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Anti-flubodies

By Michael J. Haas, Senior Writer

Two separate teams have isolated human antibodies that neutralize many variants of influenza antigen hemagglutinin A and prevent viral entry and infection. Both teams are developing their antibodies as therapeutics but are going after different markets: one is targeting pandemic flu outbreaks whereas the other wants to use its antibodies to treat or prevent seasonal flu in immunocompromised populations.

Though potentially useful additions to the anti-influenza armamentarium, the antibodies are not likely to replace vaccines as the primary defense against the disease.

Influenza antigen hemagglutinin A is a protein expressed on the viral surface that has two main regions: a globular head that enables viral attachment and fusion and a stem region that is buried in the viral envelope and undergoes a conformational change to allow viral entry and infection after fusion. There are 16 different hemagglutinin subtypes that fall into two groups. The 10 subtypes in Group 1 include H1, which occurs most frequently in seasonal influenza strains but also includes the pandemic 1918 flu virus, and H5, which includes the highly pathogenic strains of avian flu. Among the six Group 2 subtypes is H3, which also occurs in seasonal flu strains.

Another surface protein, neuraminidase, helps mature virus exit the infected host cell so that it can infect a new cell. Neuraminidase has nine subtypes, each of which can occur in combination with any hemagglutinin subtype. Thus, influenza viruses are designated according to their specific hemagglutinin and neuraminidase subtype—for example, H5N1.

Influenza infections and vaccinations mostly stimulate antibodies against the hemagglutinin head because it is exposed to the immune system during viral fusion and entry. The problem is that the head region mutates readily without losing its attachment or fusion function, thereby evading anti-hemagglutinin antibodies.

Such mutations also make it difficult to predict which influenza strain will be in circulation from one season to the next. Thus, there is a market for therapeutics or prophylactics that are simultaneously effective against many strains of seasonal and/or pandemic strains.

Flu screens

A team from the **Dana-Farber Cancer Institute**, the **Burnham Institute for Medical Research** and the **Centers for Disease Control and Prevention** (CDC) set out to isolate antibodies that would block multiple strains of H5N1 influenza.

They screened a human antibody library against recombinant H5 and isolated three leads that neutralized all nine Group 1 subtypes that they tested against in human cell lines.

Mice injected with any of three antibodies one hour before challenge with H5N1 or up to 48 hours after viral challenge survived and showed few or no clinical signs of infection. The antibodies also protected mice from two lethal strains of H1N1.

X-ray crystallography of an H5 hemagglutinin-antibody cocrystal showed that the antibody bound an epitope on the hemagglutinin stem that is conserved across all Group 1 subtypes.

All six Group 2 subtypes had a different conserved epitope at this stem position.

In a report in *Nature Structural and Molecular Biology*, the team said their results suggest that the antibodies could provide broad-spectrum protection against pandemic and seasonal flu viruses.[1]

On a conference call, team coleader Wayne Marasco, associate professor of medicine at Dana-Farber and Harvard Medical School, said the group is developing the antibodies primarily to contain pandemic outbreaks. The antibodies would be given to family members, co-workers and healthcare workers who come in close contact with an infected individual, he said.

"These antibodies are ready to go," team coleader Robert Liddington told *SciBX*. They could "snuff out a pandemic."

Liddington is professor and director of the infectious disease program at Burnham. Ruben Donis, chief of the CDC's molecular virology and vaccines branch, also co-led the *Nature Structural and Molecular Biology* study.

Another flu in the ointment

In addition to the *Nature Structural and Molecular Biology* paper, two papers from **Crucell N.V.** and others described the identification of human antibodies that could neutralize different hemagglutinin subtypes and detailed the underlying mechanism of how the neutralization occurred.[2,3]

In a paper published in *PLoS One*, the team constructed combinatorial display libraries using antibodies recovered from the serum of individuals inoculated against H1N1 virus and then screened those libraries against recombinant H5 hemagglutinin. The lead antibody from that screen, CR6261, neutralized all six Group 1 subtypes of hemagglutinin it was tested against; the team did not test the other four subtypes.

The antibody also protected mice from challenge with lethal strains of H5N1 or H1N1.

In a follow-on study in *Science*, another Crucell-led team reported structural studies of an H5 hemagglutinin–CR6261 cocrystal that showed that the antibody targeted the same conserved epitope identified in the *Nature Structural and Molecular Biology* study.

The *PLoS One* team, led by Crucell CSO Jaap Goudsmit, also included researchers from **The University of Hong Kong**'s Queen Mary Hospital, **Algonomics N.V.**, **Wageningen University**, **Johann Wolfgang Goethe University** and **Bambino Gesú Children's Hospital**.

The *Science* team was led by Ian Wilson, professor of molecular biology at **The Scripps Research Institute**, and included Goudsmit and other

researchers from both Crucell and Scripps.

Scripps' Wilson told *SciBX* that a team of researchers from Scripps and **Sea Lane Biotechnologies LLC** had previously reported antibodies that neutralized both the H1 and H5 subtypes.[4] However, the researchers could not identify the actual hemagglutinin epitope targeted by the antibodies because they did not have a hemagglutinin-antibody cocrystal structure.

New flu tools

Although the antibodies identified by the *Nature Structural and Molecular Biology* and Crucell teams could help treat or prevent both seasonal and pandemic influenza, logistical and commercial drawbacks might limit utility.

