Commentary

A paradigm shift in vaccine production for pandemic influenza

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The recent introduction of an H5N8 subtype highly pathogenic avian influenza virus (HPAIV) into the United States from Asia is a timely reminder of the propensity of avian influenza viruses to undergo rapid global movement. The subsequent reassortment of this virus to generate an H5N2 subtype variant, which has caused significant mortality in commercial poultry across America (1), similarly highlights the potential of HPAIV to change quickly and unpredictably. Concurrent with the introduction of H5N2 subtype influenza into the US, H7N9 subtype avian influenza virus continues to circulate in birds in China and to cause associated zoonotic infection of humans, with a high case fatality rate in confirmed cases (2,3). Although the incidence of human infections with H7N9 is relatively low, the outbreak has persisted for more than 2 years and spread throughout Eastern China, a region with large areas of high human population density. Given the current high case fatality rate, if the H7N9 virus were to acquire the ability to transmit efficiently in humans, its subsequent emergence into an immunologically naïve population would likely require a major global public health response.

Influenza A virus (IAV) has long posed challenges to public health: seasonal influenza viruses of H1N1 and H3N2 subtypes exert a substantial global burden of morbidity and mortality in human populations with an estimated 1 million deaths annually (4). In the United States alone, the annual burden of seasonal influenza virus is estimated at between 25 and 50 million cases annually, which results in approximately 200,000 hospitalizations per year (5). In addition to seasonal epidemics, the emergence and establishment of pandemic IAVs in humans are characterized by high mortality, including excess mortality in age groups relatively less affected by seasonal influenza. The 1918 H1N1 pandemic influenza virus is reported to have caused on the order of 50 million fatalities worldwide in the immediate aftermath of its emergence (6).

Against this backdrop, the report by Bart and colleagues in *Science Translational Medicine* is a welcome and timely examination of the safety and immunogenicity of an experimental influenza A (H7N9) subtype vaccine in human volunteers. The work is interesting on several fronts. Firstly, the demonstration of safety and immunogenicity of an H7N9 vaccine is clearly desirable, given the potential public health impact of the virus. Secondly, the authors demonstrate the potential dose sparing capacity of adjuvanting the H7N9 vaccine with MF59. Lastly, to generate the vaccine used in their report, Bart and colleagues utilized state of the art technologies and novel production systems, allowing shortened production times and potentially increased antigenic specificity relative to classical methods. In this respect, the study highlights the fruits of ongoing efforts by research and development groups within the influenza vaccine-manufacturing sector to introduce innovation into the vaccine production process.

Currently licensed trivalent inactivated influenza vaccines (TIV) play a major role in protecting public health from influenza related disease (7,8). These vaccines are clearly important, but are also recognized to be suboptimal in certain respects. Many studies of TIV efficacy have been performed (9,10), and while there is considerable debate over the exact numbers, influenza vaccines that are well matched to circulating seasonal strains can provide protection from influenza mortality and morbidity in the general population with approximately 50% effectiveness. Certain populations, such as the elderly, may exhibit lower levels of protection. Thus, while influenza vaccines are efficacious, there is considerable room for improvement.

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Moreover, currently licensed influenza vaccines are typically strain-specific and, in seasons where the vaccine strain differs antigenically from a predominant circulating strain, vaccine efficacy can drop (11). Antigenic drift of influenza viruses necessitates regular vaccine strain updates. Due to the long lead time required for the generation of influenza vaccines and the rapid mutation rate of influenza viruses, however, vaccine mismatches can and do occur.

While a reduction in lead-time of influenza vaccine production is clearly desirable, this aim is currently hampered by major bottlenecks in the manufacturing process. To generate an influenza vaccine, a committee of international experts coordinated by the WHO first identifies the antigenic variants most likely to predominate in an upcoming influenza season by analyzing viral sequence data obtained from national surveillance laboratories. Since 2013-2014, one subtype H1N1, one subtype H3N2 and one or two type B influenza strains are selected for inclusion in the WHO recommended vaccine. The committee convenes months before the onset of an influenza season to allow sufficient lead-time for vaccine production. Following selection, strains are shipped to specified laboratories where they are cultured and reassorted with a high growth influenza virus [A/PR/8/34 (H1N1)] in embryonated chicken eggs. The progeny viruses are tested for appropriate growth properties and antigenic match with circulating strains. Seed strains with the desired characteristics are then shipped onwards to vaccine manufacturing facilities, where the vaccine viruses are inoculated into embryonated eggs, amplified, harvested, pooled, processed to enrich for major neutralizing antigenic proteins, tested again for antigenic match, filled, finished and distributed. Even in the absence of unforeseen production difficulties, this process can take approximately 6 months to complete. If circulating influenza viruses undergo antigenic drift during this time period, vaccine mismatch and associated loss of protection will result. Competition between a putative emergent pandemic strain (e.g., A/H7N9) and currently circulating seasonal strains for growth substrates and vaccine manufacturing facilities significantly complicates decision making in the event of a zoonotic outbreak. Due to long lead times, commitment to any vaccine strain is furthermore an essentially irreversible decision.

