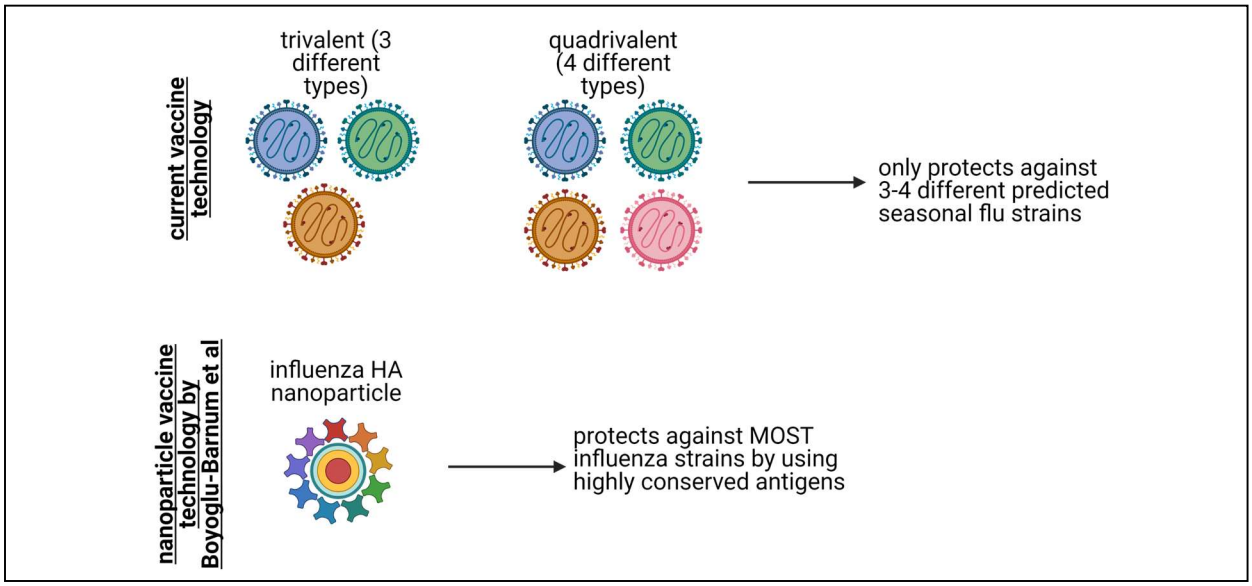
Making a better flu vaccine to provide broader protection against multiple flu strains
by Brendan Finicle

Recent advances in 2021 by immunologists at National Institutes of Allergy and Infectious Diseases in Bethesda, Maryland have produced a novel flu vaccine candidate that could provide more broad protection against the flu than the conventional seasonal vaccines that are currently on the market.



The flu can be a serious disease that can send you and your loved ones to the hospital and can sometimes even lead to death. On average, about 8% of the population in the United States gets sick from flu each season. As such, hundreds of thousands of people are hospitalized and tens of thousands die from the flu every year. As with many viral borne diseases, the best way to protect you, your family, and your community from the flu is to get vaccinated! Vaccination has been shown to not only reduce the risk of contracting the flu, but also, if infected, to protect from severe illness and to reduce the risk of spreading the virus to others in your community. However, the current flu vaccine technology only protects against the strains that are predicted by immunologists to be dominant each season.

To understand how flu vaccines work, we must first understand the viruses themselves. There are two types of influenza viruses that produce seasonal illnesses in humans: type A and type B. Within each of these types include multiple subtypes that arose due to mutagenesis during successive rounds of replication in humans. The subtypes of influenza A viruses are divided based on what viral proteins are present on the outside of the virus. We have all heard of the 2009 H1N1 pandemic. The H1N1 virus is an influenza A virus that is identified due to the presence of a type 1 HA protein and a type 1 NA protein on the surface of the virus, hence H1N1. Because there are 18 different types of HA proteins and 11 different types of NA proteins, there are potentially 198 different subtypes of influenza A that could exist. Currently, only 131 subtypes have been identified in human populations. Influenza B viruses are different from the A type because they change more slowly and therefore there are much fewer subtypes that exist in human populations. Therefore, only two different lineages of influenza B viruses are tracked:

B/Yamagata and B/Victoria. Every year, different influenza types and subtypes become dominant therefore producing seasonal variation. In summary, there are many types of influenza viruses which can complicate therapeutic intervention by applying vaccines to prevent illness in human populations.