Penny Heaton, CMO of **Novavax Inc.**, noted that more than one dose probably would be required during the course of a season or pandemic because the typical antibody has a half-life of four to six weeks.

Thus, she said, "antibodies are not the be-all or end-all treatment for pandemic or seasonal flu."

Novavax has virus-like particle (VLP)-based vaccines against pandemic influenza in Phase II testing and expects to begin two Phase IIa trials of a VLP-based vaccine against seasonal flu this year.

Bill Enright, president and CEO of **Vaxin Inc.**, agreed that half-life is important.

Vaxin is developing seasonal and pandemic influenza vaccines that express H1 or H5 in an adenovirus vector. The pandemic vaccine is in Phase I testing; the company hopes to begin Phase II testing of the seasonal vaccine this year. The vaccines are delivered by intranasal inhalation rather than intramuscular injection.

> **"Antibodies are not the be-all or end-all treatment for pandemic or seasonal flu."**
> ***—Penny Heaton, Novavax Inc.***

"We have seen immune responses to our vaccines that last more than one year," Enright said.

Alan Shaw, president and CEO of **VaxInnate Corp.**, also thinks an influenza antibody would face several hurdles. He said the doses needed to protect mice in both the *Nature Structural and Molecular Biology* and Crucell studies translated to huge doses in humans: about 1,500 mg for a 100-kg person. Thus, Shaw thinks it would be very hard to produce and distribute sufficient quantities of the antibodies and that production and prescription costs would be prohibitive.

VaxInnate has H1- and H5-based vaccines in Phase I testing for seasonal and pandemic influenza.

Both Larry Smith, VP of vaccine research at **Vical Inc.**, and George Kemble, VP of R&D and general manager of vaccines at the **MedImmune Inc.** subsidiary of **AstraZeneca plc**, agreed that the antibodies faced major logistical hurdles.

Smith pointed to the unknown treatment dose, unknown duration of protection and potentially high cost of antibody protection as significant disadvantages.

Last year Vical completed Phase I testing of a plasmid DNA vaccine encoding H5 to prevent pandemic influenza.

"The quantity of material required to protect an adult population from infection for three to six months or more during a pandemic is staggering," Kemble said.

He suggested that using the antibodies to treat individual cases of influenza would be a more manageable option. "Having another tool at the bedside to intervene in what could be a severe or lethal infection would complement, but not replace," prophylactic vaccinations, he said.

MedImmune markets FluMist, a live attenuated influenza vaccine (LAIV) to prevent seasonal flu. The company also has an LAIV in Phase I testing to prevent pandemic flu.

Goudsmit noted that Crucell's CR6261 also protected ferrets from influenza up to six days after infection at doses that are feasible in humans. The company plans to develop CR6261 to treat or prevent seasonal influenza in at-risk populations. These include children, the elderly and immunocompromised individuals who are not always well protected by marketed vaccines.

According to Goudsmit, seasonal flu infects about 200,000 elderly people in the U.S.—of which about 35,000 die—and the U.S. represents about half of the $2.2 billion global market of elderly patients. "The size of the problem observed in these populations with seasonal flu vaccines indicates a major unmet medical need," he said.

On the pandemic front, Heaton said antibodies might not be the best approach.

"Pandemic infection is so aggressive that the early-stage immune response causes respiratory problems" and eventually pneumonia, she noted. The reason is the virus spreads through the respiratory system quickly—a process that is inhibited primarily by the immune response to neuraminidase. Thus, an anti-hemagglutinin antibody might not halt respiratory spread of a pandemic virus.

Heaton said Novavax's pandemic and seasonal influenza vaccines offer broader protection than antibodies because they are based on recombinant hemagglutinin, neuraminidase and one other viral surface protein, matrix protein 1. She said this combination induces three distinct immune responses in a vaccinated individual: antibodies against hemagglutinin, a cell-mediated response to neuraminidase that slows the progression to pneumonia and the induction of cytotoxic lymphocytes that kill infected cells.

Heaton added that it was almost impossible to completely prevent viral entry. Thus, "depending on an anti-hemagglutinin antibody alone is a very tall order," she said.

Vical's Smith agreed. Although developing a vaccine from the findings of the *Nature Structural and Molecular Biology* and Crucell teams would require more work than developing the antibodies, "vaccination ultimately would be a better strategy for controlling the morbidity and mortality of influenza viruses," he said.

Antibody flight plans

Marasco said the *Nature Structural and Molecular Biology* team will seek partners in government or industry to develop the antibodies. The team hopes to take the antibodies into the clinic by the winter of 2011–2012.

Liddington said the team's other priorities include isolating antibodies against the Group 2 epitope and performing additional screens to identify back-up antibodies against Group 1 subtypes. A combination of antibodies targeting Group 1 and Group 2 subtypes could be used against any flu virus, he said.

Indeed, Goudsmit said Crucell has already isolated an antibody that targets the conserved Group 2 epitope and plans to develop it in combination with CR6261 to treat or prevent seasonal flu in at-risk populations.

The company expects to start clinical trials within the next two years, he said.

The findings reported in *Nature Structural and Molecular Biology* are patented by Dana-Farber and Burnham and are available for licensing, according to Ruth Emyanitoff, senior licensing manager at Dana-Farber. The findings reported in *PLoS One* and *Science* are patented by Crucell.

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COMPANIES AND INSTITUTIONS MENTIONED

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