Influenza – New Strategies for Defeating an Old Enemy

Peter Palese, PhD

Scientia

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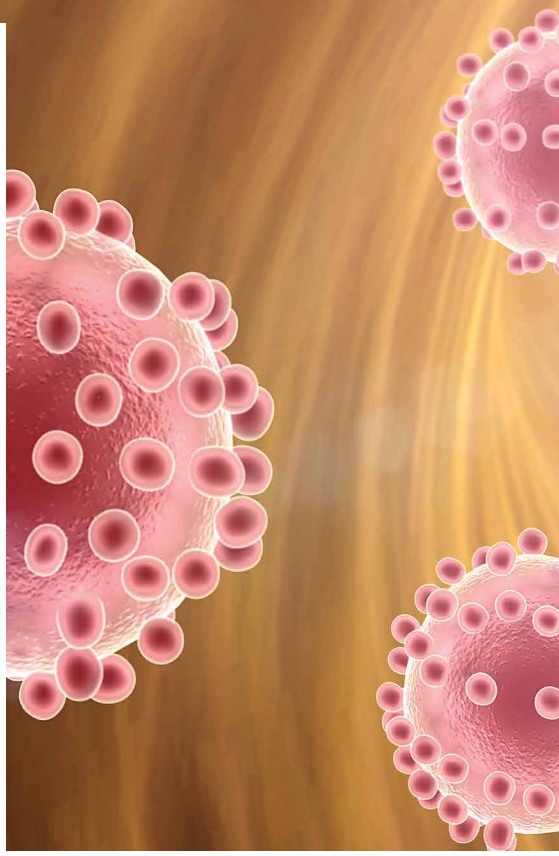

Influenza continues to be one of the most common respiratory diseases in humans and represents a significant public health burden, due to its associated morbidity and mortality. **Dr. Peter Palese** and his colleagues at the Icahn School of Medicine at Mount Sinai in New York are spearheading a ground-breaking project that aims to design a novel influenza virus vaccine. This vaccine will overcome many of the limitations faced by available vaccination approaches and, ultimately, provide universal and long-lasting protection against seasonal and pandemic influenza.

The Need for a Universal Influenza Virus Vaccine

Catching "the flu" is something we all dread and go to great lengths to avoid. However, influenza viruses are tricky characters, prone to fast evolutionary modification and a high mutation rate. This changeable nature allows their evasion of our body's humoral (antibody-mediated) immune response. Causing a wide range of symptoms, influenza viruses are considered significant human respiratory pathogens. They are one of the most common causes of acute respiratory disease and can result in high mortality and morbidity, particularly in high-risk populations such as infants, the elderly, and those with chronic illnesses. Surveillance data show that influenza viruses result in 2–5 million cases of severe illness and up to 250,000–500,000 deaths per year worldwide.

Furthermore, influenza viruses are responsible for seasonal, endemic infections and can emerge periodically to cause unpredictable and devastating pandemics. Vaccination is currently the best, and most cost-effective, countermeasure against influenza virus infections in humans. Although available vaccines offer effective prophylactic protection, particularly in healthy individuals and children, they are limited in scope and are effective against only the circulating strains that are closely related to the vaccine strain. Through a mechanism called "antigenic drift", the genetic variation of influenza viruses allows them to escape immune responses quickly. Therefore, revaccination with reformulated vaccines is required annually to ensure continued protection – an inconvenient, impractical and costly undertaking. In addition, because vaccine strain selection is based on surveillance and prediction, mismatches between vaccine strains and circulating viruses frequently occur, resulting in reduced vaccine efficacy.

Four distinct types of influenza viruses are currently circulating in the human population: two influenza A viruses (the 2009 H1N1 pandemic strain and H3N2) and two divergent lineages of the influenza B virus. Consequently, effective vaccine formulations need to contain at least the two influenza A virus strains and one influenza B virus strain, which further complicates the manufacturing process.