In the United States, current flu vaccines consist of either inactivated or live attenuated influenza viruses. To increase the number of strains that the vaccine can protect against, most inactivated vaccines consist of split viruses or multiple antigens. There are trivalent vaccines available that protect against two predicted influenza A strains and one predicted influenza B strain. There is also a quadrivalent vaccine that adds an additional B strain. Both of these vaccines must be reformulated annually based on the prevalent circulating strains in each season. Predicting the dominant strains each season can be challenging. Just as all vaccines do, the flu vaccines stimulate our bodies to produce molecules called antibodies that recognize and neutralize virus particles and infected cells in our body. Because the flu vaccines are made from inactivated or live attenuated virus particles, the antibodies produced in response to these vaccines only offer protection against the viruses used to make the vaccine. Importantly, these current vaccines that are on the market are designed to produce antibodies that target the head of the H protein. This head of the H protein is frequently mutated and changes frequently between each influenza strain and subtype. Therefore, new vaccines that are designed to target more conserved regions of the H protein could offer more broad protection and could eliminate the need to reformulate vaccines annually for each season.

In 2021, scientists working at the National Institute of Allergy and Infectious Diseases have made a strong step forward to meeting the goal of developing a supraseasonal flu vaccine that would not need to be re-developed annually. By combining the novel technology of nanoparticles with recombinant protein technology, scientists developed a flu vaccine candidate that could incorporate 20-different combinations of the HA protein found on the influenza virus surface. In essence, this flu vaccine candidate not only could protect against the 4 main different influenza types and subtypes (two different A types and two different B types), but also could produce immune responses targeted against influenza viruses whose HA proteins were not used in the vaccine formulations. This could happen because the vaccine nanoparticle also exposed regions of the HA protein that are highly conserved in all influenza strains and types. In summary, the scientists at the NIAID developed flu vaccine candidates that could protect mice from a wide variety of seasonal and pandemic influenza strains. Currently, this flu vaccine nanoparticle is being advanced toward phase I clinical trials. If the same immune responses seen in mice and other animals are also seen in humans, then this new vaccine nanoparticle could provide protection against nearly all influenza strains without the need to reformulate the vaccine annually unlike current flu vaccine technology.

In summary, this novel flu vaccine nanoparticle technology could eliminate the conventional seasonal vaccines for influenza and therefore provide a supraseasonal influenza vaccine option. This same vaccine nanoparticle technology could also be used to combat other viruses and potentially even develop vaccines against cancer.

