Plasmid Rescue System Injects New Life Into Flu Vaccines

St Jude Children's Research Hospital

In November 1918, as World War 1 was ending, a new battle emerged — one with a microscopic foe. The 1918 influenza pandemic stretched into 1920, and when it was over, nearly one-third of the world's population had been infected. The war claimed nearly 17 million military and civilian lives. The 1918 influenza pandemic killed about50 million people, with some estimates reaching nearly 100 million.

Today, there are better defenses against influenza, in the form of vaccines that prime immune systems to fight the virus (the first U.S.-approved flu vaccine arrived in 1945). But influenza viruses mutate constantly, which means vaccines need to keep changing too.

To that end, researchers at St. Jude Children's Research Hospital developed a technique called the 8-plasmid reverse genetics system. Using precise genetic modification, it quickly and effectively reduces the time involved for vaccine development when influenza pandemics emerge.

The Fundamentals of Flu Vaccine

Based in Memphis, Tenn., St. Jude Children's Research Hospital is known as a research center for childhood cancers. But St. Jude also has a long history of flu vaccine research, dating back about 40 years.

As it turns out, influenza research has plenty of relevance for St. Jude's cancer patients. "A lot of our oncology patients die from infectious diseases — including influenza — because their immune systems are just so weak," says Shawn Hawkins, associate director of technology licensing for St. Jude. So you have to try to find ways to treat those infectious diseases better.

To appreciate how St. Jude scientists did this, it helps to understand the basics about how vaccines are made.

Before vaccine manufacturers can produce millions of doses, they need to know exactly what to produce. For that, they require a vaccine seed strain — a benign strain of the influenza virus that will build immunity without causing infection. These seed strains are created in a handful of laboratories approved by the World Health Organization, and St. Jude Children's Research Hospital is one of those labs.

Historically, scientists have created seed strains by injecting chicken eggs with two flu strains: the target strain (which the vaccine builds immunity against) and also another flu strain that's proven to grow quickly and therefore enhances seed strain growth (because if the seed strain fails to reproduce well, it can lead to vaccine shortages). Inside the eggs, the genetic material of the two flu strains combines naturally and randomly. Scientists must test the eggs to find the hybrid virus that's best-suited for seed strains, then purify them and send them to manufacturers for vaccine production. With this approach, it can take up to 4 months for scientists to identify the right seed strain and an additional 4 to 6 months for manufacturers to produce large quantities of vaccine. That gives a pandemic virus plenty of time to wreak havoc.

Targeting the Troublemakers

> *At St. Jude, researchers developed a system that can develop seed strains for influenza vaccine in about 2 weeks instead of up to 4 months.*

Called the 8-plasmid reverse genetic system, this technique still combines the target flu strain with a fast-growing strain, but in a more precise way. With funding from the National Institutes of Health, Robert Webster, Ph.D., and Eric Hoffman, Ph.D., began developing the 8-plasmid reserve genetic system around 1998, building on recent vaccine advances.

Instead of adding the entire target flu strain into the mix, the researchers selected specific parts of the virus, using a reverse genetics technique that improved on earlier versions developed in the 1990s at Mount Sinai School of Medicine. This allowed the St. Jude researchers to focus on two genes that make the proteins: hemagglutinin and neuraminidase. These two proteins are the main culprits behind influenza infection. They represent the H and N in flu viruses' names (such as H1N1 and H5N1). By isolating the genes for those troublemaker proteins — something the conventional vaccine system doesn't allow — the scientists can develop an effective seed strain more quickly and reproducibly.

To combine the hemagglutinin and neuraminidase genes with the fast-growing flu strain, the researchers use one of genetic engineering's main workhorses: plasmids. Plasmids are DNA molecules that can transfer genes between cells. Since the 1970s, laboratories have used plasmids to combine DNA from more than one source. Because the influenza virus has eight genetic segments, Webster and his colleagues used eight plasmids — one for each genetic segment. Of the eight plasmids, two contain genes for the troublemaker proteins (hemagglutinin and neuraminidase), and the other six plasmids contain genes from the fast-growing virus strain. The plasmids are added to cultured mammalian cells (instead of chicken eggs), where their genetic material combines to make seed strains for vaccines.

St. Jude's system improved on work done at University of Wisconsin-Madison, where researchers had developed a 12-plasmid system during the late 1990s.

"The 8-plasmid system hadn't been developed before because the molecular biology wasn't in place to allow it to happen," says Webster. "As the right molecular biology became available, we used it. We needed the simplest possible system. It was sort of a no-brainer."

