Researchers getting closer to a "universal" flu vaccine

With new vaccine targets and more powerful delivery platforms, researchers are making inroads toward an influenza vaccine that could offer better, longer-lasting protection.

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When urgent coronavirus disease 2019 (COVID-19) vaccine development efforts began in earnest in early 2020, researchers were by no means starting from scratch. That's in part attributable to the decades of research dedicated to creating better influenza vaccines. Indeed, many flu vaccinologists pivoted to COVID-19 two years ago, bringing to bear the knowledge and tools they'd developed to fight a seasonal menace that has the potential to spark pandemics.

But these vaccinologists haven't turned away from their longstanding goal: an influenza vaccine that protects against all strains. Such an achievement could save hundreds of thousands of lives every year. And COVID-19 vaccine efforts may end up helping to accelerate that work.

A universal influenza vaccine represents a game changer that could take the threat of both seasonal and pandemic influenza "off the table," according to a November 2021 report, one of four from the

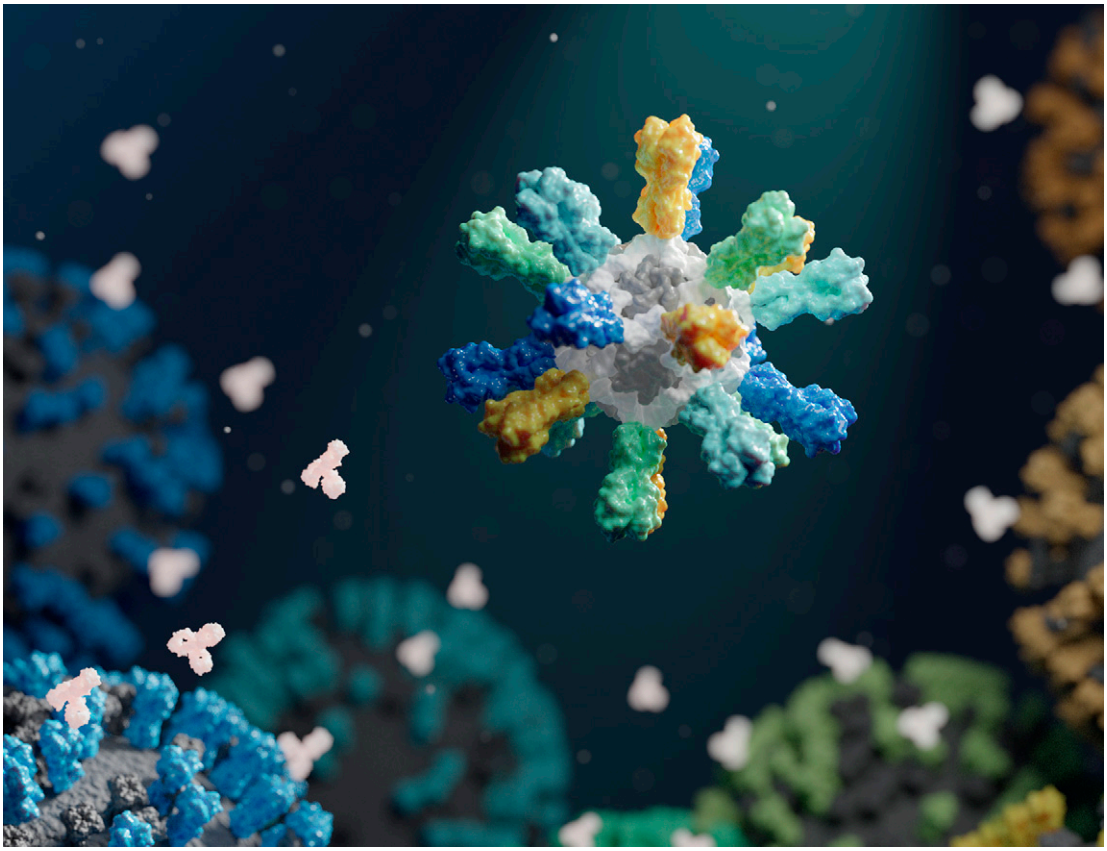

Fig. 1. Researchers are using nanoparticles to build multi-antigen influenza vaccines like the one illustrated here. Image credit: Ian C. Haydon / UW Institute for Protein Design.

