Bird Flu Needs Better, Modern Vaccine Production Methods (2005)

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This is part three of a series on human and avian flu vaccines.

Washington – The threat of a global pandemic from the H5N1 bird flu virus is prompting scientists to move away from the 50-year-old influenza vaccine production methods in use today and turn to techniques perfected in the current age of biotechnology.

In a 2004 National Academy of Sciences report, The Threat of Pandemic Influenza: Are We Ready?, experts estimated that if a pandemic were to strike today, it would take six months to eight months to identify the viral strain and release initial doses of vaccine, using standard methods.

"Given existing manufacturing capacity," the report says, "vaccine availability would fall far short of projected demand, especially in countries without vaccine manufacturing facilities."

Standard methods, developed more than 50 years ago, involve identifying which virus strains should go into a vaccine, then growing those strains in millions of fertilized (embryonated) chicken eggs before harvesting, purifying and killing the viruses and using them in vaccines.

The egg-based method is especially problematic for a potential H5N1 vaccine because the virus kills chicken embryos before much of the virus can grow.

Preliminary clinical trials at the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health, have shown that relatively large doses of the H5N1 vaccine are needed to generate an effective antibody response.

"So you've got this double problem," says Andrew Pekosz, assistant professor of molecular microbiology at the Washington University School of Medicine in Missouri. "You can't make a lot of the vaccine, but you need more to induce a good immune response."

MODERN METHODS

Several faster, more flexible methods exist for producing flu vaccines, Pekosz said, including cell culture and reverse genetics.

Cell culture involves growing animal or human cells in the laboratory in a nutrient solution. The virus is injected into the cells, and cells and viruses multiply. Then the cells' outer walls are removed and the virus is harvested, purified and inactivated.

Reverse genetics, Pekosz said, "allows us to more rapidly and efficiently generate viruses containing current HA and NA proteins."

There are three kinds of flu viruses – influenza types A, B and C. Influenza A viruses mutate much more rapidly than influenza types B and C and are divided into subtypes based on two proteins on the virus surface – hemagglutinin (HA) and neuraminidase (NA).

Influenza A subtypes are named according to their HA and NA surface proteins, which is what the letters H and N refer to in subtype names like H5N1. This subtype has an HA 5 (H5) protein and an NA 1 (N1) protein on its surface.

These ever-changing surface proteins are targets for vaccines.

“Instead of isolating viruses, growing them in eggs and inactivating them,” Pekosz said, “which is how the current vaccine is made, we can simply identify the HA and NA genes, genetically engineer viruses to express [produce] them and then use cell culture to grow those viruses up.”

The process of reverse genetics and cell-culture growth of influenza virus, he added, “is one that every basic research laboratory that works in influenza does every day.

“As a process,” he said, “it can be scaled up [to produce large quantities of vaccine], as long as the regulatory changes take place so those processes can be validated for the production of a human vaccine.”

Putting more of an emphasis on more modern methods, Pekosz said, would take a concerted effort from the U.S. Food and Drug Administration, which approves such processes, and the NIH and vaccine manufacturers “because there are some regulatory issues that need to be addressed.”

SOMETHING COMPLETELY DIFFERENT

Methods like cell culture and reverse genetics are more modern than the egg-based method for producing vaccines, but they still involve making a virus, inactivating it and inoculating people.

But there are completely different ways to generate the appropriate antibodies, and Pekosz’s laboratory is working on one of them, with funding from NIAID.

His vaccine targets a cell-membrane protein called M2 of the influenza A virus.

“Unlike the hemagglutinin or HA and the neuraminidase or NA proteins, the sequence of the M2 protein has been very highly conserved in all human influenza virus isolates [samples from people] in this century,” Pekosz said. “It’s almost invariable.”

A vaccine that targets a stable protein like M2 would not have to be reformulated every year. Other groups are working on a similar idea, but using different approaches.

Pekosz’s group now is testing vaccine candidates in mice, and it has generated candidates that protect mice from human and avian influenza viruses. The group is trying to understand how the antibodies are protecting the mice and how the virus will respond to the presence of anti-M2 antibodies.

"The M2 protein hasn't mutated much in the past century," Pekosz said. "If we suddenly start to immunize people with the protein, will the virus then begin to change, and what's the consequence of those changes?"

To find that out, his group is immunizing animals and looking for mutants of the virus that grow or respond differently to the antibody treatment.

Pekosz and his colleagues still are characterizing how effective the vaccines will be and how the virus will respond, but he says within six months to 12 months they may be ready to test the vaccine candidates in people.

VIRUS VERSUS VIRUS

Another new way to protect people against influenza is by using a harmless virus, such as an adenovirus, as a carrier, or vector, for a vaccine.

Molecular virologist Mittal Suresh and collaborators at Purdue University in Indiana are working on an adenovirus vector with funding from NIAID.

"Our approach is to use an adenovirus to deliver some components of the bird flu virus in a vaccine formulation," Mittal said. "We already know how to grow large amounts of adenovirus and how to purify it because adenoviruses already are used in clinical trials for gene therapy as vectors."

"In an adenovirus vector," Pekosz said, "you can simply inoculate a person. That virus doesn't cause disease but it will make the HA protein and your body will respond, make the HA antibodies and then protect you from the flu."

Some of the adenovirus processes already are approved for human use, he added, so using them for people would be a matter of having an already-approved process approved for a new use, which takes less time than changing the process to generate a vaccine.

Such processes can be used to create vaccines in a much shorter time than with egg-based methods, and they would be more useful in fighting a pandemic.

"Our M2 strategy relies on protein expressed in the bacteria that we purify," Pekosz said. "In my laboratory here I could probably brew up enough for 1,000 doses in 24 to 48 hours."

THE FUTURE

Vaccines are one of the best ways to protect against a global influenza epidemic, but they are not the only way to slow it down.

NIAID Director Dr. Anthony Fauci says handling an influenza pandemic requires a comprehensive strategy that includes surveillance, vaccines, anti-viral drugs, international collaboration and transparency in reporting cases, and public health measures.

Pekosz agrees.

“We need to put more of an emphasis these days on public health and epidemiology,” he said. “We need to follow the H5N1 situation very carefully and not just rely on vaccines and anti-virals, but emphasize basic public health measures, such as standard hygiene, including issues of respiratory masks and hand sanitizers.”

When should people start putting on respiratory masks?

“Especially in this day of world travel and the rapid movement of people,” Pekosz said, “as soon as a pandemic strain emerges, you want to try to instill some level of public health measures almost immediately.”

In a worst-case scenario, if a population has weeks rather than months or years to protect itself against a pandemic, he said, “we’re going to need all of our tools to try to control the infection.”