

# Emerging Technologies for Influenza Vaccination

Aniket Schneider

## Abstract

Mounting concerns about the pandemic potential of H5N1 avian influenza make the development of more effective vaccines an urgent need. Because of the high pathogenicity of the H5N1 viruses, conventional vaccines cannot be produced against these strains. New vaccines based on reverse genetics will soon become licensed, but suffer from limitations in production capacity. New, more robust techniques, however, are still in the early stages of development. This review discusses the current status of influenza vaccination technology and the drawbacks and benefits of various emerging techniques.

## Introduction

The influenza virus spreads across the world in seasonal epidemics, causing approximately 250,000 to 500,000 deaths per year. While most people recover within one to two weeks without medical attention, the very young, the elderly, and people suffering from various co-morbidities experience more serious complications<sup>1</sup>. On rare occasions, influenza strains arise to which humans have no innate immunity. These strains have the potential to create pandemics such as the Spanish Flu pandemic in 1918 which killed over 40 million people<sup>2</sup>.

The classification of a strain of influenza virus depends on the various antigenic proteins expressed by the virus. Differences between the nucleoprotein and matrix protein of the virus are used to classify virus strains into three broad categories, types A, B and C, with type A being the most virulent in humans. Type A strains are further subdivided by differences in their surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). These two proteins, particularly hemagglutinin, provide the major targets for the host immune response<sup>3</sup>. Currently, the H1N1 (hemagglutinin 1, neuraminidase 1) and H3N2 strains of influenza A circulate most widely in humans<sup>1</sup>.

Recently, however, a new strain of influenza, with many of the characteristics of a pandemic virus, has been discovered in birds. This virus, classified as an H5N1 strain of influenza A, has a very high pathogenicity, and of the 291 confirmed cases of infection to date, 172 (59%) have resulted in the death of the patient<sup>4</sup>. Luckily, the virus cannot yet be transmitted from human to human, but health care officials are concerned that it may reassort with another influenza strain and become a major pandemic threat<sup>5</sup>.

Apart from its high pathogenicity and mortality rates, H5N1 avian flu has several features that make it a particularly potent pandemic threat. Because of the strain's novel H5 surface antigen, humans have no preexisting immunity to the virus. Additionally, there are no known H5N1 strains with low pathogenicity from which to produce a vaccine. Finally, due to extensive use of antiviral drugs to control the spread of illness in poultry during a 1997 outbreak of H5N1 in Hong Kong, much of the H5N1 virus in circulation is already resistant to one of the major influenza antiviral drug types, adamantane<sup>6</sup>. Because of these factors, development of a vaccine against H5N1 avian flu has become a high priority.

## Conventional Vaccines

The standard method for creation of influenza vaccines involves growing a virus in embryonated chicken eggs. Allantoic fluids of the eggs are harvested and the virus is purified out, after which it can be injected directly, and function as a live virus vaccine, or it can be chemically inactivated, and function as an inactivated whole virus or inactivated subvirion vaccine<sup>3</sup>. However, the high pathogenicity of H5N1 strains presents a challenge to this traditional approach of vaccine production in an egg: not only does the virus present an unusually great danger to the people working on the vaccine, requiring a higher biosafety level than is present in most production plants, but it also infects the eggs so effectively that it kills the eggs before the virus reaches a sufficiently high concentration for harvesting<sup>5</sup>.

Substances called adjuvants can greatly increase the potency of vaccines administered at low doses, a technique that is especially useful when very little vaccine is available<sup>7</sup>. Many types of compounds can be used as adjuvants, but the ones most commonly effective with influenza vaccines are an aluminum salt called alum, and a proprietary emulsion of squalene called MF59<sup>7, 8</sup>. The use of both types of adjuvants in humans is currently being tested in large-scale clinical trials in the US, and MF59 is already in use in European vaccines<sup>7</sup>. Despite their promise, though, adjuvants have not solved the problem of creating a pandemic flu vaccine, and United States regulatory agencies have been slow to approve their use<sup>7</sup>.