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National Academy of Medicine (NAM) on how to prepare for an influenza pandemic with lessons learned from COVID-19. As defined by the National Institute of Allergy and Infectious Diseases (NIAID) in 2018, a "universal" vaccine implies at least 75 percent effectiveness protecting all age groups for a minimum of one year against all strains of influenza A (1). Ideally, says the NAM report, a universal vaccine could also work against influenza B and offer protection for three to five years.

Seasonal flu vaccines—although valuable tools in mitigating flu—protect against only the narrow range of strains projected to be most problematic in a given year. When projections are off, the mismatch has led to vaccine effectiveness as low as 10 percent; even in a good year, vaccine effectiveness barely reaches 60 percent (2). There's plenty at stake: Each year, influenza results in an estimated 290,000 to 650,000 deaths globally despite seasonal vaccines (3). A novel flu virus for which there's no vaccine could lead to a pandemic that kills millions more.

A broadly protective flu vaccine has been a goal for decades, but it's become more attainable in recent years thanks to new vaccine targets and more effective delivery platforms. When the coronavirus pandemic hit, influenza researchers were already making progress with platforms such as mRNA and viral vectors that have led to successful coronavirus vaccines.

All this means there's good reason to believe that a universal flu vaccine is possible within a decade. But hurdles remain—from regulations designed to evaluate more traditional flu vaccines, to overcoming puzzling quirks of the human immune system.

A Moving Target

An influenza virus particle looks much like the now infamous image of coronavirus. Proteins point outward from a sphere of lipids, forming a spikey ball. The most abundant of these protruding proteins is hemagglutinin, which, along with another surface protein called neuraminidase, is where influenza A viruses get their "H" and "N" designations (see Fig. 2).

Hemagglutinin is the key that unlocks host cells, letting the virus in. That makes it a main focus of the human immune system and the primary target of most flu vaccines. Flu vaccines aim to elicit long- and short-term immune responses, including antibodies that recognize specific locations on the hemagglutinin and attach to those spots, blocking the virus.

But hemagglutinin is a moving target. It consists of a stalk topped by a head that is especially prone to evolve, causing small changes to accumulate within each circulating strain. Since the 1970s, the World Health Organization has tried to stay ahead of these changes by recommending, months in advance, which of the circulating strains to include in seasonal flu vaccines (4). In recent years, those recommendations have included two influenza A and two influenza B strains, which are all incorporated into the single "quadrivalent" vaccine given in the United States (5).

In the late 2000s, several research groups made a key discovery that suggested it might be possible to end the race against evolving strains: Humans, it turned out, can generate flu-neutralizing antibodies against parts of the virus that remain largely unchanged (6). Since then, the search was on for the best of these "conserved" regions.

Viruses contain many proteins, all covered in antigenic sites, or epitopes, that trigger matching antibodies. But although hitting one antigenic site may deal the virus a lethal blow, hitting another may leave it relatively unscathed. "Your immune system doesn't know the difference between what's protective and what's not," explains immunologist Jenna Guthmiller, a postdoctoral fellow at the University of Chicago, IL, and an incoming assistant professor at the University of Colorado Anschutz Medical Campus in Aurora. Vaccination, she says, can teach our antibody-generating B cells to focus more on attacking critical regions of the virus. But first you have to get the cells' attention.

Redirecting Attention

Many researchers developing vaccines against conserved parts of the flu virus have focused on the hemagglutinin stalk, which typically changes less than the head. Unfortunately, the stalk doesn't generate as strong an immune response. The reason for the head's "immunodominance" is not entirely clear, explains virologist and vaccinologist Florian Krammer of the Icahn School of Medicine at Mount Sinai in New York.

