

MODIFICATION OF ENGINEERED INFLUENZA HEMAGGLUTININ POLYPEPTIDES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application 62/345,502 filed on June 3, 2016, the entirety of which is hereby incorporated by reference.

SEQUENCE LISTING

[0001.1] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on August 4, 2017, is named SPR-003WO_SL.txt and is 121,848 bytes in size.

BACKGROUND

[0002] Influenza has a long standing history of pandemics, epidemics, resurgences and outbreaks. Vaccines have been the most effective defense against influenza. However, the effort to design and manufacture vaccines that induce strain-specific immunity year-over-year has been difficult as influenza continues to cause significant health problems across the globe. Indeed, currently marketed influenza vaccines must be updated annually based on predicted strains that will be present in human populations in the impending season.

[0003] Current influenza vaccines are based on inducing immunity to the hemagglutinin antigen present on the surface of influenza viruses. Hemagglutinin (HA) is a glycoprotein responsible for the binding of the influenza virus to cells through interaction with sialic acid-containing structures on their membranes. It is highly variable across influenza virus strains due to on-going mutation of the virus and immune pressure by the host. Variability (also known as antigenic drift) in the HA molecule results in a HA polypeptide-based vaccine generally reactive only to a small subset of related circulating viruses. Over time, the number of cross-reactive strains decreases as the virus continues to mutate. Consequently, the HA compositions of influenza vaccines are modified on regular basis when the variation in the HA molecule is such that the existing vaccine is no longer effective. Among the current strategies for vaccination against influenza, the development of a universal vaccine holds the promise to increase the breadth of current strain-specific vaccines and tolerance to antigenic drift. Universal influenza vaccines have the potential to protect humans and animals against a broad range of influenza types, subtypes and strains, including pandemic and/or seasonal strains. Several approaches to designing universal influenza antigens are known in the art. However, the existing approaches often result in an HA polypeptide that remains biased towards seasonal or pandemic strains.

[0040] **Figure 6** shows a graphical representation of the putative N-Linked glycosylation sites (SEQ ID NOS 32-35, respectively, in order of appearance), lysine loop insertion sites (SEQ ID NOS 36-37, respectively, in order of appearance) and modification of residues around the RBS site.

[0041] **Figure 7** shows flow cytometry assay results demonstrating the proper folding and expression of recombinant HA polypeptides generated by modifications to putative N-linked glycosylation sites, lysine loop insertions, or amino acid residues in the globular head region.

[0042] **Figure 8** shows flow cytometry assay results demonstrating the increased immunological breadth of recombinant HA polypeptides generated by modifications to putative N-linked glycosylation sites, lysine loop insertions or amino acid residues in the globular head region.

[0043] **Figure 9** shows a graphical representation of amino acid residues in the globular head region that can be modified to alter the immunological breadth of a recombinant HA polypeptide.

[0044] **Figure 10** shows flow cytometry assay results demonstrating the increased immunological breadth of recombinant HA polypeptides demonstrated by a gain of 4K8 binding to de-glycosylated constructs.

[0045] **Figure 11** shows an exemplary timeline for immunizations and subsequent *in vivo* evaluation.

[0046] **Figure 12** shows a representative Kaplan-Meier survival curve of animals immunized with next generation DO2a modifications compared to original SMARTDO2a.

[0047] **Figure 13** shows representative weight loss curves of animals immunized with next generation DO2a modifications compared to original SMARTDO2a.

[0048] **Figure 14** shows representative viral lung titers of animals immunized with SMARTDO2a constructs compared to PBS on day 4 post viral challenge.

[0049] **Figure 15** shows a representative hemagglutination inhibition (HAI) assay results of serum from animals immunized with SMARTDO2a constructs.

Table 9

construct	Modification	Residue indices (CA09 numbering)	Original sequence of engineered HA (SMART_DO2a)	Modified sequences
1	E137T (charge change)	137	E	T
	antigenic site Ca	154-159	SHNGKS (SEQ ID NO: 28)	PHAGAK (SEQ ID NO: 29)
	antigenic site Sa	210-214	HTEN (SEQ ID NO: 30)	QNAD (SEQ ID NO: 31)
	N262T	262	N	T
2	antigenic site Ca	154-159	SHNGKS (SEQ ID NO: 28)	PHAGAK (SEQ ID NO: 29)
3	antigenic site Sa	210-214	HTEN (SEQ ID NO: 30)	QNAD (SEQ ID NO: 31)
4	E137T (charge change)	137	E	T
	Ngly142	144-145	TV	NT
	antigenic site Ca	154-159	SHNGKS (SEQ ID NO: 28)	PHAGAK (SEQ ID NO: 29)
	Ngly177	177	N	T
	antigenic site Sa	210-214	HTEN (SEQ ID NO: 30)	QNAD (SEQ ID NO: 31)
	N262T	262	N	T
5	Ngly142	144-145	TV	NT
	antigenic site Ca	154-159	SHNGKS (SEQ ID NO: 28)	PHAGAK (SEQ ID NO: 29)
	Ngly177	177	N	T
6	Ngly142	144-145	TV	NT
	Ngly177	177	N	T
	antigenic site Sa	210-214	HTEN (SEQ ID NO: 30)	QNAD (SEQ ID NO: 31)

Example 4. *In vivo* efficacy of engineered HA polypeptides

[0194] This Example illustrates that engineered HA polypeptides modified in accordance with methods described herein elicit immune responses in the form of broad antibody responses against several influenza strains.

Preparation of virus-like particles (VLPs) containing engineered mosaic hemagglutinins (HAs)

[0195] Influenza VLPs are prepared by three-plasmid transient transfection of HEK293T cells in serum-free Freestyle293 medium. Plasmids encoding engineered HA polypeptide sequence as well as those for NA, and HIVgag are mixed at 1:1:1 ratio and used