Nanoparticles for the flu

By Tracey Baas, Senior Editor

An NIH team has created a self-assembling influenza nanoparticle that induces the production of antibodies against a wider range of flu strains than traditional vaccines.¹ The team hopes to test the immunogenicity of the vaccine in humans once a GMP manufacturing process is developed.

The nanoparticle consists of a fusion of the influenza A virus hemagglutinin (HA) from New Caledonia H1N1 1999 strain and ferritin, an iron-storage protein from *Helicobacter pylori*.

Ferritin self-assembles into nanoparticles, and previous work has shown nanoparticles made up of 24 ferritin monomers could be used to display 24 peptides on the capsid surface by engineering an N-terminal fusion peptide with ferritin. The researchers used the peptide-ferritin nanoparticles in rats to generate high titers of antibody to an HIV Tat protein antigen.²

The NIH team hypothesized that the spacing between the ferritin units in the nanoparticle could yield HA proteins in the form of spike-like trimers that would mimic exactly the presentation of the protein on the surface of the influenza virus.

The group expressed the HA-ferritin fusion protein in mammalian cells and showed using electron microscopy that the self-assembled spherical nanoparticle presented eight trimeric viral spikes. To confirm the antigenicity of the HA trimer, the group showed that two mAbs against the H1N1 New Caledonia 1999 HA—one against the stem and one against the head—bound to the HA-ferritin nanoparticle.

In mice, an adjuvant plus the HA-ferritin nanoparticle led to about seven times more neutralizing antibodies than adjuvant plus a conventional trivalent inactivated influenza vaccine containing the same strain.

The HA-ferritin vaccine also induced broadly neutralizing antibodies that cross-reacted with mismatched virus.

The team then used the strategy to create a trivalent vaccine formulated as a single dose. They incorporated three HA-ferritin nanoparticles, presenting HA from the H1N1 California 2009 strain, the H3N1 Perth 2009 strain and the Florida 2006 strain.

Mice vaccinated with the trivalent HA-ferritin nanoparticle formulation plus adjuvant showed higher levels of neutralizing antibodies than mice vaccinated with a conventional trivalent vaccine plus adjuvant.

Ferrets immunized with the HA-ferritin nanoparticle plus adjuvant produced about ten times more neutralizing antibody than ferrets immunized with a conventional vaccine plus adjuvant. In addition, sera from the nanoparticle-immunized ferrets neutralized four of six different types of H1N1 viruses, whereas sera from conventionally immunized ferrets neutralized only one type of H1N1 virus.

Ferrets that were immunized with adjuvant plus HA-ferritin nanoparticles containing HA from New Caledonia H1N1 1999 and then challenged with a different strain of the virus showed less viral shedding and weight loss than ferrets immunized with adjuvant plus a regular trivalent vaccine.

Finally, the researchers performed detailed neutralization assays to help explain the broadly neutralizing capability of the antibodies induced by the HA-ferritin.

Those studies showed that the HA-ferritin nanoparticle vaccine induced antibodies targeting conserved regions of both the HA stem and the HA head in all the nanoparticle-immunized ferrets, in contrast to those given the conventional trivalent vaccine.

Antibodies that target conserved regions of the HA stem and the receptor binding site on the HA head should help limit viral escape.

Results were published in *Nature*.

“The studies are quite impressive to show that the vaccine can generate antibodies that recognize a conserved epitope within the receptor binding site of HA,” said JoAnn Suzich, VP and head of infectious disease and vaccines research at MedImmune LLC, the global biologics research and development arm of AstraZeneca plc.

The protein sequence of the HA head allows the virus to bind and enter host cells and is made up of conserved and variable regions that are used to categorize the different strains of influenza A virus. A vaccine that generates broadly neutralizing antibodies against the conserved receptor binding site on the HA head could thus protect against multiple viral strains.

HA's humors

The NIH team now plans to test the safety and immunogenicity of the HA-ferritin nanoparticle in humans.

“Immunogenicity can be assessed both with hemagglutination-inhibition and virus-neutralization assays. These tests will determine whether the nanoparticle vaccine can elicit similar cross-protective antibodies in humans,” said Gary Nabel, who led the NIH team and was previously director of the NIH Vaccine Research Center. “Typically as you test immune responses in higher species, vaccine potency decreases, but the potent responses seen in both mice and ferrets give us hope that the potency will also be seen in humans.”