But Krammer, along with fellow virologist collaborators, is developing hemagglutinin proteins that attract the immune system's attention to the stalk. They do so by taking advantage of another immune system tendency: to respond most readily to what it already knows. Even the strains included in seasonal flu vaccines from year to year, which may have sufficiently different hemagglutinin heads to evade antibodies tailored to a past version, also include many of the same or similar antigenic sites. So, the head is both immunodominant and very familiar. "The head domain has all of the advantages," says Krammer.

Krammer's group takes a hemagglutinin protein and swaps the familiar head for one from a distantly related strain. The head of this chimeric hemagglutinin is still immunodominant, but the stalk is the only familiar portion. "So you redistribute the advantages," says Krammer. By delivering a second vaccination with the same stalk and another unfamiliar head, the stalk's advantage grows.

The team recently designed a vaccine to protect against group 1 influenza A viruses—one of two groupings within influenza A based on the relatedness of their hemagglutinins (7). To make the H1 stalk the most familiar target presented in the vaccine, they topped their chimeric hemagglutinins with heads from avian H5 and H8 viruses. The results of their phase 1 clinical trial, published in January 2021, showed the vaccine induced a broad, durable immune response against the stalk (8). The team is

similarly developing vaccines to protect against the more distantly related influenza A group 2 viruses and against influenza B, with the ultimate goal of creating a trivalent universal flu vaccine.

Despite much potential in the stalk, the head may hold some promise yet, says Guthmiller. She recently isolated antibodies produced by volunteers vaccinated against the 2009 H1N1 virus. Structural virologist Julianna Han of The Scripps Research Institute in La Jolla, CA, then used electron microscopy to reveal precisely how and where each antibody attached to the hemagglutinin head. Of the 66 individual antibodies identified, fully half targeted conserved head regions (9). In an in vitro study, these antibodies blocked nearly all human H1N1 viruses. And in mice, a representative sample of the antibodies provided 100% protection against weight loss and death from a mouse-adapted 2009 H1N1 virus.

Others are bringing computational brute force to the search for the best epitopes in an effort to "build" a better hemagglutinin for use as a vaccine antigen (10). Vaccinologist Eric Weaver, director of the Nebraska Center for Virology at the University of Nebraska–Lincoln, mines public databases to collect hemagglutinin gene sequence data for flu strains recorded over time. He and his team enter those data into the Epigraph vaccine designer, a computer algorithm that builds a new hemagglutinin based on the most common variations in the structure of the protein. To fill in potential gaps in protection, the algorithm can create another hemagglutinin protein using the most frequently occurring forms of each variable region not captured in the first protein, and so on for additional designs.

Working in pigs, which can serve as mixing vessels for avian, human, and pig strains to swap genome segments, Weaver's team recently developed a vaccine aimed at protecting North American swine from H3 strains—a diverse influenza A subtype that circulates in both humans and pigs (11). Pigs vaccinated with a mixture of three synthetic hemagglutinin proteins generated antibodies that protected against 11 of 13 North American H3 swine strains tested. "If we can protect swine from humans, and if we can protect humans from swine," Weaver says, "we'll eliminate this mixing vessel."

