Creating the Next Generations of Influenza Vaccines

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Which comes first – the chicken or the vaccine? If you know your vaccine technology, it's an easy answer. But if you don't, you might wonder what the two have in common. It is true that in the post-molecular era, most of our seasonal and pandemic influenza vaccines are made using technology that dates back to the mid 1900s. Though these vaccines have had a high measure of success in influenza prevention over the past 30 years or so, the opportunity—some would say need—to create more effective, safer vaccines more quickly against flu and similar viruses, like the current H1N1, is technologically within reach.

"For influenza and similar viruses, the vaccines that will replace the current older technology will be produced through recombinant technology, using genetic material to generate vaccines from cell culture or a high production throughput system," says Ted Ross, Ph.D., Center for Vaccine Research (CVR) at the University of Pittsburgh. "One of the things we need to do is to get vaccines made more quickly especially against pandemic outbreaks, such as the current H1N1 experience," he says.

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The manufacturers licensed to manufacture the H1N1 vaccines fell short of delivering the original number of 195 million doses the U.S. government ordered this summer. At the time this article went to press, the Centers for Disease Control (CDC) confirmed that about 64 million doses have been shipped thus far. This shortfall created the triaged system now in place in which select groups have been receiving the vaccines ahead of the general public.

Chickens and Their Eggs

"The H1N1 vaccine supply we make for the United States is grown entirely in chicken eggs," says Philip R. Dormitzer, M.D., Ph.D., senior project leader, Viral Vaccine Research at Novartis. Novartis is one of the large companies involved in seasonal flu and H1N1 vaccine production. "You need an awful lot of chickens producing literally millions of eggs," he explains. "That creates an element of vulnerability in the vaccine supply where you need chickens to make more chickens to get enough eggs." Each egg must be inoculated and harvested individually, making the process labor intensive. And as Dormitzer puts it subtly, "Chickens and their eggs are far from sterile, and it introduces a potential for a bioburden which can be eliminated with a cell-culture based or other more advanced vaccine."

Not only are chicken eggs needed to function as tiny incubators, but Dormitzer explains that flu viruses generally do not grow terribly well in eggs. "Flu viruses are adapted to grow in human respiratory cells," says Dormitzer. "For them to grow in eggs, they have to be adapted to those eggs, because eggs bear different receptors on their surface than those that flu virus use to get into humans." The consequences include the need to choose a strain for production that grows in eggs thereby placing a restriction on the range of strains that can be used in a vaccine. Second, to grow in eggs, flu viruses have to undergo an adaptive mutation to bind the egg receptors. Explains Dormitzer, "It turns out that the receptor binding site is in a key antigenic region, so those viruses will always have a slight difference from the viruses that actually circulate in people because they have acquired a mutation right where many neutralizing antibodies bind."

Clearly, there is room for improvement. Molecular biology now makes it possible to produce a safe immunogen that will not replicate and is noninfectious but contains all the proteins or epitopes needed to stimulate the body's immune system. There are a variety of different mechanisms to pursue this goal. Says Ross, "It is like we've decided we like ice cream but haven't figured out which flavor."

Cell Culture Vaccines

Much of the core science that will deliver the next level of efficiency has been available since the mid 1980s. "The innovation is really how you adapt it to an industrial setting to really make effective vaccine," says Ross. "With cell-based production methods, such as in mammalian cell culture, bacteria, insect cell culture, or a yeast system, you can scale them up into very large bioreactors to safely make a lot of product."

Novartis already markets a cell culture-based H1N1 vaccine, Celtura, and has licensed a seasonal flu vaccine, Optaflu, in Europe. And on November 24, 2009, it inaugurated the U.S. Flu Cell Culture Facility in North Carolina with the mission of being able to produce 150 million doses of flu vaccine within 6 months of the declaration of a pandemic. When operational in 2011, the facility, which is a partnership between Novartis and the Department of Health and Human Services, will use large tanks of Madin-Darby canine kidney (MDCK) cells as a substrate in which to grow flu virus.

Cell culture technologies also offer the possibility of producing vaccines without the need for live whole virus. Novavax is developing both seasonal flu and H1N1 vaccines using cell culture of virus-like particles. "Virus-like particles (VPLs) look like a virus, have all the right structures, but contains none of the genetic information, so they can't replicate," says Ross, who has been involved in conducting a trial of a VPL vaccine from Novavax. The first VPL vaccines licensed are those for hepatitis B virus (HBV) and the human papillomavirus (HPV) vaccine, Gardasil.

"There are several of them going through clinical trials now and I would expect a few to be licensed over the next 10 years—particularly for influenza, rotavirus, and respiratory synctial virus," says Ross. "Another advantage with VPL vaccines is that they can be made very quickly compared with current technology, on the order of 3–4 months."