Quadrivalent influenza nanoparticle vaccines induce broad protection

Lily Li

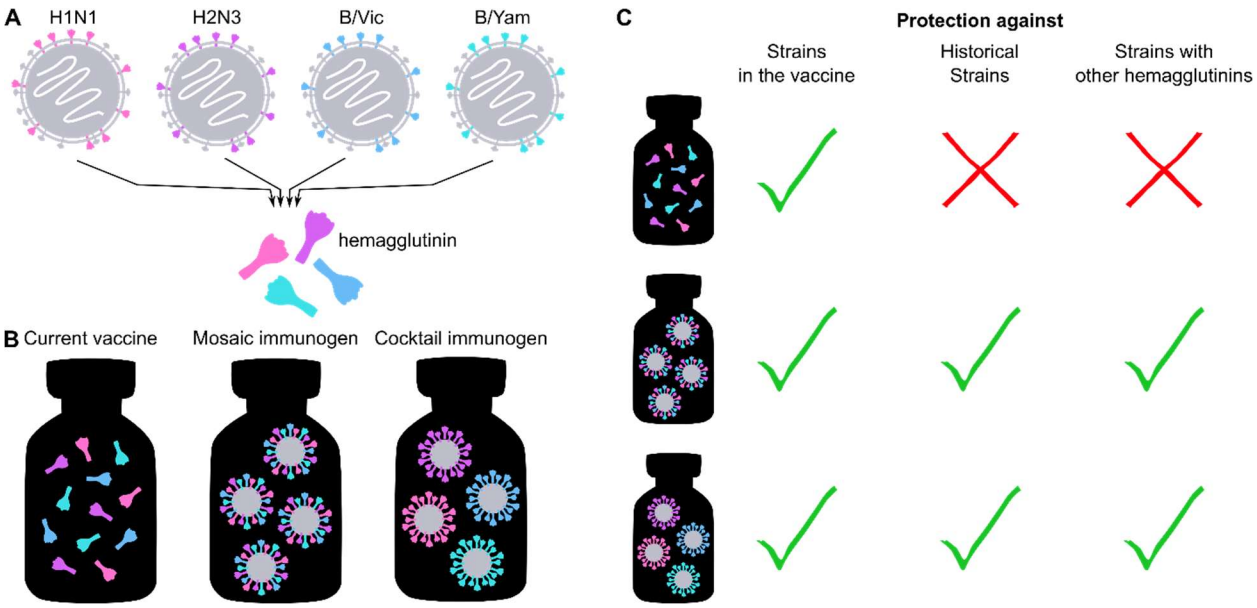


Figure 1. Improvements on the current flu vaccine. (A) The current flu vaccine depends on the identification of the most pathogenic and transmissible strains of flu for that year, and most flu vaccines target the head of hemagglutinin proteins. **(B)** Compared with the current flu vaccines which are a cocktail of the hemagglutinin proteins of the four strains of flu of each variety (H1N1, H2N3, B/Vic, and B/Yam), Boyoglu-Barnum et al. created self-assembling nanoparticle scaffolds which will display multiple hemagglutinin proteins at once. They created two versions, one mosaic version that has equal numbers of each hemagglutinin protein, and a cocktail version that contains four immunogens, each with only one type of hemagglutinin protein. **(C)** The mosaic and cocktail nanoparticle immunogens produce protection against the strains chosen to make the vaccine, historical viral strains, as well, as strains with other hemagglutinins (e.g. H5N1, H7N9, etc.) unlike the current flu vaccine, which mainly gives protection against the strains used to make it.

Introduction

Despite the wide availability of vaccines, the flu (combined with pneumonia) is ranked as the 9th leading cause of death in the United States.[1] And globally, the flu kills around 650,000 people each year. The current vaccines provide from 10-60% protection against symptomatic infection, depending on the year, and must be remade each year based on the prediction of the strains most likely to cause disease. Consequently, scientists have been working towards a "universal" vaccine that would provide broad protection against many flu strains and thus would hopefully need to be reformulated and administered less often.

A primer on the influenza virus and current vaccines

There are four types of flu virus (A, B, C, and D); however, the two that are responsible for seasonal epidemics are A and B.[2] Influenza A virus (IAV) has two main proteins that are present on the surface of the viral envelope, hemagglutinin (H) and neuraminidase (N). These proteins play crucial roles in recognizing, binding, and infecting cells and lysing the infected host cells, respectively. As these are the proteins that a host's immune cells can bind to and thus trigger an immune response, they are also the target of flu vaccines. While there exist 18 types of hemagglutinin and 11 of neuraminidase, only H1-3 and N1-2 are commonly found in humans. Currently, the two that routinely circulate in humans are H1N1 and H3N2, and thus strains of each of these are included in the flu vaccine each year. The other main flu virus, influenza B virus (IBV), is divided into two lineages, B/Yamagata and B/Victoria. IBVs tend to evolve more slowly than IAVs.

