Virologists developing more potent vaccine technology

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Virginia Tech virologist Chris Roberts' goal is to develop a platform for a flu vaccine that allows rapid modifications to meet new strains of flu.

Since 90 percent of complicated flu cases occur among those over 65, the associate professor in biomedical sciences and pathobiology has been working on a novel flu vaccine for the elderly. That is still his aim, but he is now more motivated than ever to speed development of his cell culture-based vaccine technology that is more rapid than the egg-based growth system presently used to create vaccines.

Influenza is an enveloped virus. It obtains its envelope or membrane as it buds from the surface of the host cell it has invaded. Roberts is using this practice against the virus - introducing membrane-bound immune-system stimulatory molecules such as cytokines into cells in such a way that the virus will incorporate them as part of its envelope. "Using this approach, inactivated influenza vaccines can be created that have enhanced immunogenicity, meaning they can boost our immune response to the vaccine and hopefully provide better protection against invading viruses," Roberts said.

Normally, cytokines are secreted proteins that boost and direct the immune system's response to inflammation and infections. When a foreign particle gets into the body, the body ultimately responds by stimulating 1) B cells to secrete anti-viral antibodies, 2) cytotoxic T cells to kill infected host cells, and 3) helper T cells to regulate and control the response of both cell types. Antibodies work by recognizing and binding

to specific components of the virus such as the glycoproteins on the surface of the virus (envelope). This serves to neutralize the ability of the virus to infect cells in the respiratory tract. A vaccination introduces weakened or killed forms of a virus so that the body recognizes the pathogen and begins producing antibodies to fight it. These antibodies are then ready to fight off infection should they encounter the virus.

Roberts' vaccine goes a step further and provides an immune-boosting signal on the surface of the vaccine.

Presently, vaccines are made from eggs and it generally takes one or two special pathogen-free eggs per dose. It also takes four to five months to prepare enough doses of the vaccine for a given year. Several companies are actively working to develop cell culture based vaccines for flu, such as is already used for polio and chickenpox vaccines, for instance. "The process could someday allow us to reduce the amount of time required to make flu vaccines," said Roberts. "Cell culture based vaccines would also help us respond more rapidly when new viral strains emerge."

Roberts' approach, to have the virus clothed in its own vaccine, capitalizes on the use of cell culture based systems for vaccine production. Roberts' group uses molecular biology techniques to fuse specific cytokines to components of the viral glycoproteins that facilitate their recognition by the virus assembly machinery. The resulting cytokine fusion proteins are then expressed in a virus permissive cell line and are actively incorporated into newly formed virus particles once those cells are infected with the virus. Now, when the virus leaves its host cell, it has cytokines bound to its outer surface and these particles are harvested, purified, and then chemically inactivated to create the vaccine. Importantly, these "killed vaccines," which Roberts' has dubbed FLU CYT-IVACs (for FLU CYTokine bearing Inactivated VACcine), still retain the bioactivity of cytokines.

The research has been tested in young adult mice and several CYT-IVAC formulations have shown promise in providing enhanced protection against viral pneumonia. Roberts noted, "Preliminary testing has also revealed that some of these FLU CYT-IVACs are better at protecting aged or old mice against viral pneumonia than non-modified vaccine."

He is already expanding this research to include the use of human specific cytokines in the FLU CYT-IVAC formulations. "Prior to being used as a human vaccine, these humanized FLU CYT-IVACs will have to undergo rigorous testing to ensure vaccine safety and this will require additional funding, which we are actively pursuing," Roberts said.

"The significance lies in the versatility of the cell culture-based vaccine platform; you can custom make a vaccine to tailor to the present need - such as swine flu," Roberts said. "And you can produce an immune-boosting response in populations with lower immunity."

Flu virus primer

The influenza virus is an RNA virus. It includes Influenza A, B, and C. Components of A and B are included in yearly vaccines. Type C is rare and sporadic. Influenza has a broad host range with swine and birds being the most important reservoirs for types that can infect humans, although most viruses are species specific.

Epidemics are caused by antigenic drift - mutations that make the virus unrecognizable to the antibodies induced by last year's vaccine, for example.

Seasonal flu usually results in an infection rate of 5 to 20 percent with about 36,000 deaths each year. But there have been three pandemics that killed millions of people, all caused by a totally new subtype of the

Influenza A virus.

Spanish flu of 1918 - H1N1
Asian flu of 1957-58 - H2N2
Hong Kong flu of 1968-69 - H3N2
The letters and numbers refer to subtype of the viral surface proteins -- hemagglutinin (H) and neuraminidase (N).

Usually the older strains disappear once new strains spread throughout the population. "Diagnosis of newly emerging strains will continue to be difficult because we still are unable to predict all the variations in influenza strains that will evolve in nature," said Roberts.

The first occurrence of the bird flu was 1997. There have been 421 confirmed cases with 257 deaths as of April 23, 2009. "So it is very lethal but not very conducive for human to human spread," Roberts said.

Swine flu

This appears to be a "mixed" strain that possesses genetic elements derived from humans, avian species, and swine species of the virus, which makes it more difficult to pinpoint the exact origin.

Through the efforts of the World Health Organization and numerous national agencies worldwide there is now a concerted surveillance effort that actually led to fairly quick assessment of this outbreak. Those agencies are to be commended, Roberts said.

It may be too soon to predict how this strain will evolve during the next several months, which makes vaccine design challenging. Existing vaccines would be unlikely to offer much protection.

More information: The results of the mice trials have been published in

the April 24, 2009 issue of *Virology Journal* ("Membrane-bound Cytokines Augment Influenca Virus Vaccines and Protect Against lethal Challenge in Mice," by Andrew S. Herbert, Lynn Heffron, Roy Sundick, and Paul C. Roberts). He and his collaborators also recently published a vaccine study in poultry in the January 2009 *Journal of Interferon and Cytokine Research* ("A Novel Method to Incorporate Bioactive Cytokines as Adjuvants on the Surface of Virus Particles," by Yufang Yang, David Leggat, Herbert, Roberts, and Sundick).

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