: (a) a recombinant hemagglutinin (rHA) from a pre-pandemic or pandemic influenza virus and (b) an adjuvant, wherein the adjuvant comprises a disaccharide having a reducing and a non-reducing terminus each independently selected from glucosyl and amino substituted glucosyl, where a carbon at a 1 position of the non-reducing terminus is linked through either an ether (-O-) or amino (-NH-) group to a carbon at a 6' position of the reducing terminus, the disaccharide being bonded to a phosphate group through a 4' carbon of the non-reducing terminus and to a plurality of lipid groups through

amide (-NH-C(O)-) and/or ester (-O-C(O)-) linkages, where the carbonyl (-C(O)-) group of the ester or amide linkage is directly linked to the lipid group, and each lipid group comprises at least 8 carbons, and wherein the composition is dosage sparing. The pharmaceutical composition for use in a method of immunizing a subject against a pre-pandemic or pandemic influenza virus, wherein the rHA is present at an amount that is dose-sparing. In various ~~embodiments~~examples, which may individually or in any combination further define the ~~invention~~disclosure: the rHA is present at a concentration that does not provide protective immunity in the absence of the adjuvant; the composition comprises a single, i.e., not more than one, recombinant protein; the amount of rHA per dose is in the range of about 15 to about 1 μg; and the adjuvant is GLA and the rH5 is from a pathogenic strain of H5N1 influenza.

[0014] These and other ~~aspects~~disclosures will become evident upon reference to the following detailed description and attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] Figures 1A-C. A single injection of rH5/GLA-SE vaccine protects mice against H5N1 infection. (A) Mice (5/group) were immunized once with 50, 150, 450, 900, or 2700 ng of rH5 (VN) formulated in 2% v/v SE emulsion or GLA-SE adjuvant (20 μg GLA) and then challenged (IN) on day 14 with H5N1 virus (1000 x LD_{50}). The mean of the maximum percentage weight loss per group was determined for each group over a two week period. Area under the curves (percent weight loss over time) was determined for each dose of rH5. (B) Percent weight loss in mice at successive days following viral challenge. Mice were vaccinated with 50 ng rH5 formulated with SE alone or with GLA-SE adjuvant, or GLA-SE or SE in the absence of protein. Each data point represents the mean weight loss per group +/- s.e.m. (C) Adjuvant-mediated rH5 protection in c57Bl/6 mice. Animals (5/group) were vaccinated once with GLA-SE alone or 50 ng rH5 (VN) formulated with 2% v/v SE alone, 5 μg GLA alone, or 5μg GLA-SE. Mice were challenged (IN) on day 14 with H5N1 Viet Nam 1203 virus (1000 x LD_{50}) and monitored for survival and weight loss. Each data point represents the mean weight loss per group +/- s.e.m.