Here's one benefit of simplicity that comes from 8 versus 12. Imagine you've been given a cake recipe. If there are fewer ingredients, that can lessen the chance of a baking mishap. The same basic idea — fewer variables, more predictability — applies to the plasmid system.

The 8- plasmid and 12-plasmid systems work on the same principle," says Richard Webby, Ph.D., who joined Webster's lab during the final stages of development. "But with 8 plasmids, there are less raw ingredients you need to do the same process and less things that can go wrong, so it's a little more efficient," says Webby, who is currently a member of St. Jude Department of Infectious Diseases.

Finding a Licensee

St. Jude's Office of Technology Licensing saw the potential in this efficiency. It filed for a patent in April 2000 and began looking for licensees. The office was soon contacted by a company called Aviron, and completed negotiations on the exclusive licensing agreement by September 2001. In 2002, the company was purchased byMedImmune.

Compared to the old method of seed strain development, the 8-plasmid system has more predictable results, says Hong Jin, Ph.D., senior fellow in MedImmune's infectious disease division. "It enables rapid production, evaluation and selection of vaccine seeds.

That helped MedImmune be the first vaccine manufacturer to deliver H1N1 flu pandemic vaccine to the U.S. government in 2009, says Jin. "In addition, it allows generation and evaluation of a number of different subtypes of influenza pandemic vaccines for pandemic preparedness," she says. MedImmune has made nonexclusive licenses available for manufacturers to produce vaccines using the system. So far, 10 manufacturers have those licenses, including Novartis and GlaxoSmithKline.

With the 8-plasmid system, MedImmune has also developed a different type of seasonal flu vaccine. Most seasonal flu shots — as opposed vaccines for highly pathogenic H5N1 or pandemic flu — are developed using the older,

conventional vaccine system. They also contain inactive flu virus, which means the virus is dead.

But using the 8-plasmid system, MedImmune developed a seasonal vaccine that doesn't require injections. It's administered as a nasal spray and contains attenuated flu virus. That means the virus is alive, but weakened so it can't infect — think of an aggressive animal that's had all teeth and claws removed. For the nasal spray, the virus is attenuated by introducing specific mutations into its genome.

The nasal-spray vaccine first received U.S. approval in 2003, as FluMist. (It is trivalent, meaning it protects against three strains of flu.) In 2012, AztraZeneca — MedImmune's parent company — received U.S. approval for FluMist Quadrivalent, which protects against four strains of flu. More than 75 million doses of nasal spray flu vaccine have been distributed since 2003. Other countries have approved its use too, including Canada, South Korea, Israel and the European Union.

Pandemic Preparedness

There's widespread acceptance now for customizing influenza's genetic composition — but that's not what Webster experienced more than 15 years ago when work began on the 8-plasmid system. "I have to say that initially, there was quite a bit of resistance to genetically modifying an influenza virus," he says. "There was some opposition to the idea of manipulating that influenza virus to suit ourselves, rather than just letting mutations appear."

That sentiment changed in 2003, with the spread of a highly pathogenic avian flu, H5N1. The World Health Organization (WHO) declared a pandemic alert for H5N1 in February 2003, and the older, conventional way of producing vaccine didn't work. In addition to killing birds and people, H5N1 also killed eggs — which meant the virus had to be genetically modified (by removing the pathogenic part of the hemagglutinin gene) before a vaccine seed strain could be produced. "That really convinced people that genetic modification was the way to go," Webster says.

During that H5N1 pandemic, Webby helped lead a team at St. Jude to develop a vaccine seed virus. Using the 8-plasmid system, it took the scientists less than 3 weeks to prepare a seed strain, which they sent to the Centers for Disease Control in Atlanta and the World Influenza Center in London for additional testing. Webby notes that his lab wasn't the only one developing a vaccine seed virus to tackle the pandemic. "As the growth properties of similar vaccine viruses can vary substantially, multiple labs typically start the process simultaneously so that multiple options are available," he says.

Particularly lethal — about 60 percent of those infected have died. The virus spreads to people when they have direct contact with infected birds, but H5N1 hasn't been shown to spread from person to person, like seasonal flu does. At least, not yet.

One of biggest limitations we have in the flu world in terms of public health, is that there are flu viruses of all different shapes and sizes circulating in animal populations," says Webby, "And we don't know which of those actually pose real risks to human health — what it takes for a virus in an animal to become a human virus."

There is always a risk of nasty viruses jumping from animal to human, and then mutating to spread rapidly between people, he says: "The potential consequences are pretty catastrophic, and there's really no way of measuring that until

it happens. That's where we have to be really prepared for the very worst situation."

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