efficiently induce humoral immune responses but generally only poor cellular immune responses. Live virus vaccines cannot be administered to immunocompromised or pregnant patients due to their increased risk of infection. Thus, a need exists for a broadly protective influenza virus vaccine.

SUMMARY

Disclosed herein is the generation of optimized H1N1 influenza HA polypeptides for eliciting a broadly reactive immune response to H1N1 influenza virus isolates. The optimized HA polypeptides were developed through a series of HA protein alignments, and subsequent generation of consensus sequences, based on selected H1N1 viruses isolated from 1918-2012.

Provided herein are recombinant influenza HA polypeptides having an optimized amino acid sequence for eliciting a broadly reactive immune response against H1N1 influenza. In some embodiments, the HA polypeptide comprises an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 or SEQ ID NO: 7. In some embodiments, the amino acid sequence of the polypeptide comprises no more than 5, no more than 6, no more than 7, no more than 8, no more than 9 or no more than 10 amino acid substitutions relative to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6 or SEQ ID NO: 7. In some embodiments, the HA polypeptide comprises SEQ ID NO: 8. In some embodiments, the influenza HA polypeptide lacks the N-terminal methionine residue.

In one aspect, there is provided a recombinant influenza hemagglutinin (HA) polypeptide, comprising an amino acid sequence: (i) at least 99% identical to SEQ ID NO: 6 or at least 99% identical to residues 2-565 of SEQ ID NO: 6; (ii) at least 96% identical to SEQ ID NO: 1 or at least 96% identical to residues 2-566 of SEQ ID NO: 1; (iii) at least 99% identical to SEQ ID NO: 2 or at least 99% identical to residues 2-566 of SEQ ID NO: 2; (iv) at least 99% identical to SEQ ID NO: 3 or at least 99% identical to residues 2-566 of SEQ ID NO: 3; (v) at least 99% identical to SEQ ID NO: 4 or at least 99% identical to residues 2-566 of SEQ ID NO: 4; (vi) at least 98.4% identical to SEQ ID NO: 5 or at least 98.4% identical to residues 2-566 of SEQ ID NO: 5; (vii) at least 97% identical to SEQ ID NO: 7 or at least 97% identical to residues 2-566 of SEQ ID NO: 7; or (viii) comprising SEQ ID NO: 8.

In one aspect, there is provided a recombinant influenza HA polypeptide, comprising: (i) no more than 6 amino acid substitutions relative to SEQ ID NO: 6 (ii) no more than 10 amino acid substitutions relative to SEQ ID NO: 1; (iii) no more than 8 amino acid substitutions relative to SEQ ID NO: 2; (iv) no more than 6 amino acid substitutions relative to SEQ ID NO: 3; (v) no more than 7 amino acid substitutions relative to SEQ ID NO: 4; (vi) no more than 9 amino acid substitutions relative to SEQ ID NO: 5; or (vii) no more than 10 amino acid substitutions relative to SEQ ID NO: 7.

Isolated nucleic acid molecules and vectors encoding the recombinant HA polypeptides are also provided by the present disclosure. Further provided are isolated cells comprising such vectors.

Also provided are influenza virus-like particles (VLPs) and fusion proteins comprising the optimized HA polypeptides disclosed herein.

Further provided are compositions that include the optimized influenza HA polypeptides, fusion proteins or VLPs disclosed herein in a pharmaceutically acceptable carrier. Methods of eliciting an immune response against influenza virus in a subject by administering the disclosed compositions, fusion proteins or VLPs is also provided by the present disclosure.

Also provided are methods of immunizing a subject against influenza virus by administering to the subject a composition comprising a VLP that contains an optimized HA polypeptide.

In one aspect, there is provided a use of the influenza HA polypeptide as disclosed herein, the fusion protein as disclosed herein, the VLP as disclosed herein, or the composition as disclosed herein in the manufacture of a medicament for eliciting an immune response to influenza virus in a subject.

In one aspect, there is provided a use of a composition comprising the VLP of any one of claims 12-14 and pharmaceutically acceptable carrier in the manufacture of a medicament for immunizing a subject against influenza virus.

The foregoing and other objects, features, and advantages of the invention will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic of the process used to generate an H1N1 HA consensus sequence according to Method X-1.

FIG. 2 is a schematic of the process used to generate an H1N1 HA consensus sequence according to Method X-2.

FIG. 3 is a schematic of the process used to generate an H1N1 HA consensus sequence according to Method X-3.

FIG. 4 is a schematic of the process used to generate an H1N1 HA consensus sequence according to Method X-4.

FIG. 5 is a schematic of the process used to generate an H1N1 HA consensus sequence according to Method X-5.

