Dr. Tim Evans/Science Source

Stalking new vaccines:

Methods that target the stems of viral proteins could put universal vaccines within reach

By Carrie Arnold

In a forest in southern India in January of last year, Samil George took a cotton swab and carefully swiped the clear goo off of the back of a tiny, red-striped frog in his other hand before setting the animal back down in the leafy groundcover. Skin secretions from similar frogs were known to contain antimicrobial peptides, and George hoped that this *Hydrophylax bahuvistara* frog might contain molecules that could also fight human infections. George, a molecular ecologist at the Rajiv Gandhi Center for Biotechnology in Kerala, India, put his swabs of slime in plastic tubes, sealed them and sent the samples to the lab of his longtime collaborator, Emory University immunologist Joshy Jacob, in Atlanta. Over the next several months, Jacob isolated 32 different peptides from the *H. bahuvistara* slime, all of which were new to science, including one that seemed able to thwart the H1N1 flu virus in both cells in a dish and mice. Jacob's team named this compound urumin, after the long, curved sword historically carried by soldiers in the same region of southern India where the frog lived[1].

Jacob's experiments, published in April, revealed that urumin blocks the flu virus by targeting the lollipop-shaped viral spike protein hemagglutinin. But rather than binding to the hemagglutinin's head, which the virus uses to connect to and enter host cells, urumin interacts with hemagglutinin's stalk, which protrudes from the viral membrane.

"This is a very interesting finding that could open an avenue for novel therapeutics," says Florian Krammer, a virologist at the Icahn School of Medicine at Mount Sinai in New York. Krammer would know. He's also one of a growing number of scientists using a new strategy to make drugs that target the stalks of viral spike proteins rather than the proteins' heads.

Many spike proteins on the outside of membrane-enveloped viruses such as HIV, coronaviruses, influenza and Ebola have a key role in allowing these viruses to enter host cells. For this reason, researchers developing vaccines and antivirals have long focused on the spike protein head as a target. But this target also has a downside: with influenza, for example, the head of hemagglutinin accumulates mutations at a rapid clip. This mutation rate is what allows the virus to outsmart antivirals, the immune system, and, consequently, vaccines.

The spike protein stalk, however, is less likely to mutate over time. In influenza, for example, mutations in the stalk can reduce the virus's ability to fuse with host cell membrane, a key step in infection. The resistance of the stalk to mutation accumulation means that drugs targeting this protein segment, as urumin does, could serve as a lasting antiviral, Krammer explains. In fact, Krammer and his colleague Peter Palese, also at Mount Sinai, believe that a vaccine approach that targets the stalk of spike proteins could help produce a long-awaited universal influenza or HIV vaccine. First, though, scientists must figure out how to coax the body into making antibodies against the stalk.

Universal appeal

Palese has spent nearly four decades working to develop a universal influenza vaccine that would replace the annual immunizations required against flu. Each year, scientists have to create an updated version of the seasonal flu vaccine because these vaccines target the hemagglutinin head, which accumulates

mutations as it passes in an endless chain from person to person. The stalk, however, is less variable, and Palese has believed for decades that targeting this part of the protein could form the basis of a universal flu vaccine that doesn't need to be updated every year. In his vision, a universal flu vaccine would elicit antibodies that react against the hemagglutinin stalks of many different strains of influenza, thus neutralizing a wide range of strains.

His first experiments toward developing a hemagglutinin-stalk-based vaccine in 1983 tried to answer two initial questions: could a vaccine stimulate the production of anti-stalk antibodies in mice, and could these antibodies provide protection against flu? Palese knew the immune system has a hard time generating antibodies against the stalk because the head is physically in the way, rendering the stalk invisible. A vaccine with 'headless' hemagglutinin proteins would get around this issue. His initial attempt didn't pan out as he'd hoped. The proteins Palese and colleagues made were too unstable. Yet Palese still believed the approach was sound[2], although no one knew whether humans could even be stimulated to produce antibodies against the hemagglutinin stalk.

In 2009, Palese got a second chance to test his approach. That year, a new strain of H1N1 influenza emerged out of Mexico and spread around the world. The pandemic 'swine flu' virus appeared even as other, seasonal H1N1 flu strains continued to circulate. The arrival of the swine flu pandemic provided a perfect way for Palese to test whether humans could naturally produce antibodies against the hemagglutinin stalk. Although seasonal and swine flu strains were both H1N1 viruses, the hemagglutinin heads on these viruses were so different that antibodies generated against the seasonal flu strains didn't protect against swine flu, even though the stalks were broadly similar.

