The neglected female rodent
1571

California compromise
1574

SWINE FLU PANDEMIC

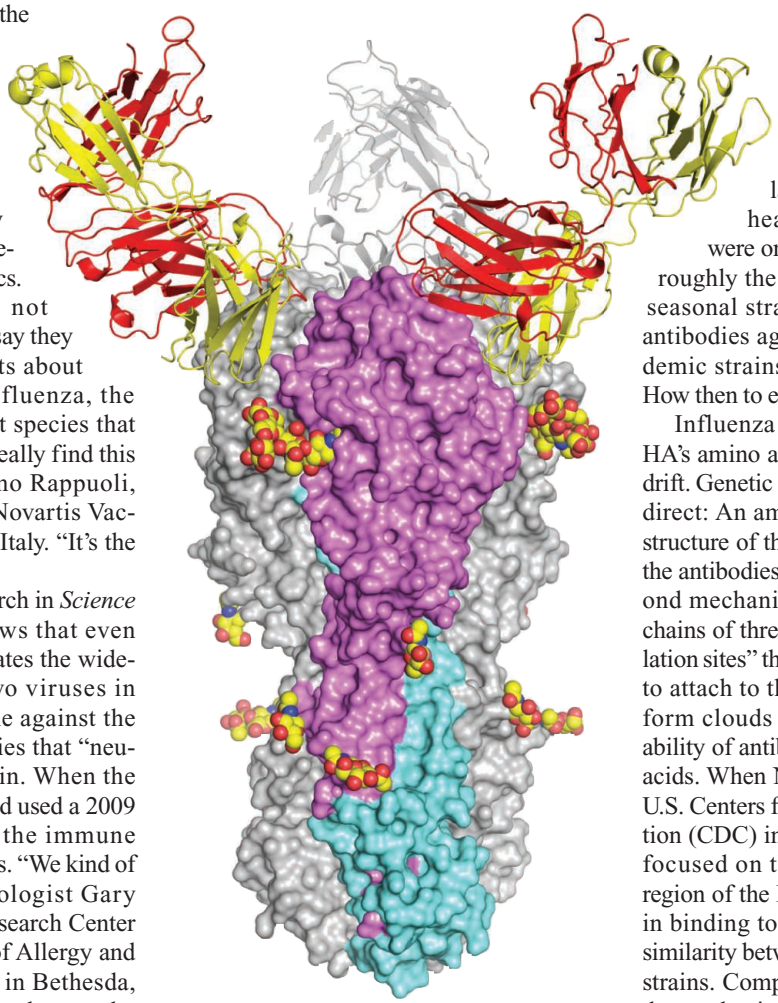
What's Old Is New: 1918 Virus Matches 2009 H1N1 Strain

The "novel" H1N1 swine influenza virus that last year caused the first human pandemic in 4 decades has one feature that is hardly novel: Its surface protein, hemagglutinin (HA)—which spikes cells and starts an infection—closely matches the HA in the H1N1 virus responsible for the 1918 pandemic. Separated by 91 years, the two strains of the highly mutable virus ought to be vastly different. This newfound similarity answers many mysteries about the 2009 pandemic, including why it largely spared the elderly. The new findings from different research groups also suggest intriguing explanations for how the 1918 influenza virus has evolved since it swept across the globe in several waves, killing more than 50 million people by the winter of 1919. And the investigators are proposing provocative—some say far-fetched—vaccination strategies to preempt future pandemics.

Influenza researchers not involved with the new studies say they pull together several concepts about the relationship between influenza, the immune system, and different species that have been gaining ground. "I really find this a fascinating story," says Rino Rappuoli, head of vaccine research at Novartis Vaccines & Diagnostics in Siena, Italy. "It's the lesson of evolution."

One study published 24 March in *Science Translational Medicine* shows that even though nearly a century separates the widespread circulation of the two viruses in humans, mice given a vaccine against the 1918 strain produced antibodies that "neutralized" the novel 2009 strain. When the team flipped the experiment and used a 2009 pandemic vaccine in mice, the immune response stopped the 1918 virus. "We kind of did a double take," says virologist Gary Nabel, head of the Vaccine Research Center at the U.S. National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, Maryland, and the lead researcher on the project. "It was an unexpected finding, but it all makes sense when you look at the data collectively." Although he acknowledges the limits of extrapolating from mouse to human immune systems, Nabel says in this restricted analysis of antibodies in response to proteins, "it's a very reasonable model."