Bacterial Fermentation

There is also work to try and decrease production times by not growing virus but simply producing antigens using other expression systems.

VaxInnate, of Cranbury, New Jersey, uses recombinant proteins in *E. coli* fermentation to produce its novel influenza and H1N1 vaccines. "We pick out the sequence for part of the viral hemagglutinin (HA) we need, convert that sequence into a physical DNA sequence, and put that into an expression plasmid in *E. coli*," explains Alan Shaw, Ph.D., VaxInnate's president and CEO. HA is a protein located on the virus envelope that is recognized and bound by respiratory cells. "We've demonstrated good immunogenicity and safety with the H1N1 human strain and have made a vaccine that will begin clinical testing in the beginning of next year," he says.

Shaw adds that one 1,000 liter fermentation batch yields about 200 million purified vaccine doses. And it takes about 10 days to do that start-to-finish. "Had we been licensed last July, given the time it takes us to build the construct and make materials, we could have been supplying H1N1 vaccine in July of this summer so there would have been enough for everyone," he says. "This technology allows us to take an emergency and turn it into a routine operation."

Other companies using a similar approach include Novavax and Protein Sciences. Both companies use insect cells instead of *E. coli* bacteria for their production platforms.

Direct DNA Injection

"The most distant approach is DNA vaccination," says CVR's Ronald Montelaro, Ph.D. "You immunize people with DNA and then that DNA incorporates into cells in the skin or elsewhere and produces those viral proteins in the body and the immune system reacts to it. The beauty about DNA is that you can produce a lot of it and it is extremely stable, eliminating the need for a cold chain."

Vical of San Diego, California, is in pursuit of an influenza vaccine using direct DNA injection. "We use *E. coli* to make gene segments that are then injected into human vaccine volunteers," says Larry Smith, Ph.D., vice president, Vaccine Research. "In our case, we take the influenza H1 hemagglutinin gene and clone it into our plasmid DNA backbone, introduce it to *E. coli*, and grow it up in mass quantities." The vaccine is then injected into muscle tissue. The company tested its vaccine strategy real-time in the wake of the Mexico City H1N1 outbreaks in April 2009. "We took a gene sequence from the CDC's GenBank and had the gene synthesized without ever touching an H1 virus or having to worry about biocontainment." Vical claims its manufacturing process would require about 10 weeks to create a vaccine from the time it receives the genetic sequence to the initial lot release.

In October 2009, the U.S. Navy committed funding to conduct clinical, regulatory, and manufacturing preparations for a phase I clinical study of Vical's H1N1 vaccine.

Mission Impossible? Universal Flu Vaccine

"If we can make one vaccine that doesn't need to change yearly to account for strain variations, that would be the Holy Grail," says Smith. But the scientific jury is still out on whether universal vaccine against influenza is feasible. Some say the research needed to fully categorize the millions of circulating influenza strains would require an investment on the order of the Human Genome Project. Because influenza virus mutates and changes so often, some suggest targeting a region of the virus that is highly conserved among strains, such as the M2e ion channel protein or the HA protein. But researchers are finding that highly conserved epitopes on HA are not the most immunogenic. "These regions probably don't change because they don't have to," says Dormitzer. "It does appear that most of the protective immune response is directed against the variable epitopes."

VaxInnate had been working on a universal flu vaccine until this summer, when it was revealed that the M2e sequence of the H1N1 virus was very different than the sequence used in the company's phase 1 universal flu vaccine study.

Vical has also pursued a universal flu vaccine incorporating the influenza nucleoprotein (NP) and M2e ion channel protein. "These are well-conserved proteins among different influenza A subtypes," says Smith. "There are 16 known influenza A hemagglutinin (HA) subtypes. Today, seasonal flu vaccines are made using strains from two of those 16 HA subtypes, as well as an influenza B strain."

Inovio Biomedical of San Diego, California, is also using direct DNA constructs, including one for HA, to make a universal flu vaccine. Its product is in preclinical testing.

"The other way to make a universal vaccine is to find the common regions on the proteins of the virus that change quite a bit and see if you can bridge many different strains," explains Ross. "I'm not sure it is possible to determine the most immunogenic epitopes on HA, for example, on the vast variety of flu strains out there." He adds that flu is a particularly tough virus to tackle fully. "We have never been able to eradicate a pathogen that affects people as long as there is an animal reservoir," Ross says. "For flu, that reservoir is wild birds, ducks, water fowl."

So, until new production practices are fully vetted, we will still rely on chickens and fowl to generate both new flu viral strains and the vaccines that target them.

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