Over the next year, however, the seasonal H1N1 flu disappeared as the pandemic strain became dominant. Palese believed that antibodies against the hemagglutinin stalk played a major role in this switch. Mouse studies supported this hypothesis, as exposure to different strains of influenza triggered the production of anti-stalk antibodies. Other researchers had identified a potential segment of the hemagglutinin stalk where human antibodies might bind[3]. In work published in PNAS, Palese synthesized chimeric hemagglutinin proteins to show that infection with pandemic H1N1 after exposure to seasonal H1N1 influenza boosted the production of hemagglutinin stalk antibodies[4]. Since the seasonal H1N1 had faded in importance, Palese knew that the anti-stalk antibodies were powerful enough to protect against later H1N1 infection.

Richard Levine/Alamy Stock Photo

Seasoned expertise: Scientists hope to replace seasonal flu shots with a universal vaccine. Opposite page: a hemagglutinin viral surface protein.

With these results, Palese finally had enough data suggesting his approach would work, and he made another attempt at building a headless hemagglutinin universal influenza vaccine. In a 2010 paper[5], he created a synthetic protein consisting of just the hemagglutinin stalk of an H2 influenza virus—circulating seasonal flu viruses had subsequently switched to H2N3 influenza—that protected mice against a broader range of flu viruses than the seasonal vaccine and also against death from disease. Together with Krammer, the pair have continued to refine their approach. In 2016, they engineered a harmless chimeric virus that in mice elicited stalk-specific antibodies able to target a broader range of influenza strains than the synthetic headless hemagglutinin vaccine[6].

"By making these strange beasts, we could show that they could redirect the immune system toward conserved regions of the virus," Palese says.

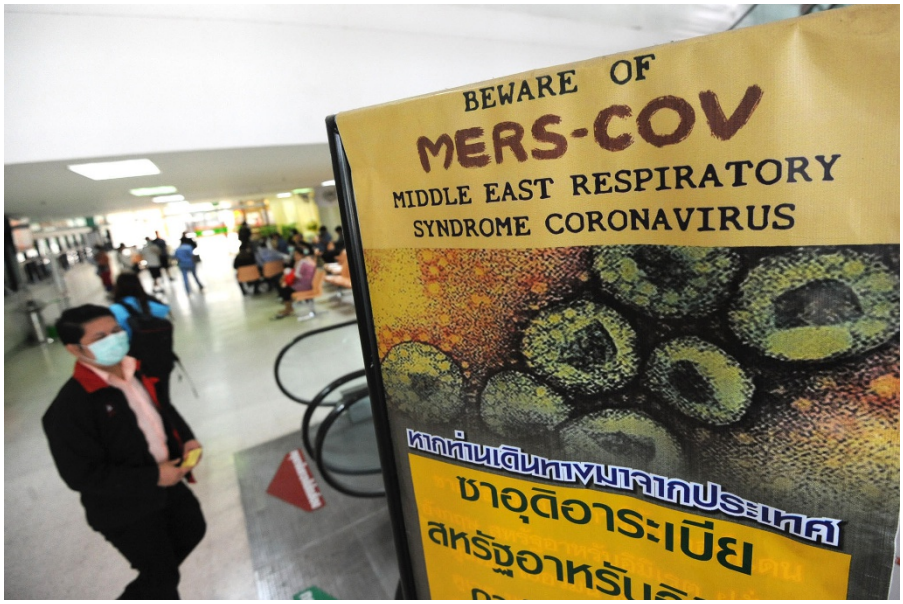
Other groups of scientists have been trying slightly different approaches to making a universal flu vaccine. The EU-funded, multinational FLUTCORE project combines input from academic scientists and pharmaceutical companies to synthesize noninfectious virus-like particles in yeast that incorporate several influenza antigens, including ones from the hemagglutinin stalk. Initial studies showed that the FLUTCORE approach protects mice from a range of influenza viruses[7], and the group is currently raising money for clinical trials. Meanwhile, the pharmaceutical startup BiondVax, based in Ness Ziona, Israel, has just completed phase 2b clinical trials for its Multimeric-001 universal influenza vaccine. The vaccine consists of a single protein built from nine different small protein components, including parts of the hemagglutinin stalk, that stimulate the production of antibodies and other protective reactions from the immune system.

Coaxing the human body to produce antibodies against the hemagglutinin stalk, however, is only the first step in the very long road to bringing a universal flu vaccine to market. No stalk-based influenza vaccines are in use, and getting any of these candidates ready for the clinic will be a long and expensive process. Krammer says it will take years to prove an influenza vaccine protects against a wide variety of strains. This, combined with the high safety of the seasonal vaccines, will make the final stages of trials challenging.

"To say you have a true universal vaccine, you'll have to wait until a pandemic hits," Krammer says, which could take decades.