Thus, despite existing vaccination programs, seasonal influenza virus epidemics represent a significant public health burden. To meet this challenge, Dr. Peter Palese, an eminent researcher in the fields of infectious diseases and virology and Chair of the Department of Microbiology at the Icahn School of Medicine at Mount Sinai in New York, believes that to overcome the shortcomings of seasonal influenza virus vaccines and to enhance our pandemic preparedness, game-changing influenza virus vaccines that confer broad, ideally universal, and long-lasting protection are needed. "We all have heard that influenza virus vaccines are not perfect. They have to be administered every year so that we are protected against the seasonal appearance of new strains. Every year, old vaccine components have to be changed. This can be

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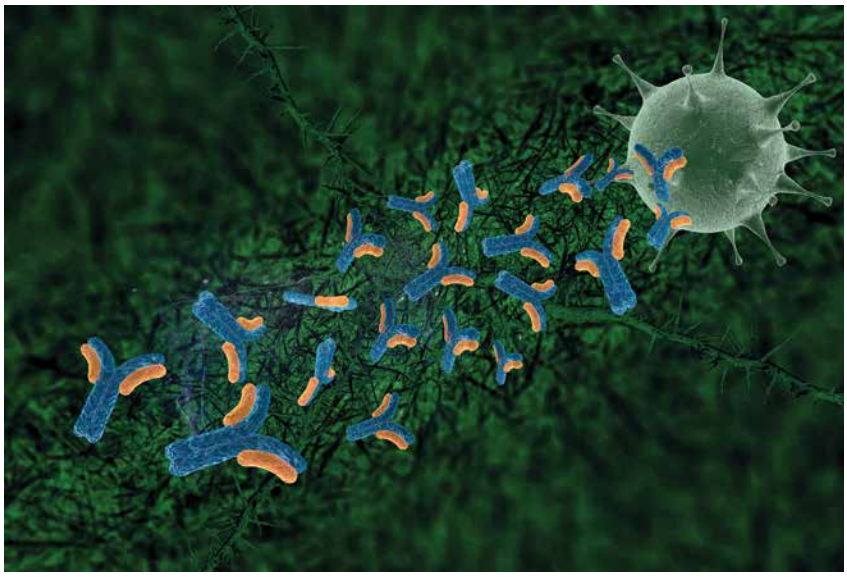
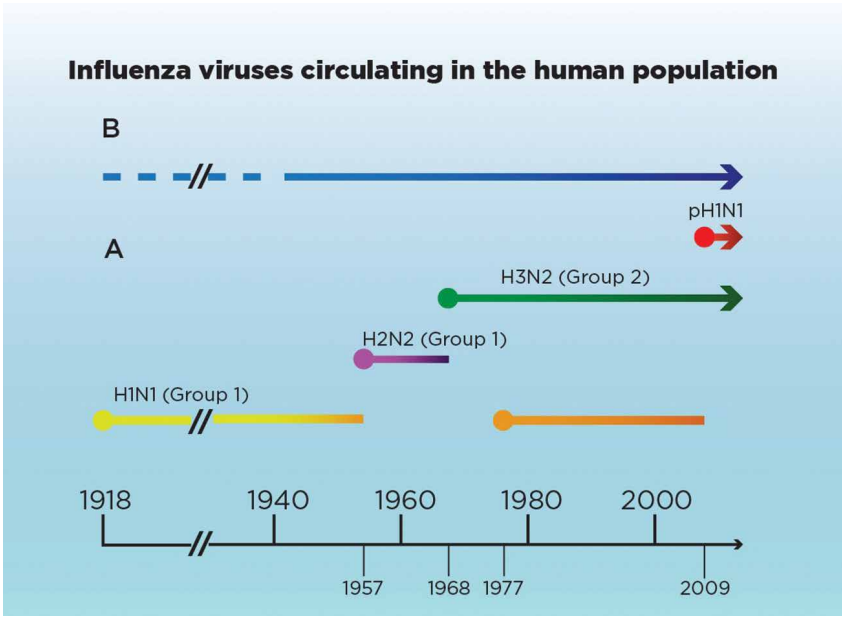




cumbersome and expensive. Our efforts are to develop a universal influenza virus vaccine that does not have to be administered every year, but will last for 20 years or even hopefully for a lifetime."

To achieve this goal, Dr. Palese, with the assistance of numerous experts, is embarking on an exciting influenza vaccine project – substantially advancing research in the field of virology. His ultimate aim is to develop a human universal influenza vaccine that will abolish the need for annual revaccination.