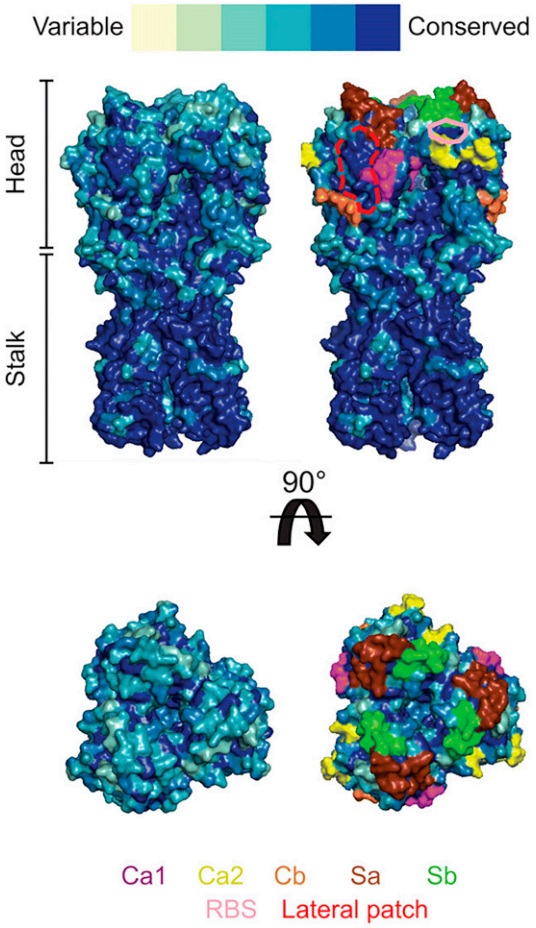

Fig. 2. The most abundant protein protruding from the flu virus is hemagglutinin, which serves as a key that unlocks host cells, letting the virus in. Influenza's hemagglutinin protein is the primary target of most influenza vaccines. But the protein is constantly evolving, especially in its head region. Image credit: Reprinted with permission from ref. 9, https://www.science.org/doi/10.1126/scitranslmed.abg4535.

More than Packaging

In the United States, most influenza vaccines contain inactivated or weakened influenza viruses, which can require high doses to generate a sufficient immune response (12). But as researchers reveal new, more specific vaccine targets, they are also finding that the vaccine platform itself—the way an antigen is delivered to the body—can have huge impacts on the strength and quality of immune responses to those targets.

Lynda Coughlan, a vaccinologist at the University of Maryland School of Medicine in Baltimore, develops vaccines that harness another virus as a delivery tool (13). Adenoviruses naturally infect humans, causing a range of illnesses (14). Researchers can turn these adenoviruses into a "vector" for flu vaccines by deleting the genes that allow the adenoviruses to replicate. Inside this viral shell, researchers insert DNA sequences encoding whatever flu antigens they'd like to present.

A big advantage of these DNA viruses as vaccine vectors is that they use the vaccine recipient's own gene-transcription and translation machinery to generate the antigen protein. And, in principle, the vaccine can keep producing antigen for weeks or longer, which researchers hypothesize could extend the immune response (15). Additionally, the way these vectors enter cells more closely mimics some real viral infections, which more actively engage the immune system.

COVID-19 vaccines currently on the market produced by AstraZeneca, China's CanSino Biologics, Russia's Gamaleya Institute, and Janssen (Johnson & Johnson) also use this adenoviral-delivery strategy, although interest in the technology long predates the pandemic. The University of Oxford's (United

Kingdom) Jenner Institute, where researchers developed the AstraZeneca COVID-19 vaccine, has tested its chimpanzee adenovirus vector against a range of diseases, including flu, although none had made it to market until the COVID vaccine. Coughlan says that the precedent set with the use of adenovirus-vector vaccines against COVID-19 in humans may help pave the way for the flu field. And despite safety issues raised by the Centers for Disease Control and Prevention (CDC) in December regarding Johnson & Johnson's adenovirus-based COVID-19 vaccine as compared with mRNA products,* Coughlan says she's confident that researchers can make modifications to adenoviral vectors to increase safety in the future.

In one recent study, Coughlan and colleagues at Mount Sinai and other institutions created a vaccine that triggered production of the hemagglutinin protein from the 2009 H1N1 virus; it protected mice from that strain (16). They also tested whether this adenoviral vaccine protected mice exposed to another virus—this one with a stalk that matched the hemagglutinin antigen in the vaccine but an entirely different head. All the mice survived, says Coughlan, compared with only a few animals that received a traditional H1N1 vaccine. When the team repeated the experiment with a still more distantly related virus, the difference was even more stark. Adenoviral-vaccinated mice survived, whereas none of the others did (16).