Thinking big

As Palese began making progress on a stalk-based vaccine for influenza, biochemist Jason McLellan watched with interest from his lab at Dartmouth College. McLellan specializes in coronaviruses, a large group of respiratory pathogens that includes the viruses that cause severe acute respiratory syndrome (SARS)



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Spike in interest: The stalk of spike proteins of MERS evolve slower than the spike proteins' head.

and Middle East respiratory syndrome (MERS). As in influenza, spike proteins stud the coronavirus membrane. As in influenza, the stalk of the spike protein changes far more slowly than the fast-evolving head. It got McLellan thinking that he might be able to try a similar approach to create stalk-based coronavirus vaccines.

The stalk of the spike protein in coronaviruses is much larger than the hemagglutinin stalk in influenza, and this larger stalk gives the immune system more target points against which to generate antibodies. McLellan needed to find the parts of the coronavirus stalk that stimulate production of the most protective antibodies to make a better vaccine. Using monoclonal antibodies from individuals who had survived SARS or MERS, McLellan and virologist Mark Denison of Vanderbilt University identified the parts of the coronavirus spike protein most likely to elicit an immune response. The researchers then turned to a new technique that enabled them to flash freeze different viral particles in an array of orientations, bombard the particles with electrons and then use supercomputers to reconstruct their 3D shape down to a resolution of four angstroms. The technique, known as single-molecule cryo-electron microscopy, revealed the detailed shape of the stalk of the coronavirus spike protein, which McLellan could then use as a guide to build a protein subunit vaccine.

But, as with the early headless hemagglutinin vaccines attempted by Palese decades ago, just lopping off the head of the spike protein and using the stalk alone yielded a protein that was too unstable for a vaccine. So the researchers returned to their 3D diagram to start tweaking individual amino acids to create a MERS coronavirus vaccine that would have enough stability to elicit a strong immune response. In August, McLellan and colleagues published a paper outlining the construction of a stable engineered spike glycoprotein from MERS coronavirus[8], although the team has yet to show whether it protects against coronavirus infection.

Different coronaviruses have many similarities in the spike glycoprotein stem, which gives McLellan an advantage in designing vaccines for other coronaviruses.

"There's a huge reservoir of coronaviruses that are waiting to emerge," he points out, and a stalk-based coronavirus vaccine may even protect against viruses that have yet to make the leap to humans, thanks to the similarities in the stalks of the coronavirus spike proteins.

The design of a stalk-based vaccine for HIV, however, has stalled, despite years of effort, owing in part to the virus's staggeringly high mutation rate. Although virologists speak of the stalk of the HIV envelope protein (Env) being conserved, that's only relative to other parts of HIV, says Julie Overbaugh, an immunologist at the Fred Hutchinson Cancer Research Center in Seattle.

"Compared to other viral proteins, the Env stalk is not very conserved," she says.

Despite the mutability of HIV, infected individuals often create broadly neutralizing antibodies that can temporarily hold the virus in check. Analysis of these antibodies from thousands of HIV-infected individuals revealed five targets for broadly neutralizing antibodies, two of which were segments of the Env stalk. Following this discovery, scientists developed a vaccine that targeted the V3 loop, a portion of which lay in the Env stalk. This attempt fizzled out after they failed to show adequate HIV protection in animal models. Current HIV vaccines in development use Env stalk antigens as only one part of a broader strategy, according to Dennis Burton, a vaccinologist at the Scripps Research Institute.

"An HIV vaccine will probably look a lot different than vaccines we're used to," Burton says. Currently, vaccine boosters consist of the same injection as the initial shot. If Burton and others have their way, an HIV vaccine will likely require multiple shots with slightly different components each time to persuade the immune system to recognize not just a small number of strains, but thousands or tens of thousands of them.

Despite the many challenges facing the development of stalk-based vaccines and antivirals, researchers remain convinced that this strategy will eventually pay off. The advantage of this approach—the ability to target a wider range of viruses with less chance of the virus mutating and thereby making the vaccine ineffective—will ultimately outweigh the time and expense of getting such therapeutics ready for human use. Although a universal flu vaccine is closest to market, it has yet to complete lengthy phase 3 trials.

Whatever the final result, the process of creating these vaccines has helped to open researchers' eyes to the tremendous complexity of interactions between viruses and the human immune system. Denison says the work has already changed scientists' thoughts on the importance of targeting parts of proteins like the stalk that provide structure as well as bind to receptors. "I'm really excited about this approach," he says, pointing out that many viral proteins have been overlooked as vaccine targets because they don't bind receptors. Work on stalk-based vaccines and antivirals may provide an approach that Denison calls "broadly applicable" to a wide variety of viruses.

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