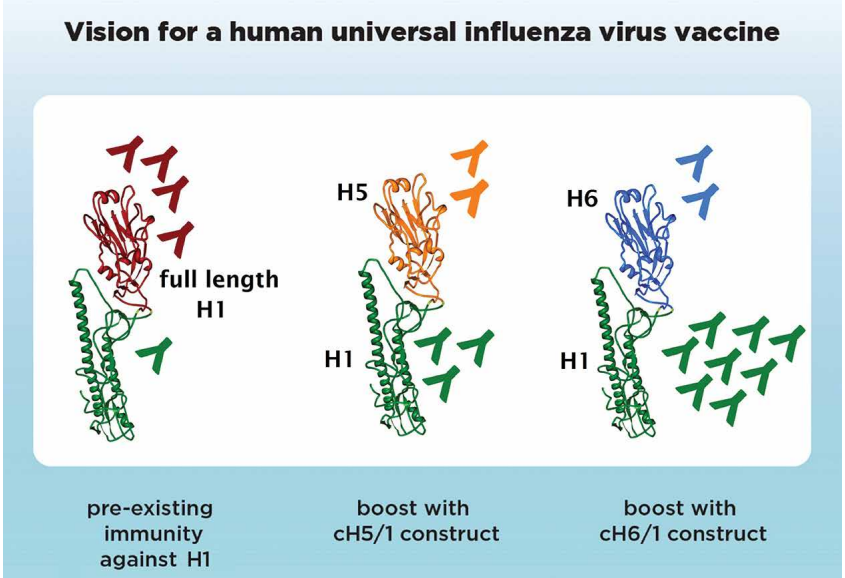
Hemagglutinin – a Viable Vaccine Target

The majority of the humoral (antibody-mediated) immune response against influenza viruses is directed against hemagglutinin (HA) – one of the major surface glycoproteins of the influenza virus. There are eighteen subtypes of HA (H1 through H18), of which only H1, H2 and H3 are found in human influenza viruses. Importantly, H5, H6, H7 and H9, which are found in highly pathogenic avian influenza viruses, can occasionally affect humans at a low rate. The HAs are responsible for binding the influenza virus to sialic acid – a widely distributed monosaccharide (sugar) that is present on the surface of host cells. This allows the influenza virus to replicate and spread infection throughout the host's tissues. Attack from antibodies directed against HA drives antigenic drift, as the head region of the HA molecule exhibits high plasticity (phenotypic adaptability). This plasticity, however, is constrained by the two functions of HA – mediation of the binding of the virus to host cell-surface receptors (through the receptor binding site) and the fusion between viral and endosomal membranes (through the stalk domain). To stay viable, the virus needs to retain the structure of both the binding site and the stalk domain. These regions are therefore conserved in the influenza virus HA.

Recognizing the importance of HA in modulating the spread of influenza virus infection, Dr. Palese and his collaborators in this universal influenza vaccine project – including Dr. Florian Krammer, also based in the Department of Microbiology at the Icahn School of Medicine at Mount Sinai, and Dr. Adolfo García-Sastre, Director of the School's Global Health and Emerging Pathogens Institute – recently developed multiple, broadly-neutralizing, monoclonal, therapeutic vaccine antibodies capable of targeting the conserved regions of HA. Two main classes of these antibodies have been proposed – those directed against the conserved, membrane proximal stalk (or stem) domain of the HA, and those directed against the membrane distal receptor binding site (the head).

Promising Vaccine Candidates Against Influenza

Dr. Palese believes that it will be possible to create a universal influenza virus vaccine by reducing the immunodominance of the influenza virus HA head domain, thereby increasing the immunogenicity of the HA stalk domain. His approach is based on the observation that sequential exposure to influenza viruses with divergent head domain but conserved stalk domain of HA refocuses the immune response towards the conserved stalk domain. He and his team have developed broadly protective vaccine candidates based on chimeric HAs – combinations of globular head domains from an HA of one influenza virus subtype or strain and the conserved stalk domain from an HA of another subtype/strain. "We are looking at



pre-existing immunity, as everyone above 2 or 3 years of age is likely to have antibodies," says Dr. Palese. "We are hoping to give a vaccine that is a chimeric construct, which, in people who have been infected at one point, will cause the immune system memory to recognize these stalk regions and amplify antibody titre against the conserved regions of the virus."

Initially, Dr. Palese and his team designed two vaccine constructs, each comprising a chimeric combination of globular head domains from exotic influenza A viruses and stalk domains from an H1 influenza virus. Both chimeric vaccine constructs were sequentially administered to mice that had been exposed (primed) to an H1 stalk antigen. The animals were then challenged four weeks after the last vaccination with a lethal dose of diverse influenza A viruses. Control groups included prime-only animals that received irrelevant protein vaccinations, vaccination-naïve animals, and standard of care (SOC) positive-control animals that received two doses of a human seasonal trivalent vaccine matched to the challenge strain – a regimen similar to the two-dose regimens recommended for the vaccination of children.