Attempts to use related low pathogenic avian influenza (LPAI) strains to create vaccines against H5N1 have met with very limited success<sup>9, 10</sup>. These vaccines achieved acceptable immunogenicity when augmented by the use of an adjuvant, demonstrating that adjuvants increase the cross-reactivity of the antibodies induced by a vaccine. Despite their low pathogenicity, however, these strains grew poorly in chicken eggs<sup>10</sup>. This type of vaccine avoids most licensing barriers by making use of previously licensed technology, but production limitations make these vaccines suitable only for high-risk patients.

## Reverse Genetics

The most promising body of research into vaccine production involves a technique called reverse genetics, which allows manipulation of the virus genome in cell culture<sup>11-15</sup>. A set of plasmids encoding the entire virus genome is transfected into a eukaryotic cell line, allowing the cells to produce entire virus particles without having been infected<sup>15</sup>. Because of the ease with which plasmid DNA can be modified, this general approach allows controlled manipulation of the features of an influenza strain, and can be used to produce desired characteristics like low pathogenicity and specific surface antigens.

Research has focused on two classes of vaccines produced with reverse genetics, whole virion and subvirion vaccines. In whole virion vaccines the inactivation process preserves the overall structure of the virus particle. Subvirion vaccines, on the other hand, include only specific purified protein subunits. Immunogenicity of whole virion vaccines generally exceeds that of subvirion vaccines<sup>16</sup>, making them good candidates for pandemic flu vaccines.

Generally, both types of vaccines are derived from two influenza strains, a pathogenic strain and an attenuated strain. The pathogenic strain supplies genes for the surface glycoproteins, allowing the vaccine to generate a specific immune response against the original strain. Genetic material from the attenuated strain reduces the pathogenicity of the recombinant virus, allowing it to grow efficiently in eggs. Additionally, in the case of H5N1, the H5 antigen actually causes some of the high pathogenicity of the strain, so the sequence of the H5 HA gene must be modified to reduce this effect<sup>3</sup>.

Treanor and colleagues developed an inactivated subvirion vaccine as a preliminary step toward a whole virion vaccine<sup>13</sup>. Using reverse genetics, they replaced the surface antigens of a common vaccine platform virus with H5N1 surface antigens, and modified the H5 HA gene to reduce pathogenicity, allowing efficient growth of the seed virus in eggs<sup>13</sup>. The resulting virus was purified out of the eggs and disrupted into its component proteins using a detergent, after which the H5 HA and N1 NA proteins were purified out and administered to patients without adjuvant<sup>13</sup>. While this vaccine was only marginally effective at high doses (two doses of 90 ug induced protective antibody levels in only 58% of patients<sup>13</sup>), use of an adjuvant could potentially increase its effectiveness.

Meanwhile, results of early clinical trials of an inactivated whole virion vaccine have shown promise. Lin and colleagues have created and tested a vaccine derived from a highly pathogenic H5N1 strain and an attenuated H1N1 strain, again with modifications to the H5 HA protein to reduce its pathogenicity<sup>12</sup>. This vaccine was prepared in the conventional way and inactivated using formalin<sup>12</sup>, a chemical which disables the virus but preserves its structure. The vaccine, administered with an aluminum-based adjuvant, induced protective levels of antibody in around 80% of patients with a dose of only 10 ug<sup>12</sup>. The trials were conducted in China, however, and approval of this vaccine by foreign regulatory agencies will have to wait for larger scale trials under their jurisdiction.

## Novel Alternative Approaches

Reverse genetics-based vaccines rely on well-established technology and tested techniques, and require no changes in the existing vaccine production infrastructure. There are disadvantages, however, to the conventional method. Drug companies base their production capacity on yearly sales, so there is no incentive for them to build the capacity for a pandemic response that may or may not come. The existing limited production capacity cannot be used to stockpile vaccine, because an emerging pandemic strain is likely to have mutated from the original vaccine targets. Finally, practical matters like egg supply and biosafety level requirements place additional limitations on vaccine production.