Influenza and the human body are like opposing Cold War spies, with the virus repeatedly donning new disguises and the human immune system racing to foil each incarnation. HA is the virus's main quick-change artist. Antibodies produced by the immune system, in turn, try to neutralize HAs by binding to them, blocking the virus from entering cells. As a rule, influenza viruses change so quickly that a vaccine against a regular "seasonal" strain circulating one year may have little impact against a similar strain a few years later. Yet the HA proteins on the 1918 and 2009 pandemic viruses look remarkably similar in close analyses done in both Nabel's study and a separate one published online this week by *Science* that includes x-ray crystallographic data (www.sciencemag.org/cgi/content/abstract/science.1186430). These two reports also clarify the evolution of seasonal strains in the decades between the two pandemics.



Crystal ball. The 2009 pandemic virus has the same amino acids at the tip of its HA as the 1918 strain shown here bound to an antibody (red and yellow ribbons) taken from a survivor of the 1918 pandemic.

CREDIT: R. XU *ET AL.*, *SCIENCE* (ADVANCED ONLINE EDITION)

The two studies focus on the top part, or the head, of the HA, which is the business end of the protein when it comes to the infection process. Each research group calculated that the amino acids in the head of the two pandemic HAs were only about 80% similar, which is roughly the divergence seen between two seasonal strains. This would suggest that antibodies against the 1918 and 2009 pandemic strains would *not* cross-neutralize. How then to explain the mouse results?

Influenza foils antibodies by changing HA's amino acids—a process called genetic drift. Genetic drift occurs in two ways. One is direct: An amino acid change can alter the structure of the protein so that the "arms" of the antibodies can't get a good grip. The second mechanism involves sugars. Specific chains of three amino acids create "glycosylation sites" that allow sugars made by the cell to attach to the viral protein. These sugars form clouds around the HA, masking the ability of antibodies to "see" the right amino acids. When Nabel, Terrence Tumpey of the U.S. Centers for Disease Control and Prevention (CDC) in Atlanta, and their co-workers focused on the amino acids in a discrete region of the HA tip that plays a critical role in binding to cells, they discovered a 95% similarity between the old and new pandemic strains. Comparisons between seasonal and the pandemic strains in this region found less than 70% similarity.

In the second study, a team led by structural biologist Ian Wilson of the Scripps ▶

Research Institute in San Diego, California, went further, linking the amino acid sequence analysis to the three-dimensional structure. Wilson's group crystallized the 1918 and 2009 pandemic viruses and showed that the HA heads had distinctly similar shapes. What's more, the few amino acid differences between the strains were mainly confined to one small region of the head. In an additional experiment, they took an antibody from survivors of the 1918 epidemic, crystallized it in a complex with the 1918 virus, determined the amino acids in HA used to bind the antibody, and then showed that the 2009 pandemic strain had the same amino acids. "The closest related structure that we have to the current 2009 swine flu is the 1918 structure," says Wilson, who also analyzed sequences from many other influenza viruses that have circulated in humans between those two pandemics. "The papers come to similar conclusions about why some people are more resistant to the current swine flu."

Both the Wilson and the Nabel studies show that the HAs of the two pandemic strains also look markedly different from seasonal viruses when it comes to sugars. All seasonal strains have at least two glycosylation sites on the top of their HAs, whereas both the pandemic strains are bald. "The absence of glycosylation at the top of these molecules is making a huge difference in the immune response," says CDC virologist Ruben Donis, who was not involved with the study.

The new studies are helping to clarify how influenza viruses have used sugars in their evolution since 1918, says NIAID virologist Jeffrey Taubenberger, a leading investigator of that devastating pandemic. "All the influenza viruses in humans are descendants of the 1918 virus," says Taubenberger, who published mouse experiments 8 March online in *Influenza and Other Respiratory Viruses* that similarly show how the 1918 virus protects against the 2009 pandemic strain. "Over the last 91 years, we've been in one large 1918 pandemic era."