Every year, the World Health Organization (WHO) selects three or four strains that are predicted to cause the most illness during that flu season based on which strains of flu virus are causing illness, how much they are spreading, and how well the previous season's vaccine protects against these strains. These strains include one influenza A(H1N1), one influenza A(H2N3), and one or two of the influenza B strains. Including both lineages of IBV has only been possible since 2012 when quadrivalent influenza vaccines (i.e. containing four flu strains) were approved.[3]

The effectiveness of these vaccines varies widely from 10-60% protection against symptomatic infection depending on successful prediction of the most highly pathogenic and transmissible strains circulating that year. Even with accurate prediction of these strains, vaccine effectiveness can be undermined by small mutations in the virus or in the vaccine virus strains during vaccine manufacture. As a result, scientists have been long desirous of developing a universal flu vaccine, which would ideally provide life-long protection against all current and future strains of seasonal and pandemic flu.

To approach this goal, scientists are targeting more highly conserved portions of the viral epitope, or the part of the protein that the immune system recognizes. The hemagglutinin protein is one of these epitopes. It looks like a broccoli floret, with a largely exposed head and a more hidden stem. Current vaccines focus on targeting this head, as it produces a stronger immune response; however, this head is constantly evolving. The stem, on the other hand, is more conserved but produces a weaker response. Focusing on this stem may be able to produce more universal immune protection. [3]

Improving vaccine effectiveness by designing scalable methods of displaying diverse epitopes

One method by which scientists have improved stem-targeted immune responses has involved displaying more than one identical epitope per molecule, or multivalent presentation of these epitopes. This appears to increase the production of epitope-specific antibodies. These epitopes are often put on protein scaffolds that mimic the shape of the virus because this triggers a stronger immune response. However, not all protein scaffolds are amenable to displaying these epitopes. To solve this problem, scientists have developed artificial scaffolds that self-assemble into various shapes (e.g. tetrahedral, octahedral, and icosahedral) to optimize the presentation of the ectodomain. [4] This is the extracellular domain of a protein that usually interacts with surfaces and leads to signal transduction. Here, this would be the ectodomain

of the hemagglutinin proteins, but another good example is the spike protein of the now well-known SARS-CoV-2.

Here, Boyoglu-Barnum et al. created two versions of these nanoparticle immunogens or artificial scaffolds with hemagglutinin ectodomains.[5] They took the ectodomains of the four strains chosen for the 2017-2018 season flu vaccine—H1 (from the H1N1 strain), H3 (from the H3N2 strain), B/Vic, and B/Yam from the two influenza B virus strains—and fused each to the N-terminal of one component of the icosahedral nanoparticle. By mixing equimolar amounts of these four hemagglutinins with the nanoparticle pentamer, they created a mosaic nanoparticle immunogen that displays all four hemagglutinins at the same time. As icosahedrons have 20 faces, each of these mosaic immunogens displays 20 hemagglutinins in total, five of each strain. They also created a cocktail immunogen that consists of equimolar amounts of four nanoparticle immunogens, each of which only displays one strain of hemagglutinin.

Effectiveness of this new strategy against different strains and viruses

They compared the immune response after inoculation with these nanoparticle immunogens and with that after inoculation with the commercial 2017-2018 flu vaccine, making sure that the total protein dose of each treatment was equivalent. Mice, ferrets, and macaques were immunized with these three vaccines, and their immune response was evaluated with two assays—a hemagglutination inhibition and microneutralization titer.

The hemagglutination inhibition assay assesses the ability of the antibody to inhibit virus-receptor interaction. It takes advantage of the fact that some viruses, like the flu virus, bind, or hemagglutinate, red blood cells, forming a lattice and preventing clumping. That means that higher concentrations of the virus, which leads to less clumping, will appear like a diffuse reddish color in contrast to a distinct pellet at lower concentrations. Thus, one can estimate the effectiveness of immune response by testing a serial dilution of immune serum incubated with the virus and then incubated with red blood cells.

A microneutralization titer measures the neutralizing activities of antibodies in animal sera against the flu. They developed viruses in which a key component was replaced with a fluorescent reporter gene. The immune serum is mixed with these reporter viruses, and the neutralizing antibodies in the serum can react. This serum-virus mixture is then inoculated into MDCK cells, a model mammalian cell line. The next day, the number of fluorescent, or virus-infected, cells is counted.