A number of new ideas are being explored in order to circumvent the weaknesses in conventional and reverse genetics-based vaccines. Increasing production capacity is one important priority, but others include creating broader-spectrum vaccines, increasing the effectiveness of small vaccine doses, and decreasing the lag time between identification of a strain and production of the vaccine. Most of these techniques are still under development, and have not yet undergone clinical trials in humans. Additionally, because they involve developing completely new technology, the licensure process for these vaccines will be longer. Nonetheless, they offer great potential for improving the effectiveness of future vaccines.

One idea is to simplify the manufacturing process by using recombinantly expressed HA protein alone as a vaccine. The gene for H5 HA is transfected into and expressed in cultured

cells, which can then be induced to overexpress the H5 antigen<sup>17</sup>. The resulting vaccine is similar to an inactivated subvirion vaccine, but does not require the cumbersome egg-based system of production. In humans, this vaccine works only at high (90 ug) doses and only in roughly 50% of patients<sup>17</sup>, but perhaps with the addition of an adjuvant to increase immunogenicity it could become a viable option.

Other researchers, attempting to broaden their range of protection, have created a vaccine which induces antibodies against the virus matrix protein, M2, which is conserved in all influenza A strains but is normally not immunogenic. The technique involves attaching M2e, the external portion of the M2 protein, to the hepatitis B virus core, which greatly increases its immunogenicity<sup>18</sup>. With the use of various adjuvants and with several copies of the M2e domain per particle, this vaccine successfully conferred protective immunity to an influenza A strain in mice, though the mechanism by which antibody production was induced is not well characterized<sup>18</sup>. This vaccine is still in the early testing stages, but it shows great promise since it would allow stockpiling of vaccine in advance of a pandemic outbreak, as well as immunization against a wide range of influenza strains with a single vaccine.

A very new type of vaccine using an adenovirus as a vector also circumvents many of the problems with conventional influenza vaccines, this time by allowing the host organism's body to do much of the manufacturing work. In this type of vaccine, a replication-defective adenovirus strain is modified to carry an antigen, in this case H5 HA. When the virus infects the host organism, the infected cells start producing large quantities of the antigen instead of producing new virus particles. Additionally, certain strains of adenovirus preferentially infect human antigen-presenting cells, which would amplify the immune response and allows use of a smaller vaccine dose<sup>19</sup>.

Adenovirus vector-based vaccines have been tested in mouse and chicken model systems, and they confer protective immunity even against antigenically distinct flu strains, provided that they carry the H5 antigen<sup>19, 20</sup>. Adenovirus vector-based methods have been used in over 100 clinical trials for other purposes, so the technology is well understood<sup>20</sup>. Additionally, Gao and colleagues managed to move from virus sequence to vaccine production in just 36 days, which would facilitate fast response in case of a pandemic<sup>20</sup>. Finally, the vaccine can be produced efficiently in an egg-free system<sup>19, 20</sup>. Unfortunately, this type of vaccine is quite far from being licensed for use, at least in the United States. For now, having been proven in chickens, adenovirus vector-based vaccines may at least be useful for controlling the rampant spread of avian flu in poultry<sup>20</sup>.

## Conclusions

The FDA recently awarded the first US license for a vaccine against avian flu in humans to Sanofi-Pasteur for an inactivated subvirion vaccine<sup>13, 21</sup>. While this step will

greatly enhance the country's ability to respond effectively to an H5N1 flu outbreak, it does not solve many of the systemic issues in conventional vaccine technology. Conventional vaccines such as this one, even those modified with reverse genetics, still suffer from production bottlenecks and shortages, as well as having relatively slow response times when facing a potential pandemic. Thanks to intensive research in recent years, a great many options for new types of vaccines now exist, but the most robust, cheap, and effective vaccines are still under development.

At the moment most governments, lacking a suitable vaccine to invest in, are stockpiling influenza antiviral drugs as their backup plan. H5N1 flu, however, has already developed considerable resistance to the adamantane class of drugs<sup>6, 19</sup>, and recently various influenza strains are exhibiting resistance to oseltamivir<sup>22, 23</sup>, one of the other two major classes of flu antiviral. This development only serves to underscore the critical importance of continuing vaccine research.



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