Influenza: Mitigation and Prevention

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Influenza Virus

Causative agent of influenza

Infection can lead to:
- pneumonia
- sinus/ear infection
- worsening of chronic conditions (asthma, diabetes, heart disease)
- Death

Electron micrograph of avian influenza H5N1 virus particles

Courtesy of R. Fleck, National Institute of Biological Standards and Control, UK
Influenza, the annual killer epidemic that won't go away...
Structure of influenza A

Tamiflu blocks activity.

Envelope
- Neuraminidase (NA)
- Ion channel (M2)
- Lipid bilayer
- Matrix protein (M1)
- Hemagglutinin (HA)

Segmented (-) strand RNA genome

Nucleocapsid protein (NP)

Cap and RNA polymerase (PB1, PB2, PA)

Nuclear packing protein (NS2)

Influenza virion

HA

100 nm

Robert A. Lamb
Influenza Virus Nomenclature

A/Fujian/411/2002 (H3N2)

- Virus Type
- Geographic Origin
- Strain Number
- Year of Isolation
- Virus Subtype
Antigenic shift and drift

**Mutation**

**Antigenic drift**

- Small Mutations

**Antigenic shift**

- New Strain
Shift and Pandemic Influenza

Avian virus

Avian reassorted virus

Reassortment in swine

Reassortment in humans

Avian-human pandemic reassorted virus

CDC
Antigenic shift – the genetic change that enables a flu strain to “hop” from one animal species to another, including humans, is not new to science – it is exactly this that brought the 1957 Asian flu pandemic and the Hong Kong flu outbreak in 1968.

Here is a look at the three ways whereby antigenic shift can produce new viral strains that our bodies have little or no defences against.

**METHOD 1**

Without undergoing genetic change, a bird strain of Influenza A can jump directly from a duck or other aquatic bird to humans.

**METHOD 2**

Without undergoing genetic change, a bird strain of Influenza A can jump directly from a duck or other aquatic bird to an intermediate host and then to humans.

**METHOD 3**

A duck or other aquatic bird passes a bird strain of Influenza A to an immediate host such as chicken or pig.

A human host passes a human strain of Influenza A to the same chicken or pig.

When the viruses infect the same cell, the genes from the bird strain mix with genes from the human strain to yield a new strain. The new strain can spread from the intermediate host to humans.

The new strain may further evolve to spread from person to person. If so, a flu pandemic could arise.
Hemagglutinin and Neuraminidase Subtypes of Influenza A Virus
The Great Pandemic

The Spanish flu pandemic of 1918

It infected 500 million, people across the world and killed 50 to 100 million of them - three to five percent of the world's population.
Over the past century, humanity has witnessed numerous emergences of zoonoses - Influenza viruses account for an important proportion of these deaths.
BIRD FLU
Is Asia hatching the next human pandemic?
Haemagglutinin (HA) is a major determinant of the pathogenicity of avian influenza viruses in poultry.
Countries in which the H5N1 HPAI virus has been detected in domesticated poultry and migratory wild birds

(Source: Wild birds and Avian Influenza: An Introduction to Applied Field Research and Disease Sampling Techniques. 2007.)
Sialic acid in the influenza receptor

- **Sialic acid**
- **Galactose**

- Human host receptor linked $\alpha_{2,6}$.
- Avian host receptor linked $\alpha_{2,3}$ as shown.
Cell barrier slows bird flu's spread among humans

Why H5N1 viruses rarely infect and spread from human to human?

Cells in the upper portions of the respiratory system lack the surface receptors that enable avian H5N1 virus to dock with the cell.
The H5N1 avian flu virus has infected and killed hundreds of people, despite the fact that, at the moment, the virus can’t spread easily between people. It thus only infects those working with infected poultry.

The death toll could become much worse if the virus gains the ability to infect in the human upper respiratory tract and becomes airborne!!!
Therapy/Prevention of Influenza

- Medications
  - Requires use of drugs such as Tamiflu early in the disease course
  - All not uniformly effective against Influenza Types A and B
  - Can be costly
  - Not all approved for children

- Vaccine(s)

- Handwashing!
Standard influenza vaccines:

Trivalent Inactivated Vaccine (TIV), intramuscular.