Messenger RNA (mRNA), a platform now famous for its use in the Moderna and Pfizer-BioNTech COVID-19 vaccines and the powerful immune responses they induce, was also in development by those companies as a flu vaccine before the coronavirus pandemic hit (17, 18).

The success of mRNA coronavirus vaccines is a testament to how well the platform can work, says vaccinologist Norbert Pardi of the University of Pennsylvania in Philadelphia, who in 2015, along with mRNA vaccine pioneers Katalin Karikó and Drew Weissman, and others, demonstrated that packaging mRNA within a protective coating of lipids prevents it from degrading too quickly and helps it enter cells (19).

mRNA vaccine technology enables researchers to quickly swap mRNA encoding for different antigens and include multiple antigens at once. In a 2020 study, Pardi, Coughlan, and others tested an mRNA vaccine that combined four influenza proteins. One of these proteins was a special hemagglutinin that contained only the stalk—another strategy for directing the immune response away from the head. In addition, the team included neuraminidase and two other viral proteins that tend to be more conserved. The idea is that incorporating multiple targets offers broader protection and also helps hedge bets. If one of these viral regions evolves to evade the immune system, says Pardi, the other targets could still potentially provide protection.

The vaccine protected mice from a broad range of group 1 influenza A viruses (20). Ultimately, the team plans to include about 10 to 12 antigens spanning influenza A and B. "This is how we believe that we can really develop a globally protective vaccine," says Pardi.

"You want people to have some level of protective immunity, even if it's not perfect, as quickly as possible without the lag phase of waiting for manufacturing of a perfectly matched vaccine."

—Lynda Coughlan

Others are similarly combining multiple antigen targets into novel platforms with the goal of ramping up both the breadth and strength of the immune response. Researchers at NIAID's Vaccine Research Center (VRC) in Bethesda, MD, are using nanoparticles to build multi-antigen influenza vaccines (see Fig. 1). It's a "very strong way to stimulate the immune system," says VRC vaccine immunologist Masaru Kanekiyo.

The team designs a genetic sequence encoding an antigen plus a nanoparticle piece at one end. They then mix this new protein with another nanoparticle piece whose shape is complementary to the first. The nanoparticles click into place like a three-dimensional puzzle, forming an "immunogen" sphere with antigens pointing outward.

In a recent test, Kanekiyo and collaborators showed that a vaccine nanoparticle displaying a total of 20 copies of four hemagglutinin proteins—one from each of the strains in a seasonal flu vaccine—generated strong immune responses in multiple animal models against these specific strains as well as or better than the commercial vaccine (21). But the nanoparticle vaccine also offered greater protection against more distantly related influenza strains, including avian strains with pandemic potential.

Kanekiyo suspects the physical spacing between antigens in the nanoparticle may create a structure that hits within the immune system's "strike zone." Whatever the mechanism, the nanoparticle platform seems to enhance the immune response, while also directing attention to the hemagglutinin stalks. The team is currently testing a similar vaccine in a phase 1 clinical trial.

Understanding the different types of immune responses induced by different vaccine platforms is a big research area, says Coughlan. Trials with COVID-19 vaccines have demonstrated that mixing and matching different platforms can provide the benefits

*On December 16, 2021, the CDC endorsed updated recommendations from the Advisory Committee on Immunization Practices (ACIP) for the prevention of COVID-19. The agency expressed a "clinical preference for individuals to receive an mRNA COVID-19 vaccine over Johnson & Johnson's COVID-19 vaccine." The agency cited ACIP's unanimous recommendation based on "the latest evidence on vaccine effectiveness, vaccine safety and rare adverse events, and consideration of the U.S. vaccine supply." https://www.cdc.gov/media/releases/2021/s1216-covid-19-vaccines.html

of each while strengthening overall responses (22). Coughlan envisions that a truly universal influenza vaccine may similarly require multiple platforms. People might, for example, receive a two-shot "universal" flu vaccination with an adenoviral-based "prime" and then an mRNA "boost."