The current egg-based vaccine-manufacturing process in the United States is also vulnerable to an insufficient egg supply, for example through disruption of the supply chain in the event of an outbreak of avian influenza, or other poultry pathogen, and a lack of capacity for a surge in production. The current H5N2 HPAIV outbreak in the US is a pertinent illustration of this problem. Since the start of 2015, approximately 40 million commercial poultry have been killed or culled due to the presence of H5N2 influenza virus in commercial facilities. The persistence of this outbreak could significantly impact the availability of chicken eggs for the production of influenza vaccine, thus extending the time of manufacture. Further potential advantages of a cell culture based approach to vaccine production have been discussed recently (12), and include maintenance in an aseptic environment, elimination of egg allergy contraindications, and freedom from seasonal constraints in egg production.

Following the experience of the H1N1 influenza pandemic of 2009, the President's Council of Science and Technology Advisors recognized the limitations of the current influenza vaccine manufacturing process and in 2010 published a report with recommendations for improving influenza vaccine production (https://www.whitehouse.gov/assets/documents/PCAST_H1N1_Report.pdf). Some of the report's key recommendations included increasing surveillance of circulating influenza virus strains, development of technologies that allow faster production of vaccine seed strains and facilitating a shift from egg-based to cell culture-based production processes.

In recent years, the use of recombinant DNA techniques has facilitated the laboratory generation of influenza viruses, including candidate vaccine strains, as soon as knowledge of the viral sequence is obtained (13-15). Using sequence data deposited in the GISAID database by Chinese Centers for Disease Control (16) and by applying DNA synthesis and influenza virus reverse genetics technologies, Bart and colleagues achieved a considerable reduction in the time required to generate an H7N9 vaccine seed strain relative to conventional methods. Their approach circumvents the need for the generation of reassortant viruses in chicken eggs, thereby shortening the time between strain selection and harvesting of sufficient virus for use in the antigenic protein enrichment step of production. A further benefit of synthesizing the vaccine seed strain directly is that handling of highly pathogenic viruses is avoided. Moreover, the group used MDCK cell culture for amplification, eliminating reliance on embryonated eggs and the potential for egg adaptive mutations during growth (17,18).

In the current *Science Translational Medicine* study (19), Bart and colleagues report the results of a phase I safety and immunogenicity trial in healthy adult volunteers which utilizes a synthetic, cell culture-derived, subunit

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vaccine containing A/PR/8/34 internal genes and the A/Shanghai/2/13 (H7N9) HA and NA genes. A prime-boost vaccine regimen with a 3-week interval was used. Three groups of volunteers received escalating doses of vaccine (3.75, 7.5, or 15 μg HA protein) combined with the Novartis MF59 adjuvant formulation, while a fourth group received a 15 ug dose without adjuvant. Only minor adverse reactions, including mild reaction site soreness and fatigue, were reported across all treatment groups, confirming an acceptable safety profile for the vaccine.

As expected for an influenza virus which has not circulated within the human population, no participants in the trial exhibited preexisting immunity to the virus, as assayed by hemagglutination inhibition (HI) or microneutralization (MN) assays. Using the standard assay for measuring correlate of protection from influenza virus disease (HI), the authors demonstrate likely protective increases in serum titers (≥40) in at least 50% of recipients in each of the groups receiving adjuvanted vaccine. Additionally, a dose response effect was observed 3 weeks post boost in these groups. In the group receiving unadjuvanted vaccine, only around 5% of individuals had serum responses associated with protection.

In summary, current seasonal vaccines are unlikely to provide protection from disease in the event of the emergence of A/H7N9 virus into human populations. Bart and colleagues have demonstrated safety and immunogenicity of a candidate pandemic vaccine in adults, with likely protective responses occurring in approximately 50% or more of individuals and have shown that dose sparing is possible through the addition of MF59 adjuvant. Reliance on a supply of embryonated chicken eggs is avoided and potentially life-saving reductions in the time for vaccine production have been demonstrated through the use of a novel cell culture based production system.

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None.

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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