Influenza Vaccine Virus Strains for 2013-14:

- Trivalent vaccines will contain:
  
  A/California/7/2009 (H1N1)-like virus,
  A/Victoria/361/2011
  B/Massachusetts/2/2012-like virus (Yamagata lineage).

- Quadrivalent vaccines, will contain, in addition:
  
  B/Brisbane/60/2008-like virus (Victoria lineage)
Traditional production of influenza virus vaccine

1. Co-infection of chicken eggs with A/PR/8/34 and epidemic strain
2. Re-assortment and selection of seed strain
3. Propagation of seed strain in the chicken eggs
4. Purification of harvested vaccine virus by zonal centrifugation
5. Treatment with ethyl ether
6. Treatment with detergents
7. Purification of HA
8. Treatment with formaldehyde
9. Inactivated vaccine
10. Split vaccine
11. Subunit vaccine
12. Evaluation in human volunteers
A flu virus contains eight gene segments. The goal is to combine the desired HA and NA genes from flu strain 1 with the six other genes from flu strain 2, which grows well in eggs and is harmless in humans.

1. After removing the dangerous part of the HA gene, scientists splice the HA and NA genes from flu strain 1 into circular pieces of DNA called plasmids.

2. Additional plasmids are created using the remaining six genes found in flu strain 2.

3. Scientists insert the HA and NA plasmids from flu strain 1 and the six plasmids carrying genes from flu strain 2 into animal cells growing in the laboratory.

4. The genes in the plasmids instruct the animal cells to make the desired new flu strain.

5. New flu strain.
Conventional Methods of Influenza Vaccine Production

Egg propagation

Time consuming, expensive and incompatible with propagating High Path avian influenza strains.
Egg-Based Production of Influenza Vaccine

Technician Working on Egg-Based Production of Influenza Vaccine
Aventis Pasteur MSD/Getty Images
Current Influenza Vaccines

- Safe
- Effectiveness is variable and depends on multiple factors
- Formulated and standardized based on HA content to induce virus neutralizing antibodies
- Egg-based technologies limit rapid response and surge capacity for pandemics
1 dose of Flu vaccine per fertile egg

Would need at least 60 million hens and 4 million roosters to produce enough vaccine each year to immunize the world population. This is about 20 to 30 times the current global vaccine production capacity.

Production of vaccine virus in tissue culture cells poses almost as many problems.
Cell-Culture–Based Production of Influenza Vaccine

Microcarriers with Vero cells are shown before (left) and after (right) infection with influenza virus, Baxter Vaccine.
Live Attenuated Influenza Vaccine

FluMist
Nasal spray; cold-adapted influenza viruses propagated by infection of cells in culture and manufactured in eggs

Vaccine induces neutralizing and mucosal antibodies but the extent to which it induces T-cell immunity is not yet known.
Salmonella: Our Friend

Recombinant Attenuated *Salmonella* Vaccine (RASV) Vector
We have now constructed three different recombinant attenuated *Salmonella* vaccines that can be grown in fermentors delivering conserved M2e, NP-HA T-cell epitopes, and a DNA vaccine encoding variable HA antigens to protect humans and poultry against diverse influenza viruses.

Our objective is to induce long-lasting immunity to conserved influenza antigens as well as to the annually variable hemagglutinin (HA) antigens.
The RASV system for delivery of multiple protective antigens to humans (and birds)

Influenza vaccine

Cross reactive immune response (M2e, NP)
- M2e displayed by a Woodchuck core (WHc) particle
- NP delivered by regulated delayed lysis vector

Protective immune response
- DNA vaccine (HA, NA)

B & T cells
- T & B cells
pYA4859 (DNA vaccine vector pYA4545 specifying influenza HA)
The synthesis of EGFP from pYA4545 harboring EGFP gene in Vero cell line

Transfection of 1 µg DNA vaccine plasmid. TO-PRO-3 iodide (642/661) nucleus staining (Blue) and fluorescence of GFP (green) were visualized 20 hours post-transfection using a confocal microscope (Leica Microsystems Heidelberg GmbH, Objective 20 X).
*Salmonella* cell lysing to release DNA vaccine vector encoding influenza HA antigen to be synthesized by cells in the immunized human or avian host.
Influenza