Original Sin and Other Hurdles

Despite these advances, the winning formula for a universal flu vaccine is far from certain. The NAM report states that it "remains a difficult scientific problem with no guarantee that a vaccine can be developed that will provide long-term protection in people of all age groups" (23).

One major challenge is posed by a phenomenon sometimes called "original antigenic sin," or imprinting (24). For reasons that are still not totally clear, the immune response to any influenza strain is launched in large part by the same B cells that developed upon a person's first flu exposure, even when the strain is mismatched (unless the new strain is so significantly different that it's beyond recognition). Guthmiller says that it's not that subsequent exposures don't matter; she likes to imagine a pyramid, with each exposure adding another level, although the B cells from that first exposure—the foundation of the pyramid—remain the most dominant.

A recent study by Guthmiller, Coughlan, Krammer, and others suggests that vaccination can sway the immune system more toward generating protective antibodies, whereas infection tends to result in more nonprotective antibodies linked to childhood infection (25). But it remains to be seen how universal vaccine candidates will perform in large numbers of people who have unique histories of exposure. Based on what we know right now, Han says, "even if you try to broaden an individual's response to multiple strains of flu, you can only get so far based on the biases already present in that individual's immune system." One possible workaround, she suggests, might be to develop different universal flu vaccines for different age groups.

Another wildcard is durability—both in terms of how long vaccine-induced immune memory will remain active, and how long a particular vaccine formulation remains "universal" enough. "In a perfect world, you would get vaccinated at the age of six months or one year and then you wouldn't need it again until you were 50," says Weaver. "It's more likely that evolution would continue to occur and that these would need to be updated."

Given that a flu vaccine that is at once long lasting, broadly protective, and highly effective could prove a tall order, at least in the short term, Coughlan also sees a place for a vaccine that could limit the severity of illness for a broad range of flu strains—even if it does not prevent infection. This stopgap vaccine could be freeze-dried, stockpiled, and rolled out only in the event of a pandemic. "You want people to have some level of protective immunity, even if it's not perfect, as quickly as possible without the lag phase of waiting for manufacturing of a perfectly matched vaccine," she says.

Universal flu vaccines could face some regulatory hurdles (26). Most candidates would be sufficiently different from existing seasonal vaccines, meaning that getting them approved under current regulatory guidelines would require more than just showing so-called correlates of protection; vaccine developers would have to perform the extra step of demonstrating that the vaccine prevents people from getting sick, says Krammer. "That might be many million dollars' difference in the cost of the clinical trial."

But momentum is building and the pace of discovery may increase with fresh funding for efforts like NIAID's Collaborative Influenza Vaccine Innovation Centers (CIVICs). Launched in fall 2019, CIVICs support collaborative research, vaccine manufacturing, and clinical trials—all at facilities within the CIVICs network. "I think that a lot of interesting vaccine approaches will come out of that structure," says Krammer, who is co-principal investigator for one of three vaccine research centers within the network.

Like much influenza research, early work out of CIVICs was slowed by the coronavirus pandemic and supply chain issues. "That has impacted a lot of lab work," says Krammer, adding that many influenza researchers—including himself—also shifted their attention to the new coronavirus for a time.

Still, he's hopeful that the coronavirus pandemic may yet play some role in advancing a universal flu vaccine, both by renewing public enthusiasm for vaccines and by demonstrating how much can be accomplished with enough political will and financial support. "The public learned that pandemics happen and we need to be prepared," says Krammer—but, he adds, researchers in the flu field "didn't need that reminder."

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