relative to SEQ ID NO: 4; no more than 2 amino acid substitutions relative to SEQ ID NO: 5; no more than 7 amino acid substitutions relative to SEQ ID NO: 6; no more than 10 amino acid substitutions relative to SEQ ID NO: 8; no more than 9 amino acid substitutions relative to SEQ ID NO: 9; no more than 10 amino acid substitutions relative to SEQ ID NO: 10; or no more than 6 amino acid substitutions relative to SEQ ID NO: 11. In some embodiments, the influenza HA polypeptide lacks the N-terminal methionine residue.

In one embodiment, there is provided a recombinant influenza hemagglutinin (HA) polypeptide, comprising: (i) an amino acid sequence at least 97.7% identical to residues 2-566 of SEQ ID NO: 8; (ii) an amino acid sequence at least 98.4% identical to residues 2-566 of SEQ ID NO: 9; (iii) an amino acid sequence at least 97.8% identical to residues 2-566 of SEQ ID NO: 10; or (iv) an amino acid sequence at least 98.9% identical to residues 2-566 of SEQ ID NO: 11.

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

In another aspect, there is provided a use of a composition comprising the VLP disclosed herein 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 summarizing the process for generating a H2N2 influenza COBRA HA sequence according to Method 2 as described in Example 1.

FIG. 2 is a schematic summarizing the process for generating a H2N2 influenza COBRA HA sequence according to Method 3 as described in Example 1.

CLAIMS

1. A recombinant influenza hemagglutinin (HA) polypeptide, comprising:

(i) an amino acid sequence at least 97.7% identical to residues 2-566 of SEQ ID NO: 8;

~~(ii) an amino acid sequence at least 99.6% identical to residues 2-562 of SEQ ID NO: 1;~~

~~(iii) an amino acid sequence at least 99.4% identical to residues 2-562 of SEQ ID NO: 2;~~

~~(iv) an amino acid sequence comprising residues 2-562 of SEQ ID NO: 3;~~

~~(v) an amino acid sequence at least 99.7% identical to residues 2-562 of SEQ ID NO: 4;~~

~~(vi) an amino acid sequence at least 99.6% identical to residues 2-584 of SEQ ID NO: 5;~~

~~(vii) an amino acid sequence at least 98.8% identical to residues 2-585 of SEQ ID NO: 6;~~

~~(viii) an amino acid sequence comprising residues 2-585 of SEQ ID NO: 7;~~

(~~ix~~ii) an amino acid sequence at least 98.4% identical to residues 2-566 of SEQ ID NO: 9;

(~~x~~iii) an amino acid sequence at least 97.8% identical to residues 2-566 of SEQ ID NO: 10; or

(~~xi~~iv) an amino acid sequence at least 98.9% identical to residues 2-566 of SEQ ID NO: 11.

2. The influenza HA polypeptide of claim 1, comprising:

(i) an amino acid sequence at least 97.7% identical to SEQ ID NO: 8;

~~(ii) an amino acid sequence at least 99.6% identical to SEQ ID NO: 1;~~

~~(iii) an amino acid sequence at least 99.4% identical to SEQ ID NO: 2;~~

~~(iv) the amino acid sequence of SEQ ID NO: 3;~~

~~(v) an amino acid sequence at least 99.7% identical to SEQ ID NO: 4;~~

~~(vi) an amino acid sequence at least 99.6% identical to SEQ ID NO: 5;~~

~~(vii) an amino acid sequence at least 98.8% identical to SEQ ID NO: 6;~~

~~(viii) the amino acid sequence of SEQ ID NO: 7;~~

(~~ix~~ii) an amino acid sequence at least 98.4% identical to SEQ ID NO: 9;

(~~x~~iii) an amino acid sequence at least 97.8% identical to SEQ ID NO: 10; or

(~~xi~~iv) an amino acid sequence at least 98.9% identical to SEQ ID NO: 11.

3. The influenza HA polypeptide of claim 1 or claim 2, wherein the amino acid sequence of the polypeptide comprises:

(i) no more than 10 amino acid substitutions relative to SEQ ID NO: 8;

~~(ii) no more than 2 amino acid substitutions relative to SEQ ID NO: 1;~~

~~(iii) no more than 3 amino acid substitutions relative to SEQ ID NO: 2;~~

~~(iv) no more than 1 amino acid substitution relative to SEQ ID NO: 4;~~

~~(v) no more than 2 amino acid substitutions relative to SEQ ID NO: 5;~~

~~(vi) no more than 7 amino acid substitutions relative to SEQ ID NO: 6;~~

(~~viii~~ii) no more than 9 amino acid substitutions relative to SEQ ID NO: 9;

(~~viiiiii~~) no more than 10 amino acid substitutions relative to SEQ ID NO: 10; or

(~~ix~~iv) no more than 6 amino acid substitutions relative to SEQ ID NO: 11.

4. The influenza HA polypeptide of claim 1, comprising the amino acid sequence of residues 2-566 of SEQ ID NO: 8, ~~residues 2-562 of SEQ ID NO: 1, residues 2-562 of SEQ ID NO: 2, residues 2-562 of SEQ ID NO: 4, residues 2-584 of SEQ ID NO: 5, residues 2-585 of SEQ ID NO: 6,~~ residues 2-566 of SEQ ID NO: 9, residues 2-566 of SEQ ID NO: 10 or residues 2-566 of SEQ ID NO: 11.

5. The influenza HA polypeptide of claim 1, consisting of the amino acid sequence of residues 2-566 of SEQ ID NO: 8, ~~residues 2-562 of SEQ ID NO: 1, residues 2-562 of SEQ ID NO: 2, residues 2-562 of SEQ ID NO: 3, residues 2-562 of SEQ ID NO: 4, residues 2-584 of SEQ ID NO: 5, residues 2-585 of SEQ ID NO:~~

~~6, residues 2-585 of SEQ ID NO: 7,~~ residues 2-566 of SEQ ID NO: 9, residues 2-566 of SEQ ID NO: 10 or residues 2-566 of SEQ ID NO: 11.

6. The influenza HA polypeptide of claim 2, comprising the amino acid sequence of SEQ ID NO: 8, ~~SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7,~~ SEQ ID NO: 9, SEQ ID NO: 10 or SEQ ID NO: 11.

7. The influenza HA polypeptide of claim 2, consisting of the amino acid sequence of SEQ ID NO: 8, ~~SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7,~~ SEQ ID NO: 9, SEQ ID NO: 10 or SEQ ID NO: 11.

8. An isolated nucleic acid molecule encoding the influenza HA polypeptide of any one of claims 1-7.

9. The isolated nucleic acid molecule of claim 8, wherein the nucleic acid molecule is codon-optimized for expression in mammalian cells.

10. A vector comprising the nucleic acid molecule of claim 8 or claim 9.

11. The vector of claim 10, further comprising a promoter operably linked to the nucleic acid sequence encoding the influenza HA polypeptide.

12. An isolated cell comprising the vector of claim 10 or claim 11.

13. An influenza virus-like particle (VLP) comprising the influenza HA polypeptide of any one of claims 1-7.

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