By analyzing the difference in the earliest available seasonal HAs from 1933 to 2009, Nabel's group found that some amino acid drift occurred and changed the structure of the HA head, but after that the bald virus started accumulating new glycosylation sites. Nabel posits that the bald 1918 virus could tolerate only a limited number of amino acid changes that altered its structure. "At a certain point, there's a fitness cost for adopting a new mutation, so the virus says, 'What else can I do?' " says Nabel.

In a perspective he co-authored in *Science Translational Medicine* about the study, Novartis's Rappuoli says people exposed to the bald 1918 virus and its sugar-free descendants that subsequently circulated for a few decades developed an immunity that later protected them from the 2009 pandemic strain. "Evolution does not necessarily bring new things," says Rappuoli. "It sometimes brings things back."

Sugar on top. Descendants of the 1918 virus dodged antibodies by mutating (red) the tips of the HA to change shape and hold glycans, but the 2009 pandemic strain (*far right*) turned back the clock.

The new evolutionary insights are leading researchers to revisit assumptions about another immune-evading trick that influenza exploits called genetic shift. Influenza viruses can swap whole genes, or reassort, with different strains. There are 16 different HAs and nine different neuraminidases, which is what the H and N numbers designate. The 1957 pandemic strain was just such a reassortant, with the H1N1 becoming an H2N2. A pandemic in 1968 saw a switch to H3N2. So many researchers assumed that the next pandemic would occur with H5, H9, or some other HA that few human immune systems had seen. "No one in the flu community predicted it would be an H1," says Taubenberger. "It took everyone by surprise, including me." Basically, immunity against the 1918 H1N1 had waned enough to create a niche for another bald H1 to return.

As Rappuoli notes, one of the lessons from the 2009 swine flu pandemic is that the reason the H1 remained bald in pigs since 1918 is because viral evolution differs dramatically in humans and other species. Humans live many decades, creating long-term relationships between the immune system in individuals and the influenza viruses they encounter during their lifetimes. "The virus is pushed to evolve quickly in humans if it wants to survive," says Rappuoli. Not so in pigs and birds, which are short-lived species—especially those on farms—that can pass influenza viruses to humans. The H1 in pigs thus has had little pressure to mutate and has remained frozen in evolution, says Donis, who calls swine "a warm freezer."

Rappuoli thinks a clearer understanding of the relationship between influenza viruses in humans and other species could be used to craft a new vaccination strategy to prevent pandemics. He proposes making vaccines against viruses that caused earlier pandemics by pulling out "archived" strains that have remained frozen for decades in pigs and birds. Chicken farmers might similarly be given an H5N1 vaccine to reduce the chances of that highly virulent "bird flu" strain, which does not spread well between people, adapting to humans.

Nabel offers his own forward-looking vaccine strategy. He predicts that the 2009 pandemic strain will follow the mutational path of the 1918 pandemic virus; as his paper went to press, he says he detected that process in four isolates. A vaccine could be developed that artificially glycosylates the novel 2009 H1N1 in a way that mimics glycosylated descendants of the 1918 strain. "We can look at how 1918 evolved in response to humans and preemptively take steps to contain and maybe even drive the 2009 pandemic strain out of existence," says Nabel.

Taubenberger notes that huge practical hurdles stand in the way of such "boutique" vaccines. But he, too, thinks the 2009 pandemic H1N1 may help humans outwit influenza in the long run. In particular, he says the 2009 pandemic virus might outcompete the H3N2 seasonal strain that now causes most of the deaths in the elderly. "It would be fantastic if a new pandemic virus, in which the elderly are somewhat protected, replaced a nasty seasonal virus that causes serious morbidity and mortality." Now, that would be a novel twist to the 2009 swine flu pandemic.

–JON COHEN

CREDIT: C.-J. WEI *ET AL.*, *SCIENCE TRANSLATIONAL MEDICINE* 2, 24 (24 MARCH 2010)