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New DNA vaccine technology poised to deliver safe and cost-effective disease protection

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New and increasingly sophisticated vaccines are taking aim at a broad range of disease-causing pathogens, targeting them with greater effectiveness at lower cost and with improved measures to ensure safety.

To advance this quest, a research team led by Roy Curtiss, director of the Center for Infectious Diseases and Vaccinology, and Wei Kong, a research assistant professor, at Arizona State University's Biodesign Institute have taken a dramatic step forward, revealing the design of a universal platform for delivering highly potent DNA vaccines, by employing a cleverly re-engineered bacterium to speed delivery to host cells in the vaccine recipient.

"The technology that we're describing in this paper can be used to develop a vaccine against any virus, any parasite, any fungus, whereas this was never possible before the development of recombinant attenuated bacterial strains like those produced in our lab," Curtiss says.

The experimental vaccine described in the new research demonstrated complete protection from influenza in mice, but Wei Kong, the leading author of the new study stresses that the innovative technique could be applied to the rapid manufacture of effective vaccines against virtually any infectious invader at dramatically reduced cost and without risk to either those vaccinated or the wider public.

"By delivering the DNA vaccine using a recombinant attenuated bacterium, we can get 10,000-100,000 doses per liter of culture," Kong says, an improvement of 3-4 orders of magnitude over use of the naked plasmid DNA, which must be painstakingly isolated from bacteria before injection.

The group's research results appear in the online Early Edition (EE) of the Proceedings of the National Academy of Sciences, the week of November 5, 2012.

Designing a vaccine that is both safe and effective presents a kind of Catch-22 for researchers. Live pathogenic strains typically generate a robust immune response, mimicking natural infection, but many challenges exist in terms of ensuring such strains do not cause illness or escape into the environment, where they have the potential to remain viable. Killed pathogen strains or vaccines produced from pathogen subunits sacrifice some of their immunogenic effectiveness for enhanced safety, and may require subsequent booster doses to ensure continued effectiveness.

The Curtiss team has worked to combine safety and effectiveness in orally administered vaccines that can be produced at a fraction of the cost of traditional methods. To do this, they have pioneered techniques using Salmonella—the notorious agent associated with food-borne illness—as a cargo vessel to deliver a suite of disease antigens to the recipient. The result has been the development and ongoing refinement of so-called RASVs (for recombinant attenuated Salmonella vaccines), capable of provoking an intense, system-wide immune response and conferring effective immunity.

One of the key innovations developed earlier by Wei Kong and other members of the Curtiss group, is a specialized Salmonella strain that can be timed to self-destruct in the body once it has carried out its

immunization duties. To create this strain, the researchers modified the bacterium in such a way that it can only survive on a non-naturally occurring form of sugar. Once the Salmonella cells exhaust their store of specialized sugar, supplied to them as part of the vaccine, they are unable to maintain the integrity of their cell walls and they essentially implode. "This crucial safety feature ensures that Salmonella are unable to persist as living organisms to survive if excreted into the environment," says Kong.

This self-destruct feature can be fine-tuned so that the bacteria fully colonize host cells, provoking a strong response from both humoral and cell-mediated arms of the immune system. Inside host tissues, recombinant Salmonella are able to synthesize protective antigens, releasing their contents when they become unstable and lyse into the intracellular fluid or cytosol.

The group demonstrated the effectiveness of this delayed-lysis bacteria in vaccine experiments with a variety of pathogens, including influenza and mycobacteria (causative agent of tuberculosis) and an RASV vaccine developed in the Curtiss lab against infant pneumonia is currently in FDA Phase I clinical trials. This earlier work focused on producing protective protein antigens in a bacterium, which would subsequently release a bolus of these antigens when the bacterial cell lysed within host cells and tissues.

In the latest research, the group sought to turn a delayed-lysis Salmonella strain into a universal DNA vaccine delivery vehicle. DNA vaccines stimulate cellular and humoral immune responses to protein antigens through the direct introduction of genetic material, prompting host cells to manufacture specific gene products. This is a crucial advance as it allows for the production of antigens that undergo host cell modification through the addition carbohydrates—a process known as glycosylation. Such modified antigens, which occur in a broad range of pathogenic

viruses, fungi and parasites require synthesis by host cells, rather than by the attenuated bacteria.

"Here, we were able to deliver a vaccine whose DNA sequence induces the immunized individual to make the protective glycoprotein the way you would during a viral infection," Curtiss says. Previous efforts to achieve this advance for delivery of DNA vacines by bacteria date to 1995, but only now has such work come to fruition.

A number of key modifications to the delayed-lysis RASV were required for this feat, and the Kong and Curtiss team has worked intensively over the past 5 years to achieve them. A hyperinvasive form of Salmonella was constructed through recombinant DNA methods in order to maximize the vaccine vector's ability to invade host cells and become internalized.

Following host cell uptake, Salmonella are encased in a membrane-bound endosome known as the Salmonella Containing Vacuole. The RASV was further modified to permit escape from the endosome so that the mature bacterium could spew its immunogenic contents into the host cell's cytosol.

Finally, further revisions to the Salmonella strain were applied to diminish the pathogen's ability to cause host cell death, which would prevent the DNA vaccine from migrating to the host cell nucleus to induce the synthesis of protective antigens necessary for the immune response.

The authors note that their orally-administered RASV is markedly superior to earlier efforts which introduced DNA vaccines by means of intramuscular injection or gene gun. These methods fail to deliver the vaccine to both mucosal tissues and certain internal lymphoid tissues, vital to a sustained, protective immunity. "We can protect mice to doses

of influenza that would be lethal were they not effectively immunized," Curtiss says, adding that "RASV safety has been established in mice just two hours old as well as in pregnant and immunodeficient mice".

Influenza spreads around the world in seasonal epidemics, resulting in about three to five million yearly cases of severe illness and about 250,000 to 500,000 yearly deaths, rising to millions in some pandemic years. Current manufacture of influenza vaccines requires use of chick embryos or cell culture methods. Global capacity is limited, making sufficient vaccine to immunize everyone impossible. Adding to concerns about managing future naturally occurring influenza epidemics is the potential for bioterrorists to produce weaponized influenza strains created using plasmid-based reverse genetics systems. "Increasing the speed of producing a matching vaccine is key in the context of response to an influenza epidemic," Kong says.

The ability to rapidly engineer and scale up effective vaccines for influenza and other potentially lethal pathogens will require innovative approaches to vaccine design, manufacture and application. The universal DNA vaccine platform outlined in the new study represents an important advance.

"The vast majority of viruses including influenza, measles, mumps and HIV all have glycosylated proteins. You could never deliver protective immunity using a bacterium to produce those protein antigens," Curtiss says. "But now we have the opportunity to produce vaccines against such pathogens," Kong says. Further, the technique permits large quantities of DNA vaccine to be produced rapidly at low cost, freeze-dried and stockpiled to be used when needed.

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