FIG. 6 is a schematic of the process used to generate an H1N1 HA consensus sequence according to Method X-6.

FIG. 7 is a schematic of the process used to generate an H1N1 HA consensus sequence according to Method A-5.

FIGS. 8A-8B show a sequence alignment of the H1N1 HA proteins set forth herein as SEQ ID NOs: 1-7.

FIGS. 9A-9F are graphs showing hemagglutination inhibition (HAI) serum antibody titers from vaccinated (week 0, 4, 12) mice against a panel of H1N1 influenza isolates. HAI titer for each vaccine group was determined at week 14 using H1N1 influenza viruses. Shown are HAI titers of mice vaccinated with VLPs containing Method X-1 HA (FIG. 9A), Method X-2 HA (FIG. 9B), Method X-3 HA (FIG. 9C), Method X-4 HA (FIG. 9D), Method X-5 HA (FIG. 9E) and Method X-

CLAIMS

1. A recombinant influenza hemagglutinin (HA) polypeptide, comprising an amino acid sequence:

(i) at least 99% identical to SEQ ID NO: 6 or at least 99% identical to residues 2-565 of SEQ ID NO: 6;

(ii) at least 96% identical to SEQ ID NO: 1 or at least 96% identical to residues 2-566 of SEQ ID NO: 1;

(iii) at least 99% identical to SEQ ID NO: 2 or at least 99% identical to residues 2-566 of SEQ ID NO: 2;

(iiiiv) at least 99% identical to SEQ ID NO: 3 or at least 99% identical to residues 2-566 of SEQ ID NO: 3;

(ivv) at least 99% identical to SEQ ID NO: 4 or at least 99% identical to residues 2-566 of SEQ ID NO: 4;

(vi) at least 98.4% identical to SEQ ID NO: 5 or at least 98.4% identical to residues 2-566 of SEQ ID NO: 5;

~~(vi) at least 99% identical to SEQ ID NO: 6 or at least 99% identical to residues 2-565 of SEQ ID NO: 6;~~

(vii) at least 97% identical to SEQ ID NO: 7 or at least 97% identical to residues 2-566 of SEQ ID NO: 7; or

(viii) comprising SEQ ID NO: 8.

2. A recombinant influenza HA polypeptide, comprising:

(i) no more than 6 amino acid substitutions relative to SEQ ID NO: 6

(ii) no more than 10 amino acid substitutions relative to SEQ ID NO: 1;

(iii) no more than 8 amino acid substitutions relative to SEQ ID NO: 2;

(iiiiv) no more than 6 amino acid substitutions relative to SEQ ID NO: 3;

(ivv) no more than 7 amino acid substitutions relative to SEQ ID NO: 4;

(vi) no more than 9 amino acid substitutions relative to SEQ ID NO: 5; or

~~(vi) no more than 6 amino acid substitutions relative to SEQ ID NO: 6; or~~

(vii) no more than 10 amino acid substitutions relative to SEQ ID NO: 7.

13. The influenza VLP of claim 12, further comprising an influenza neuraminidase (NA) protein, an influenza matrix (M1) protein, or both.

14. An influenza VLP comprising the influenza HA polypeptide of any one of claims 1-6, produced by transfecting a host cell with a vector encoding the HA polypeptide, a vector encoding an influenza NA protein and a vector encoding an influenza M1 protein under conditions sufficient to allow for expression of the HA, M1 and NA proteins.

15. A fusion protein comprising the influenza HA polypeptide of any one of claims 1-6.

16. A composition comprising the influenza HA polypeptide of any one of claims 1-6, the fusion protein of claim 15, or the VLP of any one of claims 13-15, and a pharmaceutically acceptable carrier.

17. ~~A method of eliciting an immune response to influenza virus in a subject, comprising administering~~ Use of the influenza HA polypeptide of any one of claims 1-7, the fusion protein of claim 16, the VLP of any one of claims 12-14, or the composition of claim 16 in the manufacture of a medicament for eliciting an immune response to influenza virus in a subject.

18. ~~A method of immunizing a subject against influenza virus, comprising administering to the subject~~ Use of a composition comprising the VLP of any one of claims 12-14 and pharmaceutically acceptable carrier in the manufacture of a medicament for immunizing a subject against influenza virus.

19. The ~~method~~ use of claim 18, wherein the composition further comprises an adjuvant.

20. The ~~method~~ use of claim 18 or claim 19, wherein the ~~composition~~ medicament is to be administered intramuscularly.

21. The ~~method~~ use of any one of claims 18-20, wherein the composition comprises about 1 to about 25 μg of the VLP.

22. The ~~method~~ use of claim 21, wherein the composition comprises about 15 μg of the VLP.