The study resulted in a significant difference in survival between groups vaccinated with the chimeric vaccine constructs and naïve animals, with all naïve animals and a large proportion of prime-only animals succumbing to infection. Notably, both the chimeric construct and SOC vaccination methods provided complete protection from mortality. These encouraging findings led Dr. Palese and his team to further test vaccination with the chimeric construct against challenges with antigenically distinct influenza virus strains. This allowed the comparison of the effectiveness of the novel vaccination regimen with that of the current SOC against antigenically mismatched viruses. Again, the results were promising, with the universal vaccination regimen providing complete protection from morbidity and mortality.

Broadly Protective Antibodies

To determine if sequential vaccination with chimeric vaccine constructs resulted in the production of broadly reactive antibodies, the team went on to analyze the response of the vaccines to purified influenza virions (entire virus particles, consisting of an outer protein shell and an inner core of RNA). A broad range of influenza virus strains were tested, representing strains that had circulated among the human population from 1940 to 2013. In all cases, the novel vaccination approach induced relatively constant antibody responses. This finding has significant value, in that it demonstrates that vaccination with these chimeric constructs promotes the amplification of cross-reactive, neutralizing antibodies against a wide range of diverse influenza viruses and, importantly, these antibodies could protect from future virus challenges.

This is an exciting discovery for Dr. Palese and his colleagues, and they are continuing their research in order to further understand exactly how these antibodies work. "We are now trying to understand the mechanism by which these broadly protective, stalk-specific antibodies mediate antiviral activity and how they differ from regular antibodies that just recognize the head and prevent attachment," says Dr. Palese.

Neutralizing antibodies to influenza virus HA are thought to act predominantly by inhibiting virus attachment to sialic acids on the surface of host cells. Traditionally, these antibodies were thought to interact exclusively through the antibody's variable region – the fragment antigen-binding or Fab region. However, Dr. Palese, in collaboration with Dr. Jeffrey Ravetch from The Rockefeller University, has put forward compelling evidence to suggest that to confer protection, broadly neutralizing HA stalk–specific antibodies require interactions between their tail ends (Fc region) and Fc immunoglobulin gamma receptors (FcγRs) situated on the surface of effector cells that constitute the immune system, such as B lymphocytes, macrophages, neutrophils and natural killer cells. In short, the activated effector cells destroy the viruses or the infected cells by antibody-mediated phagocytosis or antibody-dependent cell-mediated cytotoxicity.

A Vaccine for the Future

Although considerable progress has been made towards demonstrating the potential usability of headless (stem-only) and chimeric HA-based antigens, further research is needed to determine how best to direct the antibody response. Dr. Palese's universal influenza vaccine project continues in its efforts to provide a better understanding of the mechanisms underlying the immune system's response to influenza viruses, infection, and vaccination that will enhance efforts to improve vaccine design.

Development of improved vaccines, including a potentially universal vaccine, will undoubtedly offer broader protection from drifted influenza virus strains, induce long-lived immunity against seasonal strains, and allow for the rational design of vaccines that can be stockpiled for use in an arising pandemic. The use of these vaccines may also prevent, or limit, the emergence of drug resistant strains of influenza virus in human populations – redefining and reducing the negative impact of influenza.

Meet the researcher

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Dr. Peter Palese is currently Professor and Chair of the Department of Microbiology at the Icahn School of Medicine at Mount Sinai in New York, USA. His research is in the area of RNA-containing viruses with an emphasis on influenza viruses. Specifically, he established the first genetic maps for influenza A, B and C viruses, identified the function of several viral genes, and defined the mechanism of neuraminidase inhibitors (which are now FDA-approved antivirals). He pioneered the field of reverse genetics for negative strand RNA viruses, which allows the genetic manipulation of these viruses. This technique is crucial for the study of the structure and function relationships of viral genes, for investigation of viral pathogenicity, and for development and manufacturing of novel viral vaccines. At present, Dr. Palese's group works with Drs. Adolfo García-Sastre and Florian Krammer on the development of a universal influenza virus vaccine. Dr. Palese is a Member of the National Academy of Sciences, a Member of the National Academy of Medicine (formerly IOM) and a Fellow of the American Academy of Arts and Sciences.

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