Introduction to influenza A (H5N1)

Avian flu, or Influenza A (H5N1), is an RNA virus belonging to Influenzavirus A, one of the five genera classified under the Orthomyxoviridae family. Influenzavirus A has only one species, Influenza A, which has been responsible for all worldwide influenza pandemics. These viruses are further subdivided according to two important surface protein antigens, hemagglutinin and neuraminidase. Variations within these proteins have been responsible in pandemic strains of influenza such as the Spanish Flu (H1N1), which claimed upwards of 20 million lives. Human cases of Avian flu primarily belong to the H5N1 subtype, though rarer subtypes others have been noted. The presentation and vaccine development of the H5N1 subtype is contrasted to H1N1, but the same applies for H3N2, the other major subtype of human influenza virus.

H5N1 human infection was discovered in 1997 and subsequently re-emerged in 2003-2004 in poultry and human populations in several Asian countries. Patients present with symptoms 2-4 days after exposure but may be asymptomatic for up to 8 days. Unlike seasonal influenza, the nature of virus shedding in these cases is unknown at this point. Besides the common symptoms of cough, fever and shortness of breath, there are several features unique to infection with H5N1. Unilateral pneumonia progressing into a bilateral pattern within the span of 4 days is more common and can be detected on an X-ray. This is different from the cases of pneumonia which develop subsequent to H1N1 infection as they are quite rare. The mortality of patients infected with H5N1 approaches 60% and death occurs due to respiratory failure secondary to fulminant bilateral pneumonia. It should be noted that this rate has been obtained from reported cases and that the actual number might be higher. As opposed to H1N1, H5N1 might involve extrapulmonary sites as well. For example, viral RNA has been isolated from the blood of patients who died from H5N1 infection. Furthermore, viral RNA has also been detected in areas such as the liver, lymph nodes and brain. This may explain why H5N1 infection also produces gastrointestinal symptoms such as diarrhea, vomiting and abdominal pain.

In the event of an outbreak of H5N1 infection, the modes of transmission of the virus must be examined in order to initiate effective countermeasures. The H5N1 virus is present in poultry and most cases of human infection have occurred in situations where there is close contact with live or dead birds. There is also the possibility that infection occurs via the gastrointestinal tract as viral RNA has been found in feces of infected individuals. Finally, there have been very few cases of human-to-human spread at present and these cases involved lengthy contact with infected individuals. In summary, both animal-to-human and human-to-human spread remains inefficient at present but this could change if a mutation occurs in the H5N1 virus.

Vaccine development

The World Health Organization monitors influenza activity throughout the world and makes a recommendation for the seasonal influenza vaccine every year. Postinfection ferret sera are tested in an assay called the hemagglutination-inhibition (HI) test where hemagglutinin from
different strains of H1N1 are tested to see which elicits the highest immune response. This data is used to make a recommendation around February for the upcoming influenza season.

An ideal vaccine for H5N1 infection should induce a strong mucosal antibody (IgA) response as the primary site of H5N1 infection is the respiratory tract.\(^9\) Due to the extrapulmonary nature of H5N1 infection, a vaccine which induces a cell-mediated response as well would help protect from systemic manifestations of infection. This cell-mediated response needs to be generated against conserved elements of the virus such as matrix and nucleoproteins as other components mutate rapidly. Finally, this vaccine should be easy to produce as large amounts will be needed in the event of a potential or imminent pandemic.

Inactivated H1N1 influenza viruses are used for prophylaxis against seasonal influenza.\(^9\) This approach is restricted to a humoral immune response only. Live attenuated H1N1 viruses were developed to overcome this hurdle as they induce cell-mediated responses as well. These approaches are not helpful for H5N1 human infection because of the long period of time required to produce these vaccines due to the requirement of chicken eggs to complete the process. Another problem with using live attenuated viruses for H5N1 infection prophylaxis is that there is the chance of developing a deadlier strain if they undergo genetic reassortment with another strain. Live attenuated H5N1 viruses have been shown to induce immunity in chickens when challenged to H5N1 infection however and will most likely be used as a last resort during a pandemic.\(^10\)

The use of adenoviruses as vectors provides an attractive alternative to develop vaccines for H5N1 human infection.\(^11\) Specifically, adenovirus serotype 5 can be genetically engineered to express hemagglutinin specific to H5N1. The production of adenovirus vectors is faster than that used for influenza vaccines since they do not require the use of chicken eggs. These vaccines can be administered intranasally and this eliminates the need for specialized personnel to administer them in the event of a pandemic. The main drawback to this modality is that a certain segment of the world population is immune to this subtype of adenovirus and this may dampen the response to hemagglutinin required as the virus may be cleared quickly. However, it has been shown that even in people who possess natural immunity, the vaccine induces antibody production against hemagglutinin.\(^11\) Once large-scale studies examining the safety profile and efficacy of adenovirus-vectored vaccines is complete, they can be produced in large quantities to be used as prophylaxis during a pandemic.

**The role of influenza A (H5N1) vaccines in a global pandemic**

A potential H5N1 pandemic can be a serious threat to global health. Applying data from the 1918 flu pandemic, a computer model has been developed that predicts between 50 and 80 million people worldwide could be victims to H5N1.\(^12\) Preventing a pandemic of this magnitude requires controlling it and containing it as early as possible. Vaccines play an important role in controlling H5N1, and the World Health Organization (WHO) has announced that they will be stockpiling vaccine in preparation for a global pandemic.\(^13\) The current plan put forth by the WHO is to stockpile 50 million vaccines, which would protect 25 million people at two doses per person.\(^14\) The WHO is charting new territories with regards to stockpiling vaccines. Usually, vaccines for pandemics cannot be prepared until the particular strain of virus makes its way into the population. Using current vaccine development technology, this could take at least 4-6 months after the WHO declares a particular strain of virus to be a pandemic.\(^15\) The current strategy is to stockpile “prepandemic” vaccines. This involves preparing vaccines with currently circulating H5N1 strains, but with cross-reactivity to other emerging strains. For the development of an effective vaccine, it is essential to monitor H5N1 strains as well as any drift which occurs by the accumulation of mutations in H and N antigens. This would require rapid testing of patients at the level of individual communities. Cultures should be obtained from patients in
designated clinics which are representative of the community.\textsuperscript{16} This information should then be escalated to a local public health office. Patients receiving the vaccine would be primed towards H5N1, buying time until a more specific booster is developed and administered.\textsuperscript{17} To adapt to other strains, cross-reactivity is elicited by adding certain adjuvants to the vaccine, which also induce a stronger immune response and require lower doses of vaccine to be administered. GlaxoSmithKline is developing an inactivated, prepandemic vaccine for H5N1 which has undergone phase I and II clinical trials, and has been found to be safe in healthy adult volunteers.\textsuperscript{18}

There are logistical issues surrounding stockpiling not limited to distributing of millions of vaccines and making vaccines available to the developing world. It is a global responsibility to ensure that the developing world receive vaccines and will require global cooperation. The WHO has not come up with a specific plan regarding the stockpile use, and admit to still working on one. Another issue is the lack of data regarding vaccine safety and efficacy in large human clinical trials. There is little research on the vaccine in paediatric and elderly populations, which are considered to be at the highest risk for H5N1. Careful thought and planning must go into making decisions not only at the level of the laboratory, but in policy to ensure that stockpiling vaccines can be taken seriously as a solution to prevent a worldwide H5N1 pandemic.

References