

SUMMARY

[0004] The present disclosure provides improved modified recombinant HA polypeptides with broadened immunogenic profile that extends coverage (i.e., the capability of eliciting a immunogenic response against the HA polypeptide) to antigenically distinct influenza strains, for example to new or additional pandemic and/or seasonal influenza strains. The present disclosure is, in part, based on modifications deduced from *in silico* analysis of sequence variation among circulating influenza strains, mapping of the antigenic region of HA, and/or epitope patterns and structural analyses of the HA peptide. Targeted modifications can be subsequently introduced at various amino acid residue locations and/or specific regions of an HA polypeptide with a known immune profile to yield novel HA polypeptides with improved and more balanced immune profiles. As described in detail below including the Examples section, the present inventors have developed -among other things- various distinct strategies for engineering HA polypeptides to extend a seasonal response profile to cover pandemic strains, or vice versa. These strategies extend the immune profile across clusters of sequences (or clades) of antigenically distinct strains; they can be applied to an engineered recombinant HA molecule over time so that it continues to elicit an immune response against antigenically drifted circulating seasonal strains. In all cases, the strategy is designed to generally preserve specific residues of the receptor binding site (RBS) of a host HA polypeptide with modifications engineered in the region near the RBS. Similar strategies may be used to extend a pandemic response profile to cover seasonal strains. The compositions and methods of the present disclosure are applicable to a wide variety of recombinant HA polypeptides, including those engineered by a variety of methods and those comprising substantially wild-type sequences.

[0004a] In one aspect, the present disclosure provides a method of altering the immunogenic profile of an engineered hemagglutinin (HA) polypeptide, comprising:

- a) identifying the presence or absence of one or more putative N-linked glycosylation sites in a head region of an engineered HA polypeptide, wherein the head region is a segment of the engineered HA polypeptide at positions corresponding to residues 63-278, 125-277 or 135-269 of SEQ ID NO: 1 according to California 09 Numbering (CA09 Numbering);
- b) introducing into the head region of the engineered HA polypeptide one or more amino acid substitutions, deletions or insertions to disrupt the one or more putative N- linked glycosylation sites or insert additional N-linked glycosylation sites

; and c) introducing one or more amino acid substitutions in the region of or adjacent to the Receptor Binding Site (RBS) region of the engineered HA polypeptide, and/or introducing an insertion of a Lysine (K) or Arginine (R) residue into a loop or loops bounding the RBS of the engineered HA polypeptide, thereby generating a re-engineered HA polypeptide with altered immunogenic profile as compared to the engineered HA polypeptide.

[0005] Thus, in one example, the present disclosure provides a recombinant hemagglutinin (HA) polypeptide comprising an engineered head region or segment thereof derived from an engineered HA polypeptide with a predominantly seasonal immune profile and a stem region derived from a pandemic strain. In some examples, the engineered head region or segment thereof comprises a sequence at least 95%, 96%, 97%, 98%, 99% or more identical to amino acids corresponding to positions 135 – 269, 125 – 277, or 63 – 278 of SEQ ID NO:1. In some examples, the engineered head region or segment thereof comprises a sequence identical to amino acids corresponding to positions 135 – 269, 125 – 277, or 63 – 278 of SEQ ID NO:1 (SMARt_DO2a sequence).

[0006] In some examples, the stem region is derived from a naturally-occurring or wild-type pandemic strain. In some examples, a suitable naturally-occurring pandemic strain is selected from A/California/07/2009, A/New Jersey/10/1976, or A/South Carolina/1/1918. In some examples, the stem region is derived from an engineered HA polypeptide that has a pandemic immune profile.

[0007] In some examples, the engineered HA polypeptide that has a pandemic immune profile is engineered by a computationally optimized broadly reactive antigens (COBRA) technology, a mosaic technology, consensus-based combinations of influenza strains, deletion and/or rearrangement of structural domains, domain swapping, or combinations of neutralizing or cross-reactive epitopes among multiple influenza strains.

