1918 and 2009 pandemic influenza viruses lack a sugar topping

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Although they emerged more than 90 years apart, the influenza viruses responsible for the pandemics of 1918 and 2009 share a structural detail that makes both susceptible to neutralization by the same antibodies. Scientists led by Gary J. Nabel, M.D., Ph.D., of the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health, describe the molecular basis for this shared vulnerability and suggest how it might be exploited to design vaccines matched to future pandemic influenza virus strains. The research appears online today in the journal *Science Translational Medicine*.

"This study defines an unexpected similarity between two pandemic-causing strains of influenza," says NIAID Director Anthony S. Fauci, M.D. "It gives us a new understanding of how pandemic viruses evolve into seasonal strains, and, importantly, provides direction for developing vaccines to slow or prevent that transformation."

In one set of experiments, the NIAID scientists and their colleagues including Terrence M. Tumpey, Ph.D., of the Centers for Disease Control and Prevention, injected mice with a vaccine made from inactivated 1918 influenza virus. Then they exposed the mice to high levels of 2009 H1N1 virus. All of the vaccinated mice survived. The reverse was also true: Mice vaccinated with inactivated 2009 H1N1 virus and then exposed to 1918 virus were protected from death. The researchers concluded that vaccination with either pandemic virus caused the mice to produce antibodies capable of neutralizing the other virus.

"This is a surprising result," notes Dr. Nabel. "We wouldn't have expected that cross-reactive antibodies would be generated against viruses separated by so many years."

Ordinarily, he explains, antibodies made in response to one year's seasonal flu strain do not fully react with, or cross-neutralize, seasonal flu strains that come along just a few years later. This is due in part to slight, yearly changes in the amino acid sequence of hemagglutinin (HA), a viral surface protein. The amino acid sequences of the 1918 and 2009 H1N1 influenza viruses in a portion of HA called the globular head differ by about 20 percent. That difference is on a par with amino acid divergence in the HA head region among seasonal strains, so the researchers reasoned that antibody cross-neutralization in the pandemic viruses must be due to some feature besides simply the degree of amino acid variation.

In a series of experiments and computer modeling studies, Dr. Nabel and his team determined that both pandemic viruses lack a cap of sugar (glycan) molecules at two specific spots on the top of HA's globular head. Without these sugars, both the 1918 and 2009 pandemic viruses have unfettered access to the receptors that HA uses to enter human cells. This viral advantage quickly diminishes as immunity provided by neutralizing antibodies arises in people who have been infected (and recovered) or when people are vaccinated.

In contrast to the 1918 and 2009 viruses, when the investigators analyzed the structure of seasonal flu strains that had circulated between 1977 and 2008, they found that 97.8 percent had one glycan molecule covering the HA's head, while 87.8 percent of the strains had two glycans. "The glycans act like an umbrella that shields the virus from the immune system," says Dr. Nabel. "They create a physical barrier over the virus and prevent antibody neutralization."

Further analysis of influenza sequences collected prior to 1977 revealed that the HA protein in the descendants of the 1918 influenza virus acquired glycans by the early 1940's. "Our study points to the key role played by glycans in protecting hemagglutinin from neutralization as pandemic viruses evolve into seasonal ones," says Dr. Nabel.

Next, the researchers engineered mutant pandemic flu viruses by placing sugar molecules on the two critical regions at the top of the globular head. Once covered, antibodies could no longer recognize and neutralize the virus. However, says Dr. Nabel, the sugar-capped viruses did perform well as vaccines.

In their last series of experiments, the investigators added sugar molecules to a 1918 influenza virus and vaccinated mice with it. The mice produced antibodies able to neutralize the original, sugar-free version of the 1918 virus. "We can use this knowledge to preemptively design vaccines with glycosylated versions of the newly emerged 2009 H1N1 pandemic influenza virus," says Dr. Nabel. Such a vaccine, he adds, would protect against the pandemic virus and might also limit the virus's chances of acquiring a sugar shield that would allow it to entrench itself as a seasonal variant.

More information: C-J Wei et al. Cross-neutralization of 1918 and 2009 influenza viruses: Role of glycans in viral evolution and vaccine design. Science Translational Medicine. DOI:10.1126/scitranslmed.3000799 (2010).

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