Team Mutate: An mRNA Approach to Influenza Vaccines

W. Acquah, C. Amini, S. Buddula, M. Chen, N. Chintala, Q. Dang, N. Ferziger, G. Hollis, D. Jameison,
J. Jayaram, J. Manus, J. Rosenberg, J. Zhiteneva
Mentor: Stephanie A. Yarwood, PhD

UNIVERSITY OF MARYLAND HONORS COLLEGE

GEMSTONE Honors College University of Maryland

Objectives

Influenza affects millions of people each year. mRNA vaccines are a promising new method to protect populations. Eleven conserved influenza proteins were evaluated for their likelihoods as a vaccine target. Our team is currently writing a review paper advocating for the use of some of these targets in future mRNA influenza vaccines.

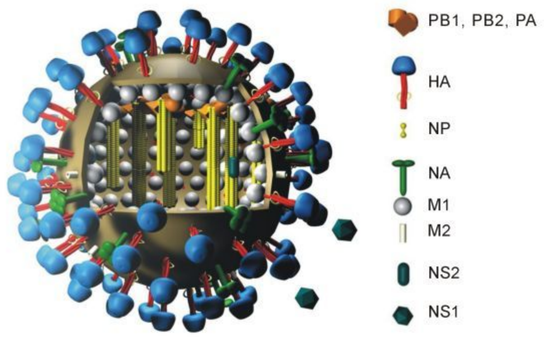

Figure 1: Influenza A virus structure and proteins. Image by Dr. Markus Eickmann.

Background

Current influenza vaccines target the head of the hemagglutinin protein. This protein is subject to high mutation rates and antigenic drift, meaning that the public must obtain an influenza vaccine every year. A more conserved protein target could increase immunogenicity of influenza vaccines. Combining a new protein target with new mRNA vaccines in light of COVID-19 could greatly improve future flu shots.

New mRNA vaccines would be better than current influenza vaccine, because they:
*Can be manufactured faster[1]
*Can be safer than other vaccine types[2]
*Can induce a greater CD4+ and CD8+ T-cell responses[3]

Methods

We investigated 11 influenza virus proteins: the hemagglutinin head (HA1), the hemagglutinin stalk (HA2), neuraminidase (NA), matrix 2 ectodomain (M2e), polymerase basic 1 (PB1), matrix 1 (M1), nucleoprotein (NP), polymerase acidic (PA), polymerase basic 2 (PB2), nonstructural 1 (NS1), and nonstructural 2 (NS2).

We used a standardized set of search terms for all proteins on two different databases: Google Scholar and PubMed. Based on our searches, the proteins were categorized as primary (green), secondary (yellow), and tertiary proteins (white). Primary proteins have been highly researched and show the most promise in a broadly protective vaccine, secondary proteins have been subject to smaller amounts of research, and tertiary proteins have the lowest amount of research.

Future Directions

Our final review paper will be submitted for publication by July 2021.

Future experiments based on our work may include creating an mRNA vaccine targeting the most conserved influenza A protein and testing its efficacy in vitro, in vivo, and in clinical trials.

Sources

Protein (primary, secondary, tertiary)	Size (amino acids AA & kiloDaltons kDa)	Quantified degree of Conservation	Individual Immunogenicity (IFN-γ and % elicited)	Immunogenicity in combination	Types of vaccines/delivery methods studied
Hemagglutinin head (HA1)	327AA (H1N1)[4]; 55 kDa[5]	Amino acids: $HA_1$98, −134, −138, −153, −183, and b−195 are conserved amongst different strains[6]	Multiple sites studied → receptor binding site seems most promising due to high conservation Antibody CH65 neutralizes infectivity of 30/36 H1N1 strains[7]	N/A	• Live-attenuated • mRNA • DNA • Inactivated
Hemagglutinin stalk (HA2)	222 AA (H1N1)[4]; 25 kDa[8]	AAs 1–11 (5%) are conserved[6]	Chimeric HA immunogens shows promise[9]	N/A	• Inactivated • DNA • mRNA
Neuraminidase (NA)	200 kDa[10]; 469 AA[11]	AAs 222-230 (2%) (N2 based) are conserved[12]	Little research has been performed on solitary NA. However, low levels of broadly protective titers have been found in humans[13,14]	N/A	• Live attenuated • Inactivated • Recombinant protein • Recombinant subunit VLP • Nanoparticles • DNA
Matrix 2 ectodomain (M2e)	23 AA; 2.5 kDa[15]	39% (AAs 1-9) nearly identical among all strains. 38% (5 AAs) in the M2e region are highly conserved[16]	Very low due to small size	Can be combined with domains from HA, NP, and other proteins, and possibly other viruses as carriers	• Recombinant protein • Recombinant VLP • Nanoparticles • Inactivated • DNA
Polymerase basic 1 (PB1)	757 AA; 87 kDA	98% AAs conserved across human influenza A	7.6-fold in IFN-gamma secretion (Goodman et al) 100% survival in murine model (Kosik et all)	can be combined with NP and M1 to elicit greater response	• DNA • Recombinant vaccinia virus • Live attenuated • Inactivated
Matrix 1 (M1)	252 AA; 28 kDa	Nearly 67%[17] of AAs are conserved, including key AAs: 76-78, 91-105, 181-193	• 100ug DNA + 100ug M1 gave 100%; IFN of ~320 pg/ml[18] • 5 vaccinations with M1 and 50ug DNA 5/20 (lethal dose challenge); IFN of ~130 spots/10^ cells[19]	• IFN of DNA +rVV ~10^5 80% survived[20] • MVA-NP+m1/MVA-NP+m1 gave 150 spot forming units per million cells[21] • IFN ~ 1200 SFC per 10^6 PMBC (tested on 6 people)[22]	By itself: DNA prime subunit, expression plasmids[18] In combination: DNA plasmid and recombinant vaccinia virus[20] modified vaccinia virus Ankara and adenovirus[21]
Nucleoprotein (NP)	498 AA; 53 kDA	59% of AAs are highly conserved.	92.9-98% immunogenicity in human epitopes of influenza A virus	Combination NP/PB1/M1 vaccines provided complete or partial protection in mice. Of the three antigens, NP-based vaccines exhibited the greatest protective effect.	• DNA
Polymerase acidic (PA)	716 AA; 83 kDa	85-93% conserved in swine strains (influenza A) and 91-94% conserved in avian strains (influenza A) [26]	Viral titers in early viral replication were lower than wild-type; CEN activity inhibited by BXA in influenza A and B and antiviral potency [27]	PA acts cohesively with PB1 and PB2 in transcription and viral replication [25]	• Cell-based
Polymerase basic 2 (PB2)	759 AA; 86 kDa	42 total AAs are 100% conserved, but scattered throughout PB2 sequence [23]	100% Survival rate with VX-787 with an efficacy quotient of 9.28[24]	Some residues (1–269 and 580–683 segments) can be combined with NP.	• Live-attenuated • Cell-based
Nonstructural 1 (NS1)	281 AA; 26 kDa	51% AA conservation across over 95% of influenza A isolates	Vaccines containing mutated NS1 proteins elicited higher than average IFN responses	N/A	• Cell-based (vero cells) • Live attenuated
Nonstructural 2 (NS2)	121 AA; 14 kDa	93.4% AA conservation across all influenza A strains[29]	N/A	N/A	• Live-attenuated (hypothetical)[30] • Cell-based (hypothetical)[31]