[0008] In another example, the present disclosure provides a recombinant influenza hemagglutinin (HA) polypeptide comprising an engineered head region derived from an HA polypeptide with a predominantly seasonal immune profile and comprising one or more amino acid substitutions, deletions or insertions at one or more putative N-linked

CLAIMS

We claim:

1. A method of altering the immunogenic profile of an engineered hemagglutinin (HA) polypeptide, comprising:
 - a) identifying the presence or absence of one or more putative N-linked glycosylation sites in a head region of an engineered HA polypeptide, wherein the head region is a segment of the engineered HA polypeptide at positions corresponding to residues 63-278, 125-277 or 135-269 of SEQ ID NO: 1 according to California 09 Numbering (CA09 Numbering);
 - b) introducing into the head region of the engineered HA polypeptide one or more amino acid substitutions, deletions or insertions to disrupt the one or more putative N-linked glycosylation sites or insert additional N-linked glycosylation sites; and
 - c) introducing one or more amino acid substitutions in the region of or adjacent to the Receptor Binding Site (RBS) region of the engineered HA polypeptide, and/or introducing an insertion of a Lysine (K) or Arginine (R) residue into a loop or loops bounding the RBS of the engineered HA polypeptide, thereby generating a re-engineered HA polypeptide with altered immunogenic profile as compared to the engineered HA polypeptide.
2. The method of claim 1, wherein the re-engineered HA polypeptide comprises a Lysine (K) or Arginine (R) residue inserted within 1-5 amino acids of the NxS/Ty consensus sequence.
3. The method of claim 1 or 2, wherein the RBS comprises all amino acids residues within 15 angstroms of a position corresponding to residue W167 according to CA09 Numbering in a three-dimensional (3-D) structure.
4. The method of claim 2 or 3, wherein the RBS comprises the epitope bound by the paratope of monoclonal antibody CH65.
5. The method of any one of claims 1-4, wherein the one or more putative N-linked glycosylation sites or additional N-linked glycosylation sites are defined by a consensus sequence of NxS/Ty, wherein x and y are not proline (P).
6. The method of claim 1, wherein the one or more putative N-linked glycosylation sites are at positions corresponding to positions 142-145 and/or 177-179 of SEQ ID NO: 1 according to CA09 Numbering.

7. The method of any one of claims 1-3, wherein the one or more amino acid substitutions, deletions or insertions to disrupt the one or more putative N-linked glycosylation sites comprise modifying the consensus sequence NxS/Ty to z1z2z3z4 wherein
- z1 is N, D, K or S;
 - z2 is Y or is unchanged;
 - z3 is E, D or N; and
 - z4 is I, L, P, S or T, or is unchanged.
8. The method of any one of claims 1-7, wherein the immunogenic profile of the re-engineered HA polypeptide is more pandemic than the engineered HA polypeptide.
9. The method of any one of claims 1-7, wherein the immunogenic profile of the re-engineered HA polypeptide is more seasonal than the engineered HA polypeptide.
10. The method of any one of claims 1-9, wherein the re-engineered HA polypeptide comprises one or more amino acid substitutions, deletions or insertions at positions corresponding to residues 137, 144, 145, 154, 155, 156, 157, 158, 159, 177, 210, 211, 212, 213, 214, 244, 245 and/or 262 according to CA09 Numbering.
11. The method of any one of claims 1-10, wherein the re-engineered HA polypeptide comprises one or more amino acid substitutions, deletions or insertions at positions corresponding to residue 137, 144, 145, 154, 155, 156, 157, 158, 159, 177, 210, 211, 212, 213 and/or 214 according to CA09 Numbering.
12. The method of any one of claims 1-11, further comprising assessing expression and conformation of the re-engineered HA polypeptide.
13. The method of any one of claims 1-12, further comprising determining if the re-engineered HA polypeptide elicits neutralizing antibodies against both seasonal and pandemic strains of influenza virus.
14. The method of any one of claims 1-13, wherein the altered immunogenic profile of the re-engineered HA polypeptide comprises increasing the binding of one or more anti-head monoclonal antibodies against seasonal and/or pandemic influenza strains.

15. The method of any one of claims 1-14, wherein the altered immunogenic profile of the re-engineered HA polypeptide comprises increasing the breadth of binding of anti-head monoclonal antibodies against pandemic influenza strains.
16. The method of claims 14 or 15, wherein increases in binding are determined by flow cytometry detection of the monoclonal antibodies bound to the re-engineered HA polypeptides expressed on the surface of mammalian cells.
17. The method of claim 16, further comprising quantitating the level of monoclonal antibody binding.
18. The method of claim 1, wherein the engineered HA polypeptide is engineered by a computationally optimized broadly reactive antigens (COBRA) technology, a mosaic technology, deletion and/or rearrangement of structural domains, domain swapping, or combinations of neutralizing or cross-reactive epitopes among multiple influenza strains.