Nabel, who became SVP and CSO of Sanofi at the end of 2012, added, “because the vaccine leads to generation of antibodies that target both the HA stem and the receptor binding site on the HA head, we think the possibility that viruses will escape neutralization from either of two different antibodies is low.”

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**—Gary Nabel,
National Institutes of Health**

Sanofi had no involvement in the *Nature* work.

Steffen Mueller, president and CSO of viral vaccine company **Codagenix Inc.**, thinks manufacturing will be a challenge.

“The big question is, will they be able to make the nanoparticles in the amounts needed in a cost-effective way,” noted Mueller. “The science is very intriguing at the research laboratory scale, but to make this commercially feasible, they are going to have to take it to GMP levels, and that may be difficult because you’re going to have to produce a lot of nanoparticles.”

Nabel agreed: “Ultimately, the Vaccine Research Center team at NIH is going to have to see if it can manufacture the HA-ferritin nanoparticles in a simple way that can be scaled up before going on to production. It’s possible because it’s essentially one gene being expressed in mammalian cell culture.”

The NIH team is also deciding which HA-ferritin nanoparticle vaccination strategy is most attractive.

“If the goal is to simply try to replace the seasonal trivalent or quadrivalent vaccines with a comparable formulation using the nanoparticles, the team can do that right now,” said Nabel. “In terms of a universal vaccination, they could use multiple, sequential vaccinations of nanoparticles with different HA strains to provide broad protection. They could also attempt to create an HA that doesn’t exist in nature but stimulates broad protection. At this stage, there is a lot of flexibility to develop different therapeutic strategies.”

“What I would really be interested in seeing is for them to move closer to providing a universal vaccine by further modifying the HA so that it would produce even more broadly neutralizing antibodies,” said Suzich.

Mueller also wanted to see whether the HA-ferritin nanoparticles provide better immunogenicity than another vaccination strategy in clinical development—influenza virus-like particles (VLPs).

Medicago Inc. and **Novavax Inc.** each have VLP-based vaccines against pandemic influenza and seasonal influenza in Phase I and II testing.

“Basically the NIH team’s HA-ferritin nanoparticle is acting like a VLP that presents only HA. Other VLPs are being developed that present not only HA but also neuraminidase and sometimes matrix proteins,” said Mueller.

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—JoAnn Suzich, MedImmune LLC

Nabel thinks nanoparticles might provide better surface protein presentation to the immune system than do VLPs.

“The HA is displayed on the particle in a regular conformation but not too tightly packed so that the immune system can better see the viral spike,” he said. “In the traditional vaccine or some VLPs, HA is more crowded and may be less accessible.”

Beyond the flu

The NIH researchers also are interested in using the nanoparticle as a template to create vaccines that present proteins from other pathogens.

“There is a lot of interest in using the nanoparticle to express proteins that have a similar intermolecular distance between monomeric units of trimers, like the team did with influenza’s HA trimers,” said Nabel. “Some possibilities would be HIV, herpes simplex virus (HSV) or respiratory syncytial virus (RSV).”

He said that the NIH team is most interested in HIV. “Preliminary structural preparations show that the trimeric structure of the HIV-1 envelope protein would be amenable to this strategy. They’ve also got their eye on malaria but would have to determine which protein might be best incorporated into nanoparticle presentation.”

A patent application has been filed and is available for licensing from the NIH Office of Technology Transfer.

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Contact: Gary Nabel, Sanofi, Cambridge, Mass.
e-mail: gary.nabel@sanofi.com
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COMPANIES AND INSTITUTIONS MENTIONED

AstraZeneca plc (LSE:AZN; NYSE:AZN), London, U.K.

Codagenix Inc., Stonybrook, N.Y.

Medicago Inc. (TSX:MDG; OTCQX:MDCGF), Quebec City, Quebec, Canada

MedImmune LLC, Gaithersburg, Md.

National Institutes of Health (NIH), Bethesda, Md.

Novavax Inc. (NASDAQ:NVAX), Rockville, Md.

Sanofi (Euronext:SAN; NYSE:SNY), Paris, France