Both assays showed that the antibody responses induced by the nanoparticle immunogens were similar or better than those induced by the standard flu vaccine. Similar results were produced when the vaccines were administered without adjuvant, a substance that increases the efficacy or potency of drugs, or when updated versions of all three vaccines using the 2018-2019 vaccine strains were used.

They then tested these vaccines against historical strains of H1N1 and H3N2 viruses dating from 1943 to 2013. The nanoparticle immunogens performed equivalently or better than the standard flu vaccine. This difference was particularly notable against the H3N2 strains tested. They then looked at survival of immunized mice against lethal challenges of H1N1 and H3N2 strains that were not represented in the

vaccine. In all cases, the mosaic immunogen led to the most survival, followed by the cocktail immune and finally the standard flu vaccine. With adjuvant, the nanoparticle immunogens both provided perfect or near-perfect protection. Without adjuvant, the mosaic immunogen provided 90% and 50% protection against the mismatched H1N1 and H3N2 strain, respectively; whereas, the cocktail immunogens provided partial protection (~50%) against both strains. In contrast, the standard flu vaccine provided partial protection (~65%) against both strains with adjuvant and only ~10% protection without. These results suggest that these nanoparticle immunogens have the potential of providing broad protection against flu strains across multiple seasons.

They then tested these vaccines against influenza A viruses with hemagglutinins that are not represented in these vaccines (H5N1, H6N1, H7H9, H10N8). The nanoparticle immunogens showed cross-reactive antibody responses to the hemagglutinins from all of these viruses, whereas the standard flu vaccine showed negligible responses. To determine if these cross-reactive responses conferred protection, they immunized mice with these three vaccines and then infected them with H5N1 or H7N9 and tracked their survival. While the mosaic immunogen provided near-perfect protection against both with or without adjuvant, the cocktail immunogen provided partial protection (85% and 50%) against H5N1 and H7N9 infection, respectively. The standard flu vaccine provided negligible protection. These results were also replicated in ferrets.

To test whether the antibodies produced in response to these vaccines in the immune sera were sufficient to provide protection, they inoculated mice with purified immunoglobin from the immune sera of macaques who were immunized with these three vaccines. The immune sera from both the nanoparticle immunogens protected mice against weight loss and disease; however, that from the standard flu vaccine did not, leading to significant weight loss and only partial protection against disease.

To test how the deliberate geometric display of the hemagglutinin on the nanoparticle scaffolds affected antibody generation, they immunized mice with a non-assembling version of these immunogens. Interestingly, these produced similar results from microneutralization assays for the viral strains used in the vaccines but orders of magnitude lower cross-reactive antibody response to H5N1 and H7N9 hemagglutinins.

Molecular basis of this vaccine's success

Both the nanoparticle immunogens produced more stem-directed antibodies than the standard flu vaccine though most of their neutralizing activity is due to target epitopes in the hemagglutinin heads, as despite their variability, they provide the strongest immune response. Interestingly, the ability of the nanoparticle immunogens to induce neutralization of the H5N1 virus is dependent on stem-directed antibodies. This was confirmed by visualizing these immunogen-induced antibodies.

Scientists have previously found that the first flu virus that you become infected with affects all your future immune responses to immune challenge by other flu strains, so they tested how a dose of the updated 2018-2019 versions of these nanoparticle immunogens affected the immune response of macaques previously immunized with the 2017-2018 versions. The macaques had maintained high levels

of neutralizing antibodies against the strains in the vaccine, and these antibodies were boosted by the new vaccine. While the standard flu vaccine produced few neutralizing antibodies targeting the hemagglutinin stem, the nanoparticle immunogens maintained high levels of these antibodies, which were also boosted by the new vaccine. This suggests that the nanoparticle immunogens can induce strong stem-targeted immune responses despite there already being strong head-targeted antibodies.

Conclusion

These nanoparticle immunogens can provide broad protection against many strains of flu, and this protective ability is due to the presentation of the hemagglutinins of interest on these geometric scaffolds that expose the more highly conserved stem of the hemagglutinin as a target for antibody binding. Based on these results, a version of the mosaic nanoparticle immunogen is being made for a